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# Hypergravity within a critical period impacts on the maturation of somatosensory cortical 1 maps and their potential for use-dependent plasticity in the adult.

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1 **Hypergravity within a critical period impacts on the maturation of somatosensory cortical**  
2 **maps and their potential for use-dependent plasticity in the adult.**

3

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19 **Author contributions**

20 Y.Z.A and C.X. conceived and designed research, performed experiments, analyzed data and

21 interpreted results, prepared figures and wrote manuscript. N.C. analyzed data and

22 prepared figures.

23

24 **Running title:** HG influence on maturation of somatosensory maps

25 **Abstract**

26

27 We investigated experience-dependent plasticity of somatosensory maps in rat S1 cortex  
28 during early development. We analyzed both short- and long-term effects of exposure to 2G  
29 hypergravity (HG) during the first 3 postnatal weeks on forepaw representations. We also  
30 examined the potential of adult somatosensory maps for experience-dependent plasticity  
31 after early HG rearing. At P22, HG was found to induce an enlargement of cortical zones  
32 driven by nail displacements and a contraction of skin sectors of the forepaw map. In these  
33 remaining zones serving the skin, neurons displayed expanded glabrous skin receptive fields  
34 (RFs). HG also induced a bias in the directional sensitivity of neuronal responses to nail  
35 displacement. HG-induced map changes were still found after 16 weeks of housing in  
36 normogravity (NG). However, the glabrous skin RFs recorded in HG rats decreased to values  
37 similar to that of NG rats, as early as the end of the 1<sup>st</sup> week of housing in normogravity.  
38 Moreover, the expansion of the glabrous skin area and RF size decrease normally induced in  
39 adults by an enriched environment (EE) did not occur in the HG rats, even after 16 weeks of  
40 EE housing in normogravity. Our findings reveal that early postnatal experience critically and  
41 durably shapes S1 forepaw maps and limits their potential to be modified by novel  
42 experience in adulthood.

43

44 **Keywords:** cortex, development, critical period, electrophysiological mapping, forepaw.

45

46 **INTRODUCTION**

47

48 Experience-dependent shaping of morphological and functional brain circuits leads to  
49 appropriately organized neural networks that subserve adult brain functions. The effect of  
50 the so-called epigenetic factors is most prominent within postnatal “critical periods” (Hensch,  
51 2004; Knudsen, 2004), during which the CNS shows a powerful structural and functional  
52 plasticity, reflecting a process of adaptation of genetically specified neural properties to  
53 environmental constraints. The barrel cortex has been extensively investigated as a model of  
54 the experience-dependent refinement of the primary somatosensory (S1) cortex during  
55 development. Damage to whisker follicles during the first few days after birth prevents the  
56 formation of barrels within layer 4 of the S1 cortex (Woolsey and Wann, 1976, O’Leary et al.,  
57 1994; for a review see Erzurumlu, 2010, Erzurumlu and Gaspar, 2012). By contrast, whisker  
58 trimming does not affect barrel-pattern formation (Simons and Land, 1987, Fox, 1992,  
59 Henderson et al., 1992, Rema et al., 2003), but impairs the neurophysiological properties of  
60 cortical neurons (Simons and Land, 1987, Fox 1992, Stern et al., 2001, Rema et al., 2003,  
61 Shoykhet et al., 2005) as well as whisker discrimination (Carvell and Simons, 1996).  
62 Surprisingly, few studies have investigated the maturation of body somatotopic maps in the  
63 S1 cortex (Armstrong-James, 1975; McCandlish et al., 1993, Seelke et al., 2012), and little is  
64 known about the experience-dependent plasticity of neural circuits underlying the postnatal  
65 development of these somatosensory maps.

66 Somatosensory cortical maps reflect a dynamic competition between proprioceptive  
67 and cutaneous inputs converging onto cortical networks (Jenkins et al., 1990; Recanzone et  
68 al., 1992; Xerri et al., 1994, Coq and Xerri, 1998). Gravity is a fundamental element in the  
69 external environment, stimulating vestibular receptors and exerting mechanical constraints

70 on the musculo-skeletal system, while providing a reference frame for posture and  
71 movements. We reasoned that changes in gravity modify the relative weight of  
72 proprioceptive and cutaneous inputs from the limbs converging on the S1 cortex and thus  
73 provide a unique opportunity to investigate the developmental emergence of  
74 somatosensory maps in an environment modifying inputs from the limbs, without sensory  
75 deprivation.

76 Decades of research have shown that environmental conditions and sensorimotor  
77 training shape the morphological and functional organization of sensory and motor cortical  
78 networks throughout life though to a lesser extent than during developmental stages (for  
79 review see Buonomano and Merzenich, 1998; Xerri, 2008; Barnes and Finnerty 2010). An  
80 important conceptual challenge is to decipher the interplay between developmental and  
81 adult experiential plasticity.

82 Our *first aim* was to examine both short- and long-term effects of early exposure to  
83 hypergravity (HG) on rat forepaw representation. HG has been shown to affect the  
84 development of the vestibular (Ross et Tomko, 1998) and motor systems (Brocard et al.,  
85 2003), and their functions such as air righting during free fall, locomotion, postural control  
86 and limb movement when the HG animals were subsequently exposed to normogravity  
87 (Bojados et al., 2013; Bouet et al., 2003, 2004). The enduring motor changes found in rats  
88 that grew up in altered gravity stress the key role of gravity during the motor development  
89 and suggest the existence of critical periods (Walton et al., 1992). However, it is still  
90 unknown whether HG has an impact on the emergence of somatosensory maps. During the  
91 developmental period, major morphological and biochemical changes take place, as  
92 behavior progresses through different stages of locomotion. The rat motor system matures  
93 slowly over the first postnatal weeks and the pups do not walk spontaneously before the

94 end of the second week of life (Altman and Sudarshan, 1975) and clear topographical  
95 organization of forelimbs begins to appear by P15 in S1 (Seelke et al., 2012). Changes in  
96 gravity induce alteration in joint and muscle proprioception (McCall et al., 2003).  
97 Consistently, HG during early development has been shown to modify postural control and  
98 locomotion in rodents (Bojados et al., 2013). Therefore, we hypothesized that HG, by  
99 modifying the pressure exerted on the ventral surfaces of the paws and muscle spindle  
100 stimulation, thereby changing the pattern of cutaneous and proprioceptive inputs to the S1  
101 cortex compared to normogravity, would shape the topographical organization of the  
102 forepaw maps in a distinctive way.

103       Our *second aim* was to determine whether early sensory experience constrains  
104 experience-dependent plasticity in the mature cortex. We reasoned that gravity-induced  
105 changes in somatosensory inputs offer insight into how early developmental experience  
106 impacts cortical map remodeling resulting from novel sensory experience at maturity. There  
107 has been no equivalent earlier study on the effect of neonatal exposure to HG on later use-  
108 dependent plasticity of the mature somatosensory cortex. Only one related study examined  
109 the impact of neonatal deprivation on use-dependent plasticity of the adult rat barrel cortex  
110 (Rema et al., 2003). These authors have shown that trimming two adjacent whiskers from  
111 birth for 21 days reduced responses of cortical layer II/III neurons at maturity. This early  
112 deprivation was also shown to impede the adult sensory plasticity induced by paired use of  
113 the neonatally trimmed whiskers in supragranular neurons. We investigated the effect of  
114 early HG rearing on experience-dependent cortical plasticity in adult animals housed in an  
115 enriched environment known to induce structural and biochemical changes in the mature  
116 cortex (see Rosenzweig and Bennett, 1996 and van Praag et al., 2000 for reviews) as well as  
117 to remodel the forepaw representation in the S1 cortex (Coq and Xerri, 1998). Our

118 hypothesis was that the potential of cortical maps for plastic changes in response to  
119 enriched environment at maturity would be constrained by early somatosensory experience  
120 in HG during a critical period.

121

## 122 **MATERIAL AND METHODS**

123

### 124 **Animals**

125 Principles of laboratory animal care were respected and all experiments have been  
126 carried out in accordance with Directive 2010/63/EU of the European Parliament and of the  
127 Council of 22 September 2010 on the protection of animals used for scientific purposes. For  
128 the sake of clarity, the labels of the experimental groups are specified in Table 1.

129 To investigate the effects of an hypergravity exposure during the developmental  
130 period on forepaw representation in the S1 cortex, three experimental groups of male Long-  
131 Evans rats were used in which cortical mapping was carried out at the 22<sup>nd</sup> postnatal day  
132 (P22): rats reared in normogravity (NG group), rats which were fertilized, born and housed in  
133 a hypergravity (HG: 2 g) environment generated by a centrifuge (see below) until the age of  
134 3 weeks (P21; HG group), and rats reared in HG with trimmed forepaw nails (HGn- group)  
135 (Fig. 1A). To constitute HG and HGn- groups, couples of rats were placed in the centrifuge  
136 generating the HG environment. After 1 week of HG exposure, adult males were taken out.  
137 After birth, the newborn rats remained with their mother and the pups were nursed  
138 normally until P22. The centrifuge was stopped once a week during half an hour for food  
139 renewal, water supply and home cage cleaning. In the HGn- group, the centrifuge was  
140 stopped every 2 days, from the 5<sup>th</sup> postnatal day until P22, and the animals were removed  
141 from the cages for about 10 min for trimming of forepaw nails. The NG animals were reared

142 in gondolas similar to those equipping the centrifuge, with identical bedding (sawdust), in  
143 the same room and under the same conditions of room temperature, dark-light cycle and  
144 ambient noise, but without centrifugation. A fan was used to generate air flow through  
145 openings in the front door of these static gondolas in order to replicate the rotation-induced  
146 airflow experienced by the HG rats.

147         The enduring effects of HG were studied in rats born and reared in HG until postnatal  
148 day 22, and then removed from the centrifuge and placed in NG under standard laboratory  
149 housing conditions for 1 week (HG1wS group), 8 weeks (HG8wS group) or 16 weeks  
150 (HG16wS group) before electrophysiological mapping. These groups were compared to age-  
151 matched groups of rats reared under NG conditions from birth, placed for 3 weeks in static  
152 gondolas, then for 1 week (NG1wS group), 8 weeks (NG8wS group) or 16 weeks (NG16wS  
153 group) in standard cages (Fig. 1A).

154         The influence of early HG rearing on the use-dependent plasticity of the adult  
155 forepaw S1 map was analyzed by comparing the effect of an enriched environment (EE) in  
156 rats raised in NG versus in HG during the first three postnatal weeks. In the EE, rats were  
157 housed in a group of 12 in two large cages (76 cm wide X 76 cm long X 40 cm high)  
158 connected by two tunnels and containing objects of different shapes, sizes, and textures to  
159 promote tactile experience; these objects were renewed daily to stimulate exploratory  
160 behavior in the rats. Animals housed in standard cages were in groups of three in Plexiglas  
161 cages (26.5 x 42.5 x 18) without objects. The rats had food and water ad libitum, and were  
162 housed on a 12-h light-dark cycle (see Coq and Xerri, 1998). Rats born and raised either in  
163 HG or NG during the first 21 postnatal days and then maintained for 16 weeks in an EE  
164 (HG16wE and NG16wE, respectively) were compared to those born and raised either in HG  
165 or NG during the first 21 postnatal days and housed in a standard environment for 16 weeks



166 (HG16wS and NG16wS, respectively) (Fig. 1B, E).

167           Electrophysiological mapping was performed in all these experimental groups (10  
168 rats per group; total: 110 rats). In order to determine the effects of hypergravity on the paw  
169 skin surfaces in contact with the floor during stance and locomotion, forepaw print  
170 measurements were performed at P22 in the NGp and HGp rats. These measurements were  
171 performed in additional animals reared in HG (HGp group) or NG (NGp group) during the  
172 first 21 postnatal days (10 rats per group; total: 20 rats) and tested under HG or NG  
173 conditions at P22 and under NG conditions after 1 and 2 weeks spent in NG. Rats attributed  
174 to the same experimental groups were taken from different litters.

175

#### 176 **Hypergravity environment**

177           The apparatus generating the HG environment (see Bouët et al., 2003, 2004)  
178 consisted of a centrifuge made of four free-swinging gondolas fixed to the four extremities  
179 of two horizontal cross-arms driven at a constant rotation speed by a DC motor (3.5kW)  
180 located in the vertical axis of the centrifuge. The gondolas (0.55/0.38/0.30 m<sup>3</sup>), located 76.5  
181 cm from the axis of rotation, were equipped with standard home cages for rats, including an  
182 aeration and lighting system providing a 12h light–12h dark cycle. Gondolas were equipped  
183 with a video system composed of a wide-angle camera connected to a monitor and used to  
184 establish the birth date of the litters. The gravito-inertial vector of 2G inside the gondola was  
185 produced with an angular velocity of 3.81rad/s, which resulted in a constant tilt angle of the  
186 gondolas of 60° from the horizontal axis. The resultant force was measured using a force  
187 sensor located in the center of the floor of the gondola. During centrifugation, rats were  
188 therefore subjected to a vector perpendicular to the gondola's floor, i.e. similar to that  
189 experienced in normal gravity (i.e. corresponding to the dorso-ventral axis of the animal)

190 (Fig. 1C). The rats were totally free-moving in the home cage. Accordingly, they were  
191 additionally exposed to variable Coriolis forces depending on the speed and direction of the  
192 animal's motion within the home cage (see Discussion). Video monitoring of the nursing rats  
193 and their pups in both NG and HG groups was performed 3 times per week during 15 min  
194 observation periods. In the HG rats exposed to normogravity, postural and locomotor  
195 behavior was observed twice a day for 15 min, during the first week.

196

197

## 198 **Electrophysiological mapping**

### 199 *Surgical procedure*

200 Anesthesia was induced with halothane and an initial injection of sodium  
201 pentobarbital (30 mg/kg, i.p.). Rats were maintained at an areflexive level of anesthesia  
202 throughout the experiment by supplemental doses of sodium pentobarbital (3 mg/kg, i.p.).  
203 The core temperature was continuously monitored by a rectal thermistor probe and  
204 maintained between 37 °C and 38 °C by a heating pad. The head was placed in a stereotaxic  
205 frame. To prevent cerebral oedema, cerebrospinal fluid was first drained through an opening  
206 in the dura covering the foramen magnum after resection of posterior neck muscles. Then, a  
207 craniotomy (about 16 mm<sup>2</sup>) was made to expose the S1 cortex to be mapped. The dura  
208 protecting the exposed part of the cortex was incised and resected. The cortical surface was  
209 bathed in a thin layer of warm silicone fluid to prevent drying. At the end of the mapping  
210 session, rats received a lethal dose of sodium pentobarbital (150 mg/kg, i.p.) and the brain  
211 was prepared for histological processing.

212

### 213 *Electrophysiological mapping procedure*

214           The mapping procedure used in the present study has been described in detail in  
215 previous papers (Xerri and Zennou-Azogui, 2003). Magnified images of the exposed parietal  
216 cortex, and the ventral and dorsal surfaces of the forepaw contralateral to the cerebral  
217 hemisphere to be mapped, were digitized by using a high-resolution camera mounted on an  
218 operating microscope. Cutaneous receptive fields (RFs) were drawn on the digitized images  
219 of the forepaw and placements of microelectrode penetrations were recorded on the  
220 digitized image of the cortex using Map 0.925 software (Peterson and Merzenich, 1995).  
221 Conventional multiunit recording and receptive field mapping techniques were used to  
222 reconstruct the forepaw representation. Sites of electrode penetration were identified  
223 relative to the vasculature of the cortical surface. Unit clusters were recorded with parylene-  
224 coated tungsten microelectrodes (about 1 M $\Omega$  at 1 kHz; WPI, UK). The electrode was moved  
225 perpendicular to the cortical surface in Cartesian coordinates by a 3D stepping  
226 micromanipulator (Märzhauser, FST; Canada). Using the recording artifact generated by the  
227 microelectrode contact with the cortex surface as a zero level, we advanced the electrode to  
228 a depth of about 650-700 microns to record responses from clusters of 2 to 4 neurons in  
229 layer IV (Waters et al., 1995; Coq and Xerri, 1998). The inter electrode penetration distance  
230 was close to 70-80 microns in all groups of rats. The amplitude of the background noise  
231 usually ranged from 15 to 20  $\mu$ V with a signal-to-noise ratio ranging from 4 to 6. The  
232 multiunit signal was amplified, filtered (bandwidth: 0.5 - 5 kHz), and displayed on an  
233 oscilloscope. This signal was also rectified and passed through a discriminator with an output  
234 signal proportional to the part of the input signal that was higher than an adjustable  
235 threshold set just above the background noise. The output of the discriminator was then  
236 delivered to an audio monitor. At each recording site, bursts of activity elicited by natural  
237 stimulation allowed us to classify neuronal responses as cutaneous or non-cutaneous. The

238 cutaneous receptive fields (RFs) of small clusters of neurons were defined at each recording  
239 site as the areas of skin where just-visible skin indentation (about 100 - 150  $\mu\text{m}$ ) elicited  
240 reliable changes in multiple-unit discharge. This stimulation, which is within the dynamic  
241 range of cutaneous receptors (Gardner and Palmer, 1989), was produced with a fine-tipped,  
242 hand-held glass probe and monitored using magnifying glasses (x4). The curvature of fingers  
243 and pads was taken into account while drawing the RFs. The ridges running along the  
244 glabrous skin of the digits and palm were used as reliable landmarks to delineate the RFs.  
245 The RF areas were transferred to the digital image of the forepaw and were subsequently  
246 measured offline using Map 0.925 software. Responses elicited by upward and downward  
247 nail displacements were examined while the tips of the digits were firmly maintained to  
248 minimize joint movement and skin deformation. As a general rule, light pressure on the nails  
249 elicited a burst of activity facilitating the assessment of neurons' directional sensitivity, i.e.  
250 the direction of nail displacement (upward: U, downward D or both U+D) eliciting the most  
251 prominent neuronal responses. These responses were categorized as cutaneous and  
252 referred to as 'nail' responses. In our classification, high-threshold responses identified by  
253 taps and pressure on tendons, intrinsic muscles or joint manipulations, while no cutaneous  
254 response was found, were classified as proprioceptive (Jenkins et al. 1990, Recanzone et al.,  
255 1992; Xerri et al., 1998, Coq and Xerri., 2000).

256         The mechanical thresholds of neuronal responses to cutaneous glabrous stimulation  
257 were determined using von Frey monofilaments (Stoelting, Semmes-Weinstein  
258 aesthesiometer) that apply indenting stimuli at a relatively constant, predetermined force.  
259 The most commonly used filaments were 2.83 (diameter: 0.127 mm; bending force: 0.068 g;  
260 bending pressure: 5.37  $\text{g}/\text{mm}^2$ ), 3.22 (0.152 mm; 0.166 g; 9.15  $\text{g}/\text{mm}^2$ ), 3.61 (0.178 mm;  
261 0.407 g; 16.36  $\text{g}/\text{mm}^2$ ), and 3.84 (0.203 mm; 0.692 g; 21.39  $\text{g}/\text{mm}^2$ ) (FIG 2B). Neuronal

262 responses elicited by von Frey filaments above 3.84, starting from 4.08 (0.229 mm; 1.202 g;  
263 29.20 g/mm<sup>2</sup>) were classified as noncutaneous presumably proprioceptive. For reproducible  
264 measurements, the filaments were used at a relatively constant room temperature (about  
265 24 °C). The stimulation consisted in pressing a filament gently against the skin, perpendicular  
266 to its surface and at the center of the RF, until the filament began to bend. This procedure  
267 was done 5 to 10 times for each filament. We used stimulus series of increasing and  
268 decreasing strengths to determine the mechanical threshold eliciting noticeable changes in  
269 neuronal discharge.

270         After the recording session, we used Canvas software (ACD Systems, Inc.) to produce  
271 maps of the forepaw representation by drawing boundaries encompassing cortical sites  
272 where corresponding RFs were restricted to a common forepaw subdivision, i.e. finger,  
273 palmar, pad. Borders were drawn midway between adjacent recording sites where RFs were  
274 located on distinct and separate skin subdivisions. The same principle was used to draw  
275 boundaries encompassing cortical recording sites where noncutaneous responses were  
276 obtained. A boundary line crossed cortical sites at which a single RF included different but  
277 adjoining skin subdivisions of the forepaw. Borders encompassing cortical sites where  
278 fingernail responses were obtained were also drawn. A boundary line crossed cortical sites  
279 at which both cutaneous and nail responses were recorded. (Fig. 2AC). The cutaneous  
280 forepaw map boundary was delimited by cortical sites exhibiting cutaneous responses to  
281 stimulation of the lower lip, wrist or non-cutaneous responses and by no stimulus-evoked  
282 sites. Canvas software was used to calculate the surface area of each cutaneous and nail  
283 region of the cortical map.

284

285 *Experimental measurements*

286           Cortical areas devoted to forepaw representation, i.e. excited by stimulation of  
287 glabrous or hairy skin areas, or by nail displacements, were calculated for each rat and  
288 described by their absolute areas (mm<sup>2</sup>). To assess relative representation of glabrous, hairy  
289 skin surface and nails as parts of the forepaw representation, the corresponding parts of the  
290 map were expressed as a percentage of the whole cutaneous map of the forepaw. Average  
291 values were computed for each group of rats. The absolute sizes of glabrous and hairy RFs  
292 were measured in mm<sup>2</sup> (Map 0.925 software), normalized relative to the ventral and dorsal  
293 skin forepaw areas, respectively, and expressed as percentages. The relative RF areas  
294 measured in each rat were averaged and the mean size of RF was calculated for each group  
295 of rats. The stimulation thresholds obtained using the von Frey filaments were processed to  
296 calculate an average force threshold for each animal (Xerri and Zennou-Azogui, 2003, 2014).  
297 Every threshold value (0.068 g, 0.168 g, 0.407 g and 0.692 g) was multiplied by the number  
298 of recording sites displaying that threshold and the sum of these weighted values was  
299 divided by the total number of sites tested. A mean threshold was then calculated for each  
300 experimental group. To assess directional sensitivity of finger nails, their displacement was  
301 induced using a hand-held electronic Von-Frey device, designed in our laboratory, to apply  
302 single stimuli through a probe (0.8 mm in diameter) (Blanc and Coq, 2007). The force  
303 envelope randomly applied (mN) by hand to induce nail extension or flexion was converted  
304 by the electronic Von-Frey device into a potential variation (0.1 mN corresponded to 10 mV)  
305 which was recorded at 1 kHz with a multichannel acquisition system (Plexon, Dallas). Stimuli  
306 and neuronal responses were synchronized using a TTL signal emitted by the Von -Frey at  
307 the onset of the nail contact. The force profile and duration varied across trials. Only  
308 relatively similar force patterns were retained to determine the units' sensitivity to nail  
309 displacement. Neuronal activity was recorded in a 600 ms time window which started from

310 the trial onset. We elicited 15 to 20 stimulus trials for each of the cortical locations recorded.  
311 The percentage of neuronal responses exhibiting clear directional sensitivity to either  
312 extension or flexion of nails, or no directional sensitivity, was calculated for each rat and  
313 averaged for each experimental group.

314

315

316

### 317 **Histology**

318 To ascertain the location of recording responses in S1 layer IV, several electrode  
319 tracks in each experimental group were marked with electrophysiological lesions in the  
320 center of the forepaw map by passing cathodal current (10  $\mu$ A DC, 10 s) through the  
321 recording electrode positioned at recording depth. After the mapping session, the rats were  
322 given a lethal dose of pentobarbital and perfused transcardially with 0.9% physiological  
323 saline followed by a solution containing 4% paraformaldehyde in 0.1% sodium phosphate-  
324 buffered (pH: 7.4). The brain was removed and post-fixed in a 4% paraformaldehyde solution  
325 containing 10% sucrose in phosphate buffer. Coronal sections 50  $\mu$ m thick were cut on a  
326 freezing microtome and processed for Nissl staining. Histological data confirmed the location  
327 of the recorded neurons in layer IV (Fig. 2B).

328

### 329 **Paw print measurements**

330 The animals stepped on an inkpad placed for 3 min in their rearing cage. Then, the  
331 animals were immediately anesthetized with halothane, and pictures of the left and right  
332 forepaws were taken using a CCD camera for subsequent measurement of inked paw  
333 surfaces. In addition, after cleaning the stained skin surfaces and one hour after recovery

334 from initial anesthesia, NGp rats were tested in hypergravity, while HGp rats were tested in  
335 normogravity. Possible long-term effects of hypergravity on forepaw prints were also  
336 evaluated under normogravity conditions, 1 and 2 weeks after NG housing, in NGp and HGp  
337 animals. Inked surfaces of the right and left forepaws were measured in square mm using  
338 Canvas software (ACD Systems, Inc.). A mean forepaw print value was calculated for each rat  
339 and then for each group of rats.

340

#### 341 **Statistical Analysis**

342 Results are presented as mean values  $\pm$  SD. Statistical treatment was done with  
343 analysis of variance (ANOVA) supplemented with multiple comparisons (Newman-Keuls  
344 post-hoc test; Statistica software, Statsoft). Repeated measures ANOVA was used to  
345 compare ipsative data consisting of the proportions of neuronal responses to nail  
346 displacement falling within the defined categories (cf. Greer and Dunlap, 1997). Repeated  
347 measures ANOVA was also used to compare forepaw prints collected over time or under  
348 different gravity conditions in the same animals. The proportions of neuronal responses to  
349 nail displacement, are ipsative data, i.e. a given set of responses always sums to the same  
350 total (100 %). We used repeated measures ANOVA to compare the proportions of responses  
351 obtained in HG and NG rats, a procedure that takes into account the inter-individual  
352 variability within each experimental group (Shaffer, 1981; Greer and Dunlap, 1997).

353

#### 354 **RESULTS**

355

#### 356 ***Organization of forepaw somatosensory maps in 3 week old NG rats***



357           This study represents the first description of high-density electrophysiological  
358 forepaw maps obtained in rat pups at P22. We also obtained a complete forepaw map for  
359 two P15 rats (Fig. 2). Interestingly, this map and those recorded at P22 appeared to be adult-  
360 like in their topographical organization and RF sizes (Coq and Xerri, 1998; Xerri and Zennou-  
361 Azogui, 2003). We found a complete representation of the glabrous and hairy forepaw skin  
362 surfaces. The cutaneous representation displayed a somatotopic order, with a clearly  
363 segregated digit representation sequentially ordered from the rostralateral to caudomedial  
364 cortical sector, and a palmar pad representation sequentially ordered along the medial edge  
365 of the map. The dorsal skin surface of the palm was contained in either a continuous or  
366 fragmented region at the lateral border of the forepaw map. Topographically organized  
367 cortical sectors displaying specific responses to nail displacements were found to be  
368 embedded within the representational regions of the forepaw skin. The borders of forepaw  
369 representation consisted of cortical sites either responsive to cutaneous stimulation of the  
370 lip or the arm, or noncutaneous inputs, or classified as unresponsive. Small islets of  
371 noncutaneous zones encountered in the maps tended to disrupt cutaneous topographical  
372 neighborhood relationships. As generally observed in adult rats, cortical cells clusters  
373 sampled in the representational cutaneous zone exhibited skin RFs covering glabrous or  
374 hairy skin surfaces generally restricted to one main subdivision of the forepaw (single digit,  
375 palmar pad).

376

### 377 ***Short-term effects of early exposure to hypergravity***

#### 378 *Organization of S1 forepaw maps*

379           This study represents the first investigation of the experience-dependent  
380 developmental plasticity of forepaw representations in the S1 cortex using high-density

381 electrophysiological mapping. A conspicuous feature of somatosensory maps derived from  
382 HG rats was a threefold enlargement of cortical sectors driven by nail displacements  
383 ( $0.58 \pm 0.12 \text{ mm}^2$ ) as compared to those of NG rats ( $0.19 \pm 0.05 \text{ mm}^2$ ) ( $F_{1,18} = 81.12$ ;  $P < 0.001$ ).  
384 In the former, nail representation was found to take over the cortical zones serving the skin  
385 surfaces (Fig. 3A). Skin representations in HG rats ( $0.76 \pm 0.14 \text{ mm}^2$ ) were significantly smaller  
386 than those of NG rats ( $1.20 \pm 0.21 \text{ mm}^2$ ) ( $F_{1,18} = 35.45$ ;  $P < 0.001$ ). This HG-induced decrease  
387 was found for both the glabrous (mean decrease: 36 %; HG:  $0.65 \pm 0.15 \text{ mm}^2$ ; NG:  $1.02 \pm 0.22$   
388  $\text{mm}^2$ ) ( $F_{1,18} = 20.05$ ;  $P < 0.001$ ) and hairy skin areas (39%; HG:  $0.11 \pm 0.04 \text{ mm}^2$ ; NG:  $0.18 \pm 0.05$   
389  $\text{mm}^2$ ) ( $F_{1,18} = 11.77$ ;  $P < 0.01$ ). No significant change in the overall area of the forepaw  
390 cutaneous map (including skin and nail representational regions) was observed in HG rats as  
391 compared to NG rats (HG:  $1.33 \pm 0.14 \text{ mm}^2$ ; NG:  $1.43 \pm 0.21 \text{ mm}^2$ ;  $F_{1,18} = 1.45$ ;  $P = 0.24$ , ns).

392 This finding led us to hypothesize that hypergravity increased sensory inputs  
393 generated by nail displacements. Therefore, we assessed the forepaw map reorganization in  
394 animals whose nails were regularly clipped (HGn<sup>-</sup> rats). The cortical area activated by nail  
395 displacement in HGn<sup>-</sup> rats ( $0.07 \pm 0.06 \text{ mm}^2$ ) was strongly reduced, compared with that of HG  
396 ( $0.58 \pm 0.12 \text{ mm}^2$ ) ( $F_{2,27} = 98.06$ ;  $P < 0.001$ ) and NG rats ( $0.19 \pm 0.05 \text{ mm}^2$ ) ( $P < 0.01$ ).  
397 Interestingly, the glabrous skin sectors were similarly reduced in the HG ( $0.65 \pm 0.15 \text{ mm}^2$ )  
398 and HGn<sup>-</sup> ( $0.74 \pm 0.08 \text{ mm}^2$ ) groups ( $F_{2,27} = 14.87$ ;  $P = 0.19$ , ns). This reduction was found to be  
399 only partially compensated for by an increased hairy skin representation in the HGn<sup>-</sup> rats  
400 ( $0.27 \pm 0.09 \text{ mm}^2$ ), which was greater than that of either NG ( $0.18 \pm 0.05 \text{ mm}^2$ ) ( $F_{2,27} = 16.34$ ;  
401  $P < 0.01$ ) or HG rats (HG:  $0.11 \pm 0.04 \text{ mm}^2$ ) ( $P < 0.001$ ). Therefore, the total surface area of  
402 cutaneous map (skin + nail regions) was smaller in the HGn<sup>-</sup> ( $1.08 \pm 0.13 \text{ mm}^2$ ) than in the HG  
403 ( $1.33 \pm 0.14 \text{ mm}^2$ ) ( $F_{2,27} = 12.06$ ; post-hoc:  $P < 0.01$ ) or NG groups ( $1.43 \pm 0.21 \text{ mm}^2$ ) ( $P < 0.001$ ).  
404 Proprioceptive zones were found to colonize the cutaneous territory in these HGn<sup>-</sup> rats (Fig.

405 3A). Overall, these findings show that early exposure to HG strongly modified the relative  
406 representation of competing cutaneous (glabrous or hairy skin, nail) and proprioceptive  
407 inputs converging onto layer IV cortical neurons.

408

#### 409 *Neuronal responsiveness*

410 We reasoned that if early rearing in HG strongly modulates sensory inputs generated  
411 by nail-movement during locomotion and changes in posture, this environment may bias the  
412 directional sensitivity of cortical neurons to upward (U) versus downward (D) nail  
413 displacement. Repeated measures ANOVA showed a main effect of directional sensitivity,  
414 i.e., the percentage of responses to U, D or both U and D displacement (U+D;  $F_{2,36} = 22.21$ ,  
415  $P < 0.001$ ), no main effect of rearing environment ( $F_{1,18} = 0.00001$ ;  $P = 0.99$ , ns) and an  
416 interaction between these two factors ( $F_{2,36} = 12.79$ ,  $P < 0.001$ ). Post-hoc tests indicated that  
417 NG rats exhibited a majority of neuronal responses elicited by both U and D nail  
418 displacement when compared to those elicited by U or D movement ( $P < 0.01$  or  $P < 0.001$ ,  
419 respectively) which were found in similar proportions ( $P = 0.39$ , ns) (Table 2). By contrast, HG  
420 rats displayed a larger proportion of neurons sensitive to U nail displacement than to D  
421 ( $P < 0.001$ ) or U+D ( $P < 0.03$ ). In HG rats, the neuronal population sensitive to U nail  
422 displacement increased ( $P < 0.001$ ) while that sensitive to U and D movements decreased  
423 ( $P < 0.03$ ) as compared to those populations recorded in NG rats. No difference in D nail  
424 response proportion was shown between NG and HG rats ( $P = 0.07$ , ns) (Fig. 4). These results  
425 suggest that the HG increase in the population of U responses resulted from an effect on  
426 U+D neurons. To assess neuronal responsiveness to glabrous skin stimulation, a weighted  
427 mean was calculated on the basis of the mechanical thresholds obtained using the von Frey  
428 filaments (see Methods). The mechanical thresholds to cutaneous stimulation recorded

429 within the reduced glabrous skin cortical sectors in the HG rats were similar to those  
430 recorded in the NG rats ( $F_{1,18} = 1.87$ ;  $P=0.19$ , ns) (Table 3).

431

432

433

#### 434 *Size of cutaneous RFs*

435 In both HG and NG groups, most of the cortical sites sampled exhibited cutaneous  
436 RFs usually covering the glabrous or the hairy skin of one or two forepaw subdivisions (digit  
437 phalange, palmar pad). The size of the glabrous RFs normalized relative to the ventral skin  
438 area of the forepaw was on average larger in HG rats ( $5.14\pm 0.48$  %) than in NG rats  
439 ( $3.58\pm 0.57$  %) ( $F_{1,18} = 43.80$ ;  $P<0.001$ ). Therefore, a lower spatial selectivity was found for  
440 the neurons within the reduced cortical regions serving the glabrous skin surfaces in the HG  
441 rats. The mean sizes of glabrous RFs were similar in HGn<sup>-</sup> ( $5.35\pm 1.32$  %) and HG rats ( $F_{2,27} =$   
442  $12.31$ ;  $P=0.59$ , ns), but larger than those of NG animals ( $P<0.001$ ). By contrast, hairy RFs sizes  
443 were similar in all groups (NG:  $6.46 \pm 2.15$  %; HG:  $5.98 \pm 1.03$ %; HGn<sup>-</sup>:  $6.77\pm 1.01$  %) ( $F_{2,27} =$   
444  $0.70$ ;  $P=0.50$ , ns) (Fig. 3B, C).

445

#### 446 *Paw print evaluation*

447 Forepaw print measurements, taken as an estimation of the ventral skin areas in  
448 contact with the floor during stance and locomotion, were obtained in HGp and NGp rats of  
449 matching body weights (HGp:  $44.49\pm 5.95$  g, NGp:  $43.01\pm 6.18$  g;  $P=0.59$ , ns) under both NG  
450 and HG conditions. Repeated measures ANOVA yielded a main effect of gravity condition ( $F_{1,18} =$   
451  $76.28$ ,  $P<0.001$ ), no effect of rearing environment when the NG and HG rats were tested  
452 under the same gravity conditions ( $F_{1,18} = 0.085$ ;  $P=0.77$ , ns) and no significant interaction  
453 between these two factors ( $F_{1,18} = 4.32$ ,  $P=0.06$ , ns). Mean HGp and NGp rat forepaw prints

454 were similar when obtained under HG testing conditions (HGp:  $40.67 \pm 9.41 \text{ mm}^2$ , NGp:  
455  $43.52 \pm 3.85 \text{ mm}^2$ ;  $P=0.06$ , ns) or under NG testing conditions (HGp:  $33.87 \pm 5.65 \text{ mm}^2$ , NGp:  
456  $32.47 \pm 2.99 \text{ mm}^2$ ;  $P=0.34$ , ns). As expected, rats from either rearing environment exhibited  
457 larger forepaw prints when tested in HG than in NG (HGp rats:  $P<0.0003$ ; NGp rats:  $P<0.001$ ).  
458 Interestingly, the ratios of HG to NG print areas recorded in each rat (HGp rats:  $1.19 \pm 0.11$   
459 and NGp rats:  $1.26 \pm 0.21$ ) appeared to be similar to the ratio of HG to NG glabrous RF areas  
460 measured in  $\text{mm}^2$  ( $1.27 \pm 0.21$ ;  $P=0.35$  and  $P=0.33$ , respectively).

461

462

### 463 ***Enduring effect of HG rearing***

#### 464 *Organization of S1 forepaw maps*

465 In order to properly describe possible enduring effects of early exposure to HG, we  
466 compared the map data recorded in 2 groups of age-matched animals (Fig. 5, 6). In the first  
467 group, rats were reared in HG for 3 weeks and then housed in standard environment in NG  
468 until the end of postnatal week 19 (HG, HG1wS, HG8wS, HG16wS rats; see Methods). In the  
469 second group, rats were reared in standard environment in NG from birth until postnatal  
470 week 19 (NG, NG1wS, NG8wS, NG16wS rats). For the total surface area of the forepaw map  
471 (Fig. 6A), the ANOVA disclosed main effects of both early rearing environment ( $F_{1, 72} = 86.63$ ;  
472  $P<0.001$ ) and subsequent NG housing duration conditions ( $F_{3, 72} = 12.55$ ;  $P<0.001$ ), as well as  
473 an interaction between these two factors ( $F_{3, 72} = 11.93$ ;  $P<0.001$ ). A gradual increase in the  
474 total area of the forepaw map was found over the 16 weeks examined in the rats reared in  
475 NG from birth (NG:  $1.43 \pm 0.21 \text{ mm}^2$ , NG16wS:  $2.30 \pm 0.35 \text{ mm}^2$ ,  $P<0.001$ ) (Fig. 5 and 6). This  
476 increase was found for the cortical territories allocated to hairy skin (NG:  $0.18 \pm 0.05 \text{ mm}^2$ ,  
477 NG16wS:  $0.47 \pm 0.12 \text{ mm}^2$ ,  $P<0.001$ ) and nail (NG:  $0.19 \pm 0.05 \text{ mm}^2$ , NG16wS:  $0.56 \pm 0.18 \text{ mm}^2$ ,

478 P<0.001) representations (Fig. 5 and 6BC), as well as for the glabrous skin representations,  
479 although to a lesser extent (NG:  $1.02 \pm 0.22 \text{ mm}^2$ , NG16wS:  $1.27 \pm 0.23 \text{ mm}^2$ , P<0.01) (Fig. 6D).  
480 By contrast, no expansion of the forepaw map was recorded in rats reared in HG for the first  
481 3 postnatal weeks and housed in NG over the following weeks (HG:  $1.33 \pm 0.14 \text{ mm}^2$ ,  
482 HG16wS:  $1.39 \pm 0.33 \text{ mm}^2$ , P=0.84, ns) (Fig. 5, 6). We examined whether this apparent  
483 “freezing effect” of the HG rearing affected similarly the different sectors of the forepaw  
484 cutaneous map. This HG-induced effect was observed for glabrous skin areas (HG:  $0.65 \pm 0.15$   
485  $\text{mm}^2$ , HG16wS:  $0.49 \pm 0.06 \text{ mm}^2$ , P=0.21, ns): ANOVA yielded no effect of duration of NG  
486 housing ( $F_{3, 72} = 0.74$ ; P=0.53 ns), but a main effect of early rearing environment ( $F_{1, 72} =$   
487  $177.70$ , P<0.001) and an interaction between these two factors ( $F_{3, 72} = 4.69$ , P<0.01) (Fig. 5  
488 and 6D). As for the hairy skin representation (HG:  $0.11 \pm 0.04 \text{ mm}^2$ , HG16wS:  $0.19 \pm 0.07 \text{ mm}^2$ ,  
489 P=0.26, ns), ANOVA showed main effects of duration of NG housing ( $F_{3, 72} = 22.77$ , P<0.001)  
490 and early rearing environment condition ( $F_{1, 72} = 90.35$ ; P<0.001) as well as an interaction  
491 between these two factors ( $F_{3, 72} = 7.17$ , P<0.001) (Fig. 5 and 6B). Similar effects were found  
492 for the nail representation (HG:  $0.58 \pm 0.12 \text{ mm}^2$ , HG16wS:  $0.59 \pm 0.09 \text{ mm}^2$ , P=0.77, ns) with  
493 ANOVA showing main effects of NG housing duration ( $F_{3, 72} = 14.49$ , P<0.001) and early  
494 rearing environment ( $F_{1, 72} = 59.93$ , P<0.001) and an interaction between these two factors  
495 ( $F_{3, 72} = 13.98$ , P<0.001) (Fig. 5, 6) (Fig. 5 and 6C). Overall, our findings indicate that early  
496 exposure to HG prevented the expansion of forepaw cutaneous representational zones  
497 normally taking place in NG over the first postnatal months, despite exposure to NG from  
498 the end of the 3<sup>rd</sup> postnatal week.  
499

500 *Neuronal responsiveness*

501 Directional sensitivity of cortical neurons to upward versus downward nail  
502 displacement was analyzed in rats reared in HG for 3 weeks and then housed in NG  
503 environment until the end of the 19<sup>th</sup> postnatal week (HG, HG1wS, HG8wS, HG16wS rats).  
504 Repeated measures ANOVA showed no main effect of duration of NG housing ( $F_{3,36} = 0.67$ ;  
505  $P=0.58$ , ns), but a main effect of directional sensitivity ( $F_{2,72} = 106.88$ ,  $P<0.001$ ) as well as an  
506 interaction between these two factors ( $F_{6,72} = 16.48$ ,  $P<0.001$ ). The HG-induced changes in  
507 directional sensitivity of cortical neurons characterized by an increased proportion of U nail  
508 displacement responses were still observed when rats reared in HG were housed for one  
509 week in NG. HG1wS rats displayed similar proportions of neurons within each category of  
510 response as compared to HG rats (U:  $P=0.68$ ; D:  $P=0.92$  and U+D:  $P=0.45$ ) (Table 2). This long  
511 lasting effect of the HG early rearing was shown to dissipate between the 2<sup>nd</sup> and the 8<sup>th</sup>  
512 week spent under normal gravity conditions: HG8wS rats displayed a majority of neuronal  
513 responses elicited by U+D nail displacement ( $P<0.001$ , when U+D is compared to U or D  
514 responses), while the rest of the neuronal populations exhibited either U or D unidirectional  
515 sensitivity in proportions that are not statistically different ( $P=0.12$ ). The proportions  
516 recorded in HG8wS rats are similar to those recorded in aged-matched NG8wS rats (U:  
517  $P=0.10$ ; D:  $P=0.86$ ; U+D:  $P=0.89$ ) and in HG16wS rats (U:  $P=0.99$ ; D:  $P=0.87$ , U+D:  $P=0.93$ ,  
518 when compared to U, D, U+D proportions found in HG8wS).

519 In the rats housed in NG from birth and in HG rats exposed to NG from the end of the  
520 3<sup>rd</sup> week until the 19<sup>th</sup> week, the mechanical thresholds of neuronal responses assessed with  
521 the von Frey filaments were found to be stable until the end of the first month (NG vs  
522 NG1wS:  $P=0.22$ ; HG vs HG1wS:  $P=0.96$ ) and to increase thereafter, reaching a maximum at  
523 the end of the 11<sup>th</sup> postnatal week (Table 3). No difference between animals reared in NG or

524 HG was observed at this time ( $P=0.12$ ) and at the end of postnatal week 19 (NG16wS vs  
525 HG16wS:  $P=0.085$ , ns). ANOVA showed a significant effect of NG housing duration ( $F_{3, 72} =$   
526  $57.59$ ;  $P<0.001$ ) but no effect of early rearing condition ( $F_{1, 72} = 3.09$ ;  $P=0.08$ , ns) on neuronal  
527 responsiveness, and no interaction between these two factors ( $F_{3, 72} = 2.56$ ;  $P=0.06$ , ns).  
528 Therefore, these age-dependent profiles of neuronal response thresholds within the  
529 glabrous skin cortical sectors were not affected by the early rearing gravity conditions.

530  
531

### 532 *Size of cutaneous RFs*

533       Regarding the size of glabrous skin RFs (Fig. 7A), ANOVA performed between our  
534 experimental groups disclosed a significant effect of both NG housing duration ( $F_{3, 72} = 17.19$ ;  
535  $P<0.001$ ) and early rearing conditions ( $F_{1, 72} = 14.17$ ;  $P<0.0001$ ) as well as an interaction  
536 between these two factors ( $F_{3, 72} = 17.32$ ;  $P<0.001$ ). Further analysis revealed that the HG-  
537 induced increase in relative glabrous RF size was reversed as early as the end of the 1<sup>st</sup> week  
538 of housing in NG environment (HG:  $5.14\pm 0.48$  %, HG1wS:  $3.76\pm 0.30$ ;  $P<0.001$ ), thus  
539 becoming similar to that recorded in NG ( $3.58\pm 0.57$  %,  $P=0.79$ , ns) or NG1ws ( $3.72\pm 0.67$  %,  $P=0.84$ , ns) rats. The hairy skin RFs, not modified by HG, were similar in all our experimental  
540 groups as shown by ANOVA which yielded no effect of NG housing duration ( $F_{3, 72} = 1.78$ ;  
541  $P=0.16$ , ns) or early rearing condition ( $F_{1, 72} = 3.40$ ;  $P=0.07$ , ns), as well as no interaction  
542 between these two factors ( $F_{3, 72} = 0.17$ ;  $P=0.92$ , ns) (Fig. 7B).

544

### 545 *Forepaw prints*

546       The enduring effect of early HG rearing on forepaw prints was evaluated under NG  
547 conditions, 1 and 2 weeks after NG housing. The weight of HGp rats after 1 week of NG  
548 ( $67.50 \pm 7.82$  g) and that of age-matched NGp rats ( $61.09 \pm 4.03$  g) was similar ( $P=0.08$ , ns).



549 The same observation applies for HGp rats exposed for 2 weeks in NG ( $97.55 \pm 8.86$  g) whose  
550 weight was similar to that of age-matched NGp rats ( $93.32 \pm 10.19$  g;  $P=0.25$ , ns). Repeated  
551 measured ANOVA performed on forepaw prints at 1 and 2 weeks after the HG rearing in  
552 HGp rats and in age-matched NGp rats disclosed no effect of early rearing environment ( $F_{1,18}$   
553  $= 0.07$ ;  $P=0.80$ , ns), but a main effect of subsequent NG housing duration ( $F_{1,18} = 18.46$ ;  
554  $P<0.001$ ), with no interaction between these two factors ( $F_{1,18} = 0.004$ ;  $P=0.95$ , ns). Post-hoc  
555 tests indicated that forepaw prints increased between the 1<sup>st</sup> and the 2<sup>nd</sup> week in both  
556 groups (HGp:  $P<0.02$ ; NGp:  $P<0.02$ ), and that they were similar in HGp rats and age-matched  
557 NGp animals when tested 1 week (HGp:  $34.30 \pm 5.09$  mm<sup>2</sup>, NGp:  $34.84 \pm 5.93$  mm<sup>2</sup>;  $P=0.82$ , ns)  
558 or 2 weeks (HGp:  $41.40 \pm 3.91$  mm<sup>2</sup>, NGp:  $41.73 \pm 5.95$  mm<sup>2</sup>;  $P=0.89$ , ns) after HG rearing.

559  
560

561 ***Influence of developmental HG experience on adult use-dependent plasticity***  
562 ***of cortical maps.***

563 ***Organization of S1 forepaw maps***

564 We sought to determine the influence of early developmental HG experience on later  
565 use-dependent plasticity. For this purpose, we used the environmental enrichment (EE)  
566 paradigm which has been shown to strongly modify the S1 forepaw map organization in  
567 adult rats (Coq and Xerri, 1998). Age-matched rats raised in NG or HG during the first 3  
568 postnatal weeks were subsequently housed in EE for 16 weeks. ANOVA performed on the  
569 data recorded in the 4 experimental groups (NG16wS, NG16wE, HG16wS, HG16wE) yielded a  
570 main effect of early rearing ( $F_{1,36} = 163.25$ ;  $P<0.001$ ), but no effect of adult housing  
571 conditions ( $F_{1,36} = 0.86$ ;  $P=0.36$ , ns) on the overall forepaw cutaneous representation, with  
572 an interaction between these two factors ( $F_{1,36} = 9.11$ ;  $P<0.01$ ). Further post hoc analysis

573 showed an EE-induced expansion in the rats reared in NG during the first 3 postnatal weeks  
574 compared to the rats housed in a standard environment during the same period (NG16wE:  
575  $2.66 \pm 0.29 \text{ mm}^2$ ; NG16wS:  $2.30 \pm 0.35 \text{ mm}^2$ ;  $P < 0.01$ ). By contrast, no such increase of the  
576 cutaneous maps was found when rats were exposed to EE after rearing in HG (HG16wS:  
577  $1.39 \pm 0.33 \text{ mm}^2$ ; HG16wE:  $1.20 \pm 0.17 \text{ mm}^2$ ;  $P = 0.15$ , ns) (Fig. 8). ANOVA yielded a main effect  
578 of both early rearing and adult housing conditions on the glabrous skin representation ( $F_{1,36}$   
579  $= 362.13$ ;  $P < 0.001$ ;  $F_{1,36} = 16.35$ ;  $P < 0.001$ , respectively), with an interaction between these  
580 two factors ( $F_{1,36} = 11.31$ ;  $P < 0.01$ ). The post-hoc tests showed an expansion of cortical zones  
581 representing the glabrous skin only in rats raised during the early postnatal period in NG  
582 (NG16wS:  $1.27 \pm 0.23 \text{ mm}^2$ , NG16wE:  $1.64 \pm 0.19 \text{ mm}^2$ ,  $P < 0.001$ ) but not in HG (HG16wS:  
583  $0.49 \pm 0.06 \text{ mm}^2$ , HG16wE:  $0.52 \pm 0.08 \text{ mm}^2$ ,  $P = 0.64$ , ns). ANOVA performed on hairy skin  
584 sectors showed an effect of both early rearing ( $F_{1,36} = 80.62$ ;  $P < 0.001$ ) and adult environment  
585 conditions ( $F_{1,36} = 5.92$ ;  $P < 0.02$ ), with no interaction between these two factors ( $F_{1,36} = 1.47$ ;  
586  $P = 0.23$ , ns). Further post hoc analysis indicated that hairy skin areas were not modified by EE  
587 in rats reared in HG (HG16wS:  $0.19 \pm 0.07 \text{ mm}^2$ , HG16wE:  $0.23 \pm 0.07 \text{ mm}^2$ ,  $P = 0.40$ , ns), while  
588 they increased in the rats reared in NG (NG16wS:  $0.47 \pm 0.12 \text{ mm}^2$ , NG16wE:  $0.60 \pm 0.17 \text{ mm}^2$ ,  
589  $P < 0.01$ ) during the first 3 weeks postnatal period, though to a lesser extent than glabrous  
590 skin sectors. Moreover, ANOVA yielded no effect of early rearing ( $F_{1,36} = 0.25$ ;  $P = 0.62$ , ns),  
591 but an effect of adult housing conditions ( $F_{1,36} = 10.38$ ;  $P < 0.01$ ) on the surface area of the  
592 nail sectors, with no interaction between these two factors ( $F_{1,36} = 0.02$ ;  $P = 0.90$ , ns). The  
593 post hoc tests showed no EE-induced changes in nail regions both in rats reared in NG  
594 (NG16wS:  $0.56 \pm 0.18 \text{ mm}^2$ , NG16wE:  $0.43 \pm 0.16 \text{ mm}^2$ ,  $P = 0.09$ , ns) or in HG (HG16wS:  
595  $0.59 \pm 0.09 \text{ mm}^2$ , HG16wE:  $0.45 \pm 0.07 \text{ mm}^2$ ,  $P = 0.06$ , ns) during the first 3 weeks postnatal  
596 period. Taken together, these findings show that the EE increased skin sectors in rats reared

597 in NG, but not in HG, during the first 3 post-natal weeks. In addition, the expansion of nail  
598 representation induced by early HG was not modified by a subsequent exposure to the EE.

599

### 600 *Neuronal responsiveness*

601 Analysis of mechanical thresholds for activating neurons with von Frey filaments  
602 assessed in our 4 experimental groups showed a main effect of the adult environment  
603 conditions ( $F_{1,36} = 19.62$ ;  $P < 0.001$ ), but no effect of the developmental rearing conditions ( $F_{1,36} = 0.30$ ;  $P = 0.59$ , ns) on neuronal responsiveness, and an interaction between these two  
604 factors ( $F_{1,36} = 5.74$ ;  $P < 0.02$ ). Post-hoc tests indicated no effect of EE on the neuronal  
605 response thresholds in rats reared in NG (NG16wS:  $0.393 \pm 0.049$  g, NG16wE:  $0.427 \pm 0.068$  g,  
606  $P = 0.16$ , ns), in contrast to the significant increase in rats reared in HG (HG16wS:  $0.343 \pm 0.025$   
607 g, HG16wE:  $0.459 \pm 0.061$  g,  $P < 0.001$ ).

609

### 610 *Size of cutaneous RFs*

611 As for the RFs recorded within the glabrous skin cortical zones, ANOVA showed a significant  
612 effect of the adult housing conditions ( $F_{1,36} = 19.87$ ;  $P < 0.001$ ) but no effect of the early  
613 rearing conditions ( $F_{1,36} = 3.96$ ;  $P = 0.06$ , ns) and no interaction between these two factors ( $F_{1,36} = 3.74$ ;  $P = 0.06$ , ns) (Fig. 9). Further analysis indicates an EE-induced decrease in glabrous  
614 RF sizes in rats reared in NG (NG16wS:  $3.39 \pm 0.31$  %, NG16wE:  $2.48 \pm 0.24$  %,  $P < 0.001$ ), but  
615 not in those reared in HG (HG16wS:  $3.40 \pm 0.32$  %, HG16wE:  $3.04 \pm 0.75$  %,  $P = 0.19$ , ns).  
616 Moreover, ANOVA yielded no significant effect of early rearing ( $F_{1,36} = 0.31$ ;  $P = 0.58$ , ns) and  
617 an effect of adult housing ( $F_{1,36} = 4.51$ ;  $P < 0.05$ ) conditions on hairy skin RFs, as well as no  
618 interaction between these two factors ( $F_{1,36} = 0.72$ ;  $P = 0.40$ , ns). Post-hoc tests indicated that  
619 the hairy skin RFs were not modified by the EE either in rats reared in NG (NG16wS:

621 7.21±1.01 %, NG16wE: 7.93±2.05 %; P=0.37, ns) or in those reared in HG (HG16wS:  
622 6.42±1.78 %, HG16wE: 8.10±2.08 %; P=0.17, ns) during the early postnatal period of  
623 development.

624 Taken together, our data indicate that the cortical map changes induced by early  
625 postnatal HG rearing impeded the remodeling of forepaw representations induced by EE in  
626 young adult animals raised in NG from birth.

627

## 628 **DISCUSSION**

### 629 **Methodological considerations**

#### 630 **1. Coriolis forces**

631 As the centrifuge used to generate HG also induced a Coriolis force, one has to consider its  
632 possible influence on the animals' behavior and effects on the forepaw. The Coriolis  
633 acceleration was computed using the following formula:

$$634 \vec{CA} = 2\vec{\omega} \times \vec{v}$$

635 where CA stands for the Coriolis acceleration,  $v$  is the rat's velocity and  $\omega$  is the angular  
636 velocity of the centrifuge and  $\times$  the vectorial product.

637 Knowing that in our experimental conditions,  $\omega = 3.81$  rad/s and the cage deviation  
638 from the horizontal was  $60^\circ$ , the maximum rat velocity in their cage being estimated at  
639 about 0.10 m/s, several cases have been computed, depending on the orientation of the  
640 rat's displacement. Obviously, the highest value of the Coriolis acceleration was obtained  
641 when the rat moved in the lengthwise direction of the cage, thus perpendicular to the  
642  $\omega$  vector. The CA value is  $\pm 0.838$  m/s<sup>2</sup> (the signs indicate the direction of the Coriolis  
643 acceleration). Thus, it appears that the value of the additional force on the rat's paws (and  
644 nails) corresponded to an acceleration of 0.085 G (0.025 N for a rat weighing 30 g) i.e., a

645 small fraction of the 2 G “vertical” acceleration. In the case of transversal direction of  
646 displacement in the cage, knowing that the speed vector was at 30° from the  $\omega$  vector, the  
647 value is lower ( $\pm 0.419 \text{ m/s}^2$ ) and corresponds to an acceleration of 0.043 G. On the basis of  
648 these calculations, in our experimental conditions, the Coriolis forces were of little  
649 significance in relation to the hypergravity force. Considering that the rat moves in various  
650 directions within the cage, the Coriolis acceleration acting on the forepaw has various  
651 orientations. Therefore, the mechanical effects of Coriolis forces on the forepaw will tend to  
652 cancel one another out. It is thus likely that the Coriolis forces relative to the 2 G  
653 hypergravity made a negligible contribution to cortical map changes reported in our study.

654

## 655 ***2. Behavioral effects of HG exposure***

656 It is worth noting that rat pups are nursed over the first 3 postnatal weeks, with a daily time  
657 devoted to lactating behavior that decreases from about 80%, during the first days, to 30%  
658 on the 18<sup>th</sup> day postnatal (Rosselet et al. 2006). The motor system matures slowly over the  
659 first postnatal weeks and the pups do not walk spontaneously before the end of the second  
660 week of life. Altman and Sudarshan (1975) reported that, in the rat, walking and running  
661 predominate by the end of the second post-natal week. Consistently, clear topographical  
662 organization of the forelimb cortical map appears by P15 in S1 (Seelke et al., 2012). These  
663 findings suggest that the impact of HG on the forces exerted on the forepaws during the  
664 locomotor behavior was predominant during the 3<sup>rd</sup> postnatal week. Regular video  
665 monitoring of the rats’ behavior during the early postnatal period in both NG and HG rats did  
666 not reveal any behavioral modification in HG rats with respect to the control NG rats housed  
667 in gondolas. Consistently with our study, Bouët et al. (2004), who used a similar  
668 experimental device to ours, did not report impaired behavior in the HG environment.

669 Importantly, no behavioral sign of stress (diminished grooming, exploratory activity,  
670 lactation or nursing) was observed in HG rats as compared to controls. Also of relevance for  
671 potential environmental stress in our study, no changes in corticosterone levels were  
672 measured in rats exposed to 2 G (Moran et al., 2001).

673       After the HG rats were exposed to NG, we did not detect any abnormalities in postural  
674 or locomotor behavior other than an intensification of the exploratory behavior that was  
675 found for the first 2 days in NG. In particular, we did not observe the enlarged support  
676 surface and lower body position reported by Bouët et al. (2003, 2004). It is important to  
677 underline that in these studies the HG-induced alterations to the rodents' posture and  
678 locomotion were reported after a long duration HG (2 G for 3 months), during a period of  
679 adaptation to NG (see also Discussion below). The available evidence suggests that the long-  
680 term cortical changes induced by the 3-week early exposure to HG were not accounted for  
681 by enduring behavioral alterations.

682

### 683 ***Summary of results***

684 In this mapping study of the maturation of somatosensory maps (see Fig. 10), we provide  
685 evidence that the forepaw cortical representations in 2 and 3 week-old rats are adult-like in  
686 their somatotopic organization, but with lower mechanical thresholds than in the adults. A  
687 continuous expansion of each of the constituent territories of the map was observed until  
688 the 19<sup>th</sup> postnatal week. Secondly, we report both short and long-term effects of early and  
689 transient HG exposure on the development of somatotopic forepaw cortical maps in rats  
690 subsequently exposed to normogravity. Despite preservation of the surface area of the  
691 forepaw map, HG was found to shape the somatotopic organization of the cortical forepaw  
692 map, by expanding the nail representation at the expense of the skin territories in which

693 ventral skin cutaneous RFs were found to be enlarged. In addition, nail responses were  
694 biased toward an increased sensitivity to nail extension. The early imprint of HG on the map  
695 organization was not modified by a subsequent exposure to normogravity during the 4  
696 month period examined. However, after 1 week of housing in normogravity, neuronal RFs  
697 recorded in HG rats displayed sizes very similar to those recorded in NG rats, while  
698 directional sensitivity to nail displacement developed within 8 weeks. Thirdly, we found that  
699 early and transient HG exposure prevented the use-dependent reorganizational changes  
700 induced by enriched environment in adult animals reared under normogravity conditions  
701 from birth.

702

703

704

#### 705 *Adult-like organization of forepaw maps in 2 or 3 week- old rats*

706 This study reports new findings on the development of forepaw somatosensory maps  
707 based on high-density electrophysiological maps. The organization and the development of  
708 representation in the forepaw barrel subfield (FBS) in the S1 cortex of the rat has been  
709 documented in very few studies, in contrast to whisker representation which has been  
710 extensively investigated. Barrel-like structures in the FBS develop around P5 (McCandlish et  
711 al., 1989; Rhoades et al., 1990). Experiments carried out to detect the earliest time when the  
712 S1 cortex is driven by somatosensory inputs indicate that responses are elicited in the snout  
713 subfield as early as 12 hours after birth, in the forepaw area by the end of the 1<sup>st</sup> postnatal  
714 day and in the hindpaw area by the middle of P2, thus following a lateral to medial gradient  
715 (McCandlish et al., 1993). However, increases in amplitude and complexity of wave shape  
716 and decreases in response latencies were described over subsequent postnatal days, until

717 P14. Seelke et al. (2012) who examined the time course of emergence of the topography of  
718 entire body maps have shown that early in development, most of S1 is occupied by  
719 vibrissae/face representations. These authors observed that from P10, representations of  
720 body parts including the forelimbs emerge with a crude topography, whereas a clear  
721 topographical organization began to appear by P15, along with a reduction of RF sizes. From  
722 P20, body maps were found to be similar to those recorded in the adult. Our results are in  
723 accordance with these data: the maps recorded at P15 in two rats and at P22 in larger  
724 samples appeared to be adult-like in their topographical organization (see Coq and Xerri,  
725 1998, 1999; Xerri and Zennou-Azogui, 2003).

726

727

728 *Short-term effects of early exposure to hypergravity on the development of*  
729 *forepaw somatosensory maps*

730 HG was found to shape the forepaw map by decreasing both the cortical zones  
731 serving the glabrous and hairy skin surfaces, whereas that of the nail expanded within  
732 cortical sectors normally allocated to skin surfaces. The specificity of the HG effect was  
733 evidenced by the fact that this expansion did not occur in rats exposed to HG and subjected  
734 to partial deprivation of movement-induced nail inputs resulting from clipping. Interestingly,  
735 proprioceptive areas that normally surround the cutaneous maps replaced the lost nail  
736 representations that were found to substitute for the skin sectors in the HG rats. In addition,  
737 HG clearly modified the overall directional sensitivity to nail displacement of the S1 neurons,  
738 as shown by a population response biased toward upward deflection, presumably resulting  
739 from counter-reaction forces exerted on the nails during upright standing and walking of the  
740 HG animals in their home cages.



741           The alteration of the organization of forepaw somatosensory maps resulting from  
742 early HG suggests that this environment strongly modifies the cortical integration of afferent  
743 signals from somatosensory receptors. Consistently with this view, previous studies have  
744 shown that changes in gravity during development influenced motor development in  
745 rodents. Bojados et al (2013) have shown that exposure to hypergravity before P10, i.e. the  
746 acquisition of locomotion, induces postural changes in mice, particularly a more extended  
747 ankle joint during locomotion (extension bias). As a mirror situation, rats exposed to  
748 microgravity from P14 to P30 exhibited changes in flexor muscle responses (Walton et al.,  
749 2005a). In rats born and reared during 100 days in hypergravity, the contractile properties  
750 and phenotype of hindlimb extensor muscle fibers were found to be modified (Picquet et al.,  
751 2002). Muscle characteristics were reinforced as the soleus, an antigravity muscle, became  
752 slower (100% expression of myosin heavy chain slow isoform I), whereas its agonist, the  
753 plantaris muscle, presented faster contractile behavior.

754           Collectively, these data sustain the view that joint and muscle proprioception is  
755 altered by gravity constraints both during development and in the adult, and that this  
756 alteration is very likely to induce enduring changes in the processing of somatosensory  
757 information within S1.

758           It is widely recognized that early sensory experience plays a critical instructive role in  
759 the structural and functional maturation of the sensory systems (Hensch, 2004; Fox and  
760 Wong, 2005). According to this view, a process of competition for cortical space between  
761 sensory inputs shapes and refines the organization of the neural circuits underlying the  
762 visual, auditory and somatosensory cortical maps (Hensch, 2004). Patterns of activity elicited  
763 by sensory experience are translated into networking of synaptic connections (Katz and  
764 Shatz, 1996). Our results confirm that the topographically organized somatosensory forepaw

765 maps are sculpted by early sensory experience. They also reveal an experience-driven  
766 competition between somatosensory sub-modalities that critically shapes cortical maps  
767 during the early sensory events of postnatal life. We propose that HG, by increasing the  
768 pressure exerted on the ventral surfaces of the paws and enhancing the antigravity muscle  
769 activity, modifies the pattern of somatosensory afferent signals, thereby leading to the  
770 emergence of a distinctive forepaw somatosensory map. The balance between cutaneous  
771 submodalities was modified, as evidenced by the contraction of the map territories allocated  
772 to skin surfaces that was paralleled by an expansion of cortical sectors serving nail areas.  
773 This expansion of nail representation can be attributed to a relative increase in nail  
774 stimulation in HG, as also suggested by a change in directional sensitivity reflected by the  
775 greater percentage of responses to upward deflection. Interestingly, drastic reduction of  
776 tactile input resulting from nail clipping in HGn- rats resulted in a prominent shrinkage of  
777 cortical sectors serving the nails that unexpectedly was compensated for, not by an increase  
778 in skin surface representations, but an expansion of proprioceptive sectors. This finding  
779 suggests that somatosensory submodalities converging on cortical neuronal targets do not  
780 have equivalent effectiveness in competing for cortical space. The propensity of  
781 proprioceptive representation for “invading” cutaneous territories of the somatosensory  
782 cortical maps has been repeatedly documented in our previous studies on adult experience-  
783 dependent plasticity after tactile impoverishment or sensorimotor restriction (Coq and Xerri,  
784 1999), as well as during aging (Coq and Xerri, 2000). An additional argument in favor of  
785 competition between cutaneous and proprioceptive inputs comes from the finding that in  
786 humans, a rapid increase in cortical proprioceptive activity elicited by stimulation of muscle  
787 afferents from the first dorsal interosseus occurs after transient cutaneous deafferentation  
788 of the cutaneous territory overlying the corresponding muscle, following anesthesia of the

789 radial nerve (Tinazzi et al., 2003). In the present study, the emergence of proprioceptive  
790 sectors could be confused with an increase in the threshold of cutaneous responses.  
791 Accordingly, a decreased response sensitivity would be expected to occur in the spared  
792 cutaneous territories of the HG maps. However, our data show a lack of change in the  
793 mechanical thresholds recorded in the shrunken glabrous skin representational zones. The  
794 reduced glabrous skin representation observed in both HG and HGN- rats more likely reveals  
795 a redistribution of effective inputs within these cortical sectors of the forepaw maps due to  
796 the HG environment. The dynamic balance between competing inputs distributed within  
797 thalamocortical and intracortical networks is underpinned by the convergence of cutaneous  
798 and proprioceptive inputs onto single layer IV neurons of the S1 cortex (Lamour and Jobert,  
799 1982; Chapin and Lin, 1984; Sievert and Neafsey, 1986; Gioanni, 1987). This convergence  
800 enables an experience-dependent, behaviorally relevant allocation and refinement of  
801 somatosensory cortex representational territories.

802         In this study, the spatial resolution of the contracted forepaw cutaneous maps was  
803 found to have deteriorated after early rearing in HG, as indicated by the increase in the sizes  
804 of the glabrous RFs, whereas hairy RF sizes were not affected. Previous studies on adult  
805 experience-dependent plasticity of somatosensory maps have described RF sharpening or  
806 expansion in response to specific task learning or changes in subject-environment  
807 interaction (see Xerri et al., 2008 for review). These studies have underscored the influence  
808 of the temporal patterning, i.e. the degree of local synchrony of concurrent sensory inputs  
809 on the representational segregation of cortical somatosensory maps through receptive field  
810 remodelling (Allard et al., 1991; Recanzone et al., 1992; Diamond et al., 1993; Armstrong-  
811 James et al., 1994; Wang et al., 1995; Godde et al., 1996; Byl et al., 1996; Rosselet et al.,  
812 2008). A consistent finding in all these studies was that temporally synchronized stimulation

813 of large skin territories induced RF enlargement, whereas temporally distributed skin  
814 stimulation resulted in RF decreasing. Along the same lines, in an earlier study, we  
815 performed severe sensorimotor deprivation by using single-forelimb casting in rats. This  
816 procedure led to an over-reliance on the non-constrained forelimb for postural balance and  
817 increased mechanical pressure on the ventral skin surfaces of the uncast forepaw (Coq and  
818 Xerri, 1999). Although the forced use of the non-cast forelimb did not affect the area of the  
819 corresponding cutaneous map, it led to enlargement of glabrous skin RFs, probably as a  
820 result of concomitant stimulation of larger than usual glabrous skin territories. In the present  
821 study, the 2G environment induced broader simultaneous contact of forepaw ventral skin  
822 surfaces with the cage floor during stance and locomotion, as confirmed by the increased  
823 paw prints. Accordingly, the glabrous skin RF enlargement recorded in HG young rats can be  
824 attributed to the spatial aggregation of formerly segregated thalamo-cortical inputs through  
825 unmasking/strengthening of formerly subthreshold synapses. The cutaneous RF  
826 enlargement found in HG rats is consistent with the decrease in GABA immunoreactivity  
827 recorded in the limb representation of the rats S1 cortex after 14 days of exposure to  
828 hypergravity (D'Amelio et al., 1998). Furthermore, an expansion of cutaneous RFs in the S1  
829 cortex was found when GABA-mediated local inhibition was antagonized (Dykes et al., 1984;  
830 Alloway et al., 1989; Tremere et al., 2001 Chowdhury and Rasmusson, 2002).

831

### 832 *Enduring effects of early exposure to hypergravity*

833 Here, we show that postnatal changes in somatosensory map organization examined  
834 after 1 week, 8 weeks and 16 weeks in NG was impacted by neonatal exposure to HG for 3  
835 weeks. Gradual increase in the area of the constituent regions of the forepaw maps was  
836 found to be typical of rats reared in NG. Interestingly, the HG-induced contraction of skin

837 representation and concomitant increase in nail areas found after HG rearing for 3 weeks  
838 were maintained over the following 16 weeks of housing in NG. Therefore, this early  
839 experience impeded normal development of somatosensory maps after exposure to NG,  
840 thereby indicating a lack of adaptation of the S1 cortex to new somatosensory experience  
841 occurring in NG.

842         These enduring effects of HG rearing on somatosensory maps could be attributed to  
843 an impaired development of posture and locomotion that would tend to maintain changes in  
844 the patterns of somatosensory signals conveyed to the S1 cortex. Consistently, previous  
845 studies on the effects of altered gravity on the development of motor functions have shown  
846 enduring changes in locomotion (Walton 1992; Bojados et al., 2013), swimming behavior  
847 (Walton et al., 2005a) and development of surface righting (Walton et al., 2005b), and thus  
848 suggested the existence of a critical period in motor development. Persisting extension bias  
849 was observed by Bojados et al. (2013) in 2 month-old mice centrifuged from conception to  
850 P10 or P30. In addition, rats born and reared until the age of 3 months in HG showed  
851 alterations in locomotor pattern when exposed to normogravity. They were found to exhibit  
852 a faster locomotor rhythm (increased number of steps/sec) (Bouët et al., 2003, 2004). They  
853 also exhibited a lower body position and an exaggerated foot dorsiflexion (Bouet et al.,  
854 2004). However, these alterations disappeared within 3 weeks, reflecting a gradual  
855 adaptation to normogravity. It is worth mentioning that in all these studies, exposure to  
856 altered gravity was longer than in the present study. Regarding descending pathways,  
857 Gimenez y Ribotta et al. (1998) showed a delayed and modified development of serotonergic  
858 projections to the spinal cord of rats exposed to HG from the 11<sup>th</sup> day after gestation to the  
859 15<sup>th</sup> day postnatal and which appeared to be smaller than controls. The organization and  
860 ultrastructure of these projections were durably affected, even after 8 months in

861 normogravity. However, no behavioral correlates were provided in this study, leaving open  
862 the question of the functional consequences of alterations in the organization of the  
863 serotonergic projections. As previously mentioned, Picquet et al. (2002) showed that soleus  
864 antigravity muscle of rats conceived, born, and reared in HG until 100 days expressed 100 %  
865 of slow myosin phenotype. In a subsequent study, the same authors showed that in these  
866 animals, normogravity from 100 days to 115 or 220 days did not transform the muscle  
867 phenotype, suggesting the existence of a critical period in muscle phenotype determination  
868 (Picquet et al., 2005). One may hypothesize that these persisting changes in descending  
869 pathways and muscle properties during subsequent exposure to NG were likely to lastingly  
870 influence the cortical integration of proprioceptive inputs and thus the corresponding  
871 representations in the S1 map. By contrast, the paw print measurements of rats exposed to  
872 NG after HG rearing were identical to those of rats reared in NG, suggesting that the flows of  
873 cutaneous information to the S1 cortex were similar in NG and in HG rats subjected to  
874 normogravity. This hypothesis is supported by the normalization of the sizes of ventral skin  
875 RFs in HG rats as early as the end of the first week of housing in NG and that of the  
876 directional sensitivity of the responses to nail displacement between the 2<sup>nd</sup> and the 8<sup>th</sup>  
877 week of normogravity. However, such normalization of neuronal responses within cortical  
878 sectors serving cutaneous inputs does not preclude the enduring imprint of early HG  
879 environment on the cortical integration of somatosensory afferents. This is suggested by  
880 persistent alterations of the forepaw map organization such as the decreased area of ventral  
881 skin sectors and the increased nail sectors, as well as the lack of age-related expansion of  
882 hairy skin zones of the forepaw maps. Furthermore, it is noteworthy that the HG-induced  
883 freezing effects lasted at least 16 weeks after exposure to normal gravity. Collectively, these  
884 findings sustain the view that the first 3 postnatal weeks represent a time window that is

885 critical for the developmental segregation of somatotopic maps. Importantly, forelimb  
886 motor maps were first detectable at the end of the second postnatal week (Young et al.,  
887 2012). Moreover, during rat locomotion development, pivoting predominates during the  
888 second half of the first week, crawling during most of the second week and walking or  
889 running by the end of the second week (Altman and Sudarshan, 1975). Therefore, the  
890 behaviorally induced influence of HG on the forepaw map formation reported in the present  
891 study was predominant during or even confined to the third postnatal week. The present  
892 findings support the view that the maturation of somatosensory maps is strongly influenced  
893 by use-dependent changes taking place within a narrow critical postnatal time window.

894         The long-lasting effects of HG reported herein may reflect enduring changes in  
895 neuronal network connectivity during early development. The enduring effects of early HG  
896 rearing are at variance with the reversibility of alteration found in adult somatosensory  
897 maps following various manipulations (see for reviews Buonomano and Merzenich, 1998;  
898 Xerri, 2008; Barnes and Finnerty, 2010), such as experimental syndactyly (Clark et al., 1988),  
899 limb immobilization (Coq and Xerri, 1999) or natural episodic behavior (nursing; Rosselet et  
900 al., 2006). These HG-induced sustained alterations provide an additional argument in favor  
901 of a critical period in the maturation of somatosensory maps, previously shown for the  
902 development of the whisker-barrel system (see Erzurumlu and Gaspar, 2012, for review).

903 Herein, we report the persistent effect of early experience on the shape and area of  
904 somatosensory map territories. By contrast, other response characteristics, namely the  
905 spatial selectivity, i.e. the size of glabrous skin RFs and the directional sensitivity to nail  
906 displacement, were found to display an adaptation to NG. This finding is in accordance with  
907 an earlier study in adult rats in which we showed that the spatial selectivity of S1 neurons  
908 resulting from ongoing segregation/desegregation of inputs converging onto neuronal

909 targets was reshaped on a time scale closely dependent upon behaviorally driven changes in  
910 somatosensory inputs (Rosselet et al., 2006). The reversibility versus retention of different  
911 neuronal response properties, after the animals were exposed to NG, shows that the use-  
912 dependent synaptic mechanisms leading to masking/unmasking of less/more effective  
913 inputs or to synaptogenesis at maturity are expressed within limits of representational  
914 malleability stabilized by somatosensory experience during a critical developmental period.  
915 This view is substantiated by our findings that the potential for experience-dependent  
916 plasticity in the adult was constrained by somatosensory experience during development. In  
917 the present study, HG rearing was found to impede the use-dependent map remodeling  
918 induced by enriched environment in adult animals reared in normogravity, originally  
919 described by Coq and Xerri (1998). It seems that early HG experience shaped the neural  
920 network underpinning the forepaw cortical maps in such a way that adult experience-  
921 dependent plasticity was limited. Interestingly, the influence of tactile interactions between  
922 parents and offspring during early life on intrinsic connections within S1 and callosal  
923 connections has been highlighted by a recent study in rodents (Seelke et al., 2016).  
924 Therefore, one cannot rule out the possibility that subtle HG-induced changes in nursing  
925 behavior that were not detected using video monitoring could have induced alteration in  
926 cortical connectivity.

927         The present study provides evidence that HG is a relevant paradigm to investigate  
928 experience-dependent cortical plasticity without sensory deprivation. Our findings advocate  
929 the view that the potential for somatotopic map remodeling induced by novel sensory  
930 experience in adulthood is constrained by construction of the underlying neuronal networks  
931 within a narrow critical period of development.

932



933

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1134

## 1135 **Captions**

1136

1137 **Fig. 1. Experimental protocols and procedure.** Synoptic representation of the experimental  
1138 protocols used to evaluate short-term and enduring effects of early exposure to hypergravity  
1139 on the development of forepaw cortical maps in S1 **(A)** and its influence on adult map use-  
1140 dependent plasticity **(B)**. Rats were born and housed in hypergravity (HG, 2G) generated by a  
1141 centrifuge **(C)** until the age of 3 weeks. Electrophysiological mapping of the forepaw  
1142 representation was performed at P22 in HG rats and HGn- rats (with trimmed forepaw nails),  
1143 and after 1 week (HG1wS rats), 8 weeks (HG8wS rats) or 16 weeks (HG16wS rats) spent in  
1144 normogravity (NG). The maps were compared to that obtained in age-matched groups of  
1145 rats reared in NG from birth (NG, NG1wS, NG8wS, NG16wS groups). Paw print  
1146 measurements were performed in 2 additional groups of rats reared in HG or NG and tested  
1147 under both NG and HG conditions **(D)**. Mapping data were also recorded in rats raised either  
1148 in HG or NG during the first 21 postnatal days, then maintained in an enriched environment  
1149 for 16 weeks (HG16wE and NG16wE, respectively) **(E)**. The results were compared to those  
1150 obtained in rats raised either in HG or NG during the first 21 postnatal days, then housed in a  
1151 standard environment (HG16wS and NG16wS, respectively).

1152

1153 **Fig. 2. Electrophysiological map of cutaneous surfaces obtained in a 2-week old rat.** Map  
1154 elaboration is based on the response characteristics (somatosensory submodality, location  
1155 and size) of the receptive fields (RFs) of neurons recorded within layer IV of the S1 cortex. **A.**  
1156 Magnified image of the forepaw area of the cortex with a superimposed drawing of the  
1157 boundaries encompassing cortical sites the RFs of which were restricted to a common



1158 forepaw subdivision. Within these boundaries, colored dots indicate cortical sites where  
1159 cutaneous hairy and glabrous skin responses were elicited while colored squares show  
1160 cortical sites where neuronal responses were elicited by nail displacements. Green and red  
1161 triangles mark cortical sites excited by lower lip and wrist stimulation, respectively. Violet  
1162 squares refer to cortical sites classified as “proprioceptive” (responses to strong pressure on  
1163 skin and tendons and/or joint movement). Black squares mark cortical sites with no  
1164 stimulus-evoked discharges. **B.** Photomicrograph of Nissl-stained section from the forepaw  
1165 area of cortex with an electrolytic lesion in layer IV (arrow) and recording trace illustrating a  
1166 typical multiunit discharge elicited by a von Frey filament (3.22: 0.166 g; 9.15 g/mm<sup>2</sup>)  
1167 applied at the center of a RF on digit 3. **C.** Colored sectors of the cutaneous map with black  
1168 dots indicating cutaneous (skin or nail sensitive) cortical sites. Maps recorded at PN15  
1169 appeared to be adult-like in their topographical organization. **D.** Samples of cutaneous RFs  
1170 on glabrous and hairy skin surfaces corresponding to the cortical sites illustrated in A and C.  
1171 1 to 5: digit 1 to 5; P: pads.

1172

1173 **Fig. 3. Effect of early exposure to hypergravity on the development of the somatotopic**  
1174 **cutaneous maps of the forepaw.** Representative maps obtained on the 22<sup>nd</sup> postnatal day in  
1175 a rat born and housed in normogravity (NG) or hypergravity (HG) and in a rat reared in HG  
1176 with forepaw nails trimmed until the age of 3 weeks (HGn-) (**A**). Glabrous (**B**) and hairy skin  
1177 (**C**) RFs of which the locations on the forepaw were used to reconstruct the cutaneous maps  
1178 illustrated in A. Note the HG-induced increase in the size of glabrous RFs recorded in HG and  
1179 HGn- rats. Note in HG and HGn- rats the HG-induced decrease in the cortical area serving  
1180 skin surfaces of the forepaw. Nail representation was shown to take over the skin zones in  
1181 the HG rat, while in the HGn- rat, proprioceptive zones colonized the cortical territory

1182 compensating for the decrease in nail and skin areas. Same conventions as in Fig.2.

1183

1184 **Fig. 4. Influence of hypergravity on neurons' directional sensitivity to nail displacement. A.**

1185 Classification of neuronal responses according to their sensitivity to nail upward (a) or

1186 downward (b) displacement induced by an electronic von Frey device generating force

1187 profiles. **B.** Percentages of neuronal responses elicited by upward (U), downward (D) or both

1188 upward and downward (U+D) movement of the nails obtained on the 22<sup>nd</sup> postnatal day in

1189 rats born and housed in normogravity (NG) or hypergravity (HG) (see Table 2 for mean

1190 percentages of neuronal responses within experimental groups). Note that hypergravity

1191 resulted in a higher percentage of neurons responding to nail upward displacement.

1192

1193 **Fig. 5. Enduring effect of early hypergravity rearing on cutaneous forepaw maps.** Forepaw

1194 representations obtained in a rat representative of the group of animals reared in NG from

1195 birth (NG, NG1wS, NG8wS, NG16wS groups) (left) and an age-matched rat representative of

1196 those born and reared in hypergravity during the first 3 postnatal weeks at P22 (HG group)

1197 and after 1 week (HG1wS group), 8 weeks (HG8wS group) or 16 weeks (HG16wS group) of

1198 housing in normogravity (NG) (right). Note the "freezing effect" of early HG rearing which

1199 prevented the expansion of forepaw skin sectors, while nail expansion was maintained,

1200 despite exposure to NG for 16 weeks.

1201

1202 **Fig. 6. Effect of early hypergravity rearing on age-dependent changes in the**

1203 **representational organization of forepaw cutaneous cortical maps.** Cortical areas (in mm<sup>2</sup>;

1204 mean ± SD) of the total cutaneous maps (A) and different sectors of the maps (B-D) as a

1205 function of age (in weeks) in rats born and reared in hypergravity until the age of 3 weeks, in

1206 rats housed for 1, 8 and 16 weeks in normogravity (empty squares) after the hypergravity

1207 rearing, and in age-matched rats reared in normogravity from birth (black dots). \* indicates  
1208 statistically significant differences ( $P < 0.05$ ) between NG and HG rats at the same time points.  
1209 Horizontal bars applies to rats reared in normogravity and indicate statistically significant  
1210 differences between time points. Note that none of the map representational zones in the  
1211 HG rats increased as a function of age, in contrast with those recorded in NG rats.

1212

1213 **Fig. 7. Reversible effect of early rearing in HG on the size of glabrous skin RFs.** A. Examples  
1214 of glabrous and hairy skin RFs of which the locations on the forepaw were used to  
1215 reconstruct the cutaneous maps shown in Fig. 5. B. Age-dependent changes in the mean  
1216 sizes of glabrous and hairy skin RFs recorded in the forepaw maps. Note that the HG-induced  
1217 increase in glabrous RF size observed at P22 was reversed as early as the 1<sup>st</sup> week of housing  
1218 in NG. Same conventions as in Fig. 5.

1219

1220 **Fig. 8. Influence of early HG rearing on use-dependent plasticity of forepaw cutaneous**  
1221 **maps in young adults.** Examples of electrophysiological maps obtained in age-matched rats  
1222 raised either in NG or HG during the first 3 postnatal weeks and subsequently housed in an  
1223 enriched environment (EE) (NG16wE and HG16wE, respectively) or a standard environment  
1224 (NG16wS and HG16wS, respectively) for 16 weeks. Note that the expansion of glabrous skin  
1225 sectors induced by the EE in rats reared in NG was not found in rats reared in HG during the  
1226 first 3 postnatal weeks. Furthermore, the HG-induced expansion of nail representation was  
1227 not reversed by subsequent EE housing.

1228

1229 **Fig. 9. Influence of early HG rearing on use-dependent plasticity of glabrous skin RFs in**  
1230 **young adults.** Examples of glabrous and hairy skin RFs corresponding to the cutaneous maps

1231 shown in Fig. 8. HG during the first 3 postnatal weeks prevented the EE-induced decrease in  
1232 glabrous RF size found in rats reared in NG.

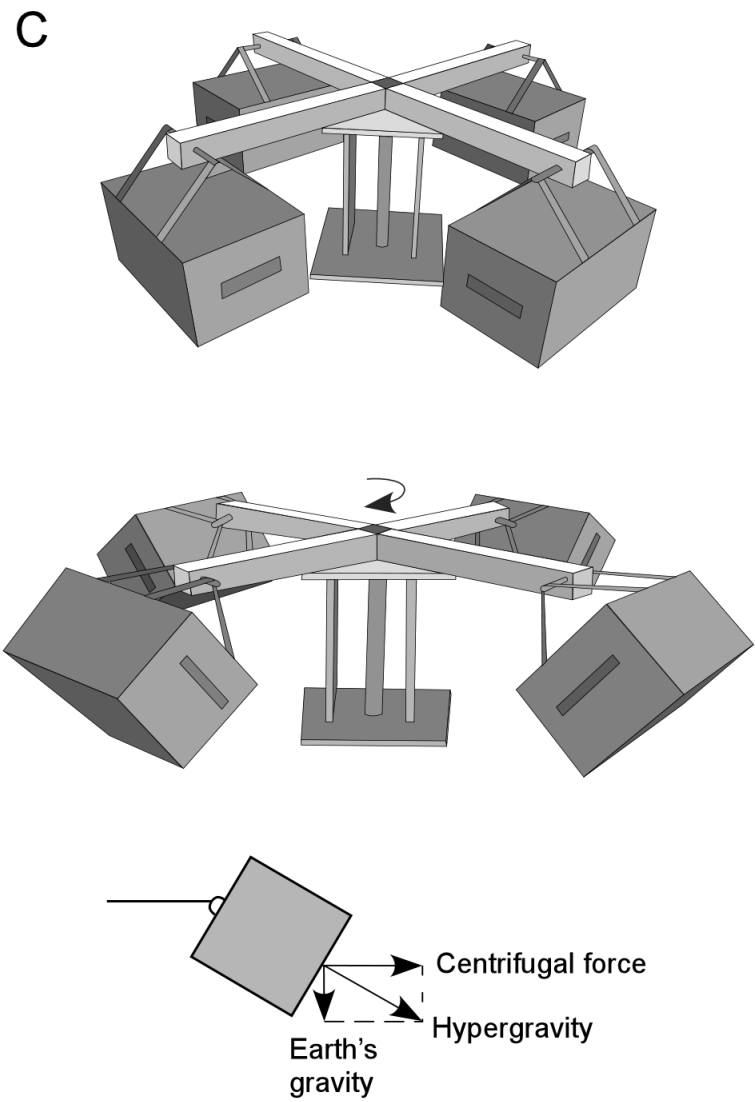
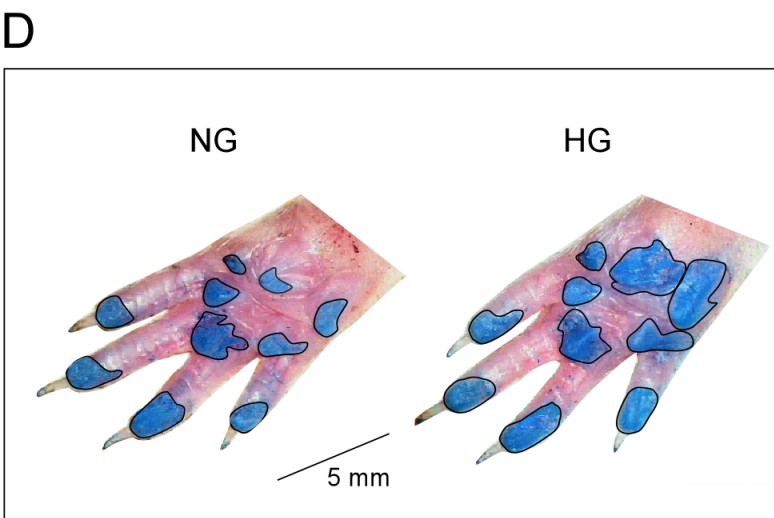
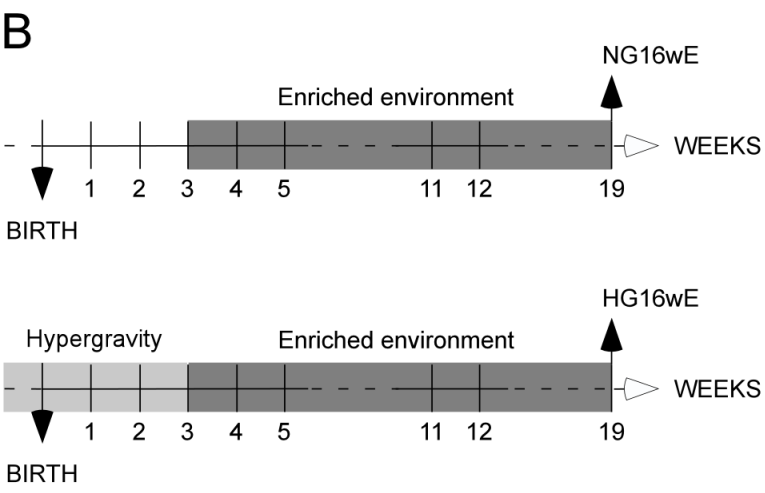
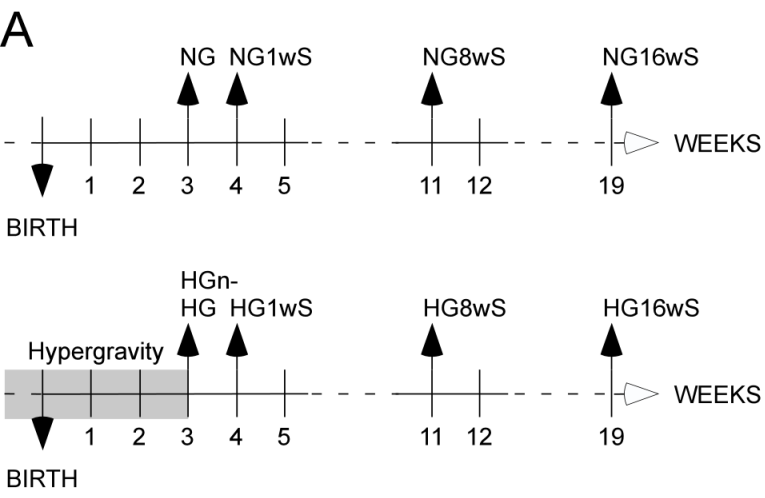
1233 **Fig. 10. Synopsis of findings on the development of the forepaw representation in the S1**  
1234 **cortex and main effects of HG.**

1235 **Table 1. Labels of the experimental groups.**

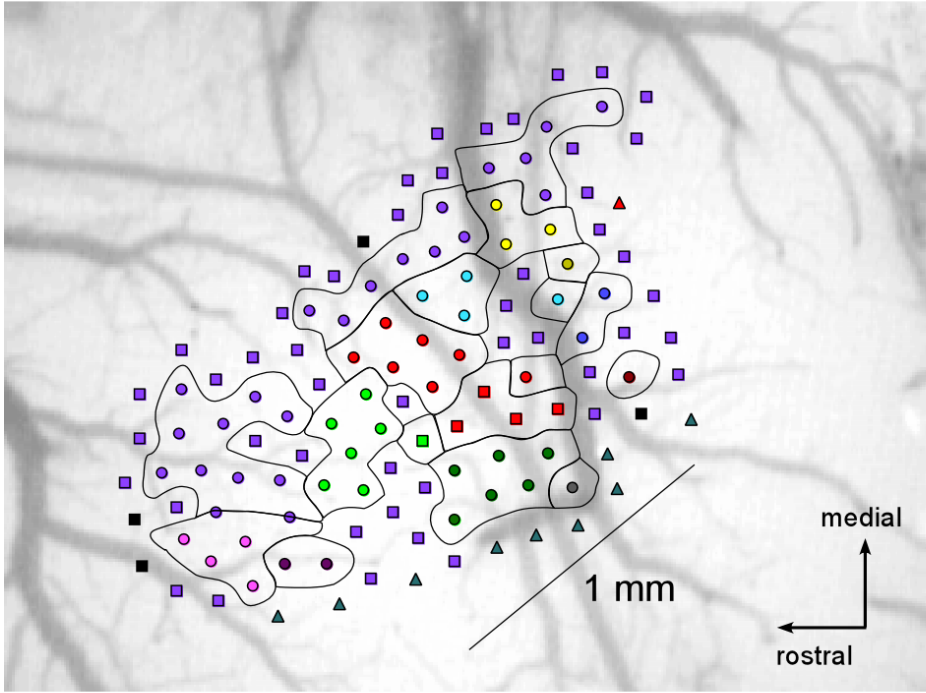
1236 **Table 2. Directional sensitivity of cortical neurons to nail displacement.** Mean percentages  
1237 and number of neuronal responses to upward (U), downward (D) or both U and D nail  
1238 displacement obtained in rats reared in NG from birth (NG, NG1wS, NG8wS, NG16wS) and in  
1239 aged-matched rats born and reared in hypergravity during the first 3 postnatal weeks at P22  
1240 (HG) and after 1 week (HG1wS), 8 weeks (HG8wS) or 16 weeks (HG16wS) of housing in  
1241 normogravity (NG). See the text for statistics.

1242 **Table 3. Neuronal responsiveness to glabrous skin stimulation.** Mean mechanical threshold  
1243 (bending force) of neuronal responses obtained using von Frey monofilaments in rats reared  
1244 in NG from birth (NG, NG1wS, NG8wS, NG16wS) and in age-matched rats born and reared in  
1245 hypergravity during the first 3 postnatal weeks at P22 (HG) and after 1 week (HG1wS), 8  
1246 weeks (HG8wS) or 16 weeks (HG16wS) of housing in normogravity (NG). Mechanical  
1247 thresholds recorded in NG and HG rats housed for 16 weeks in enriched environment  
1248 (NG16wE and HG16wE) from PN22 are also shown. See the text for statistics.

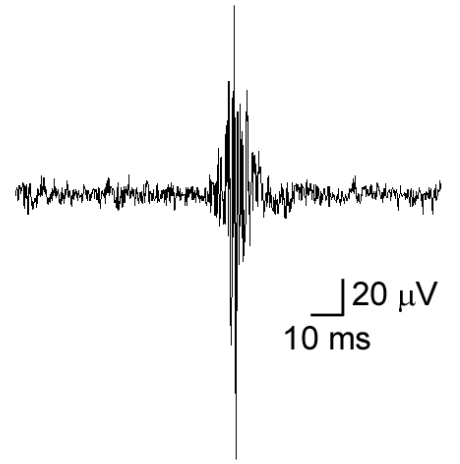
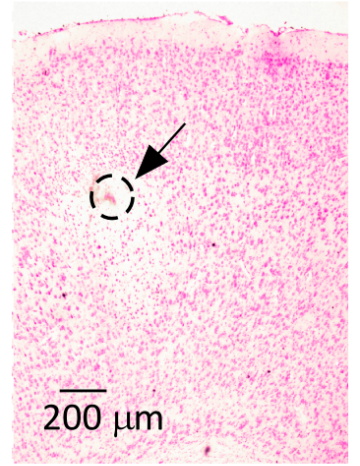
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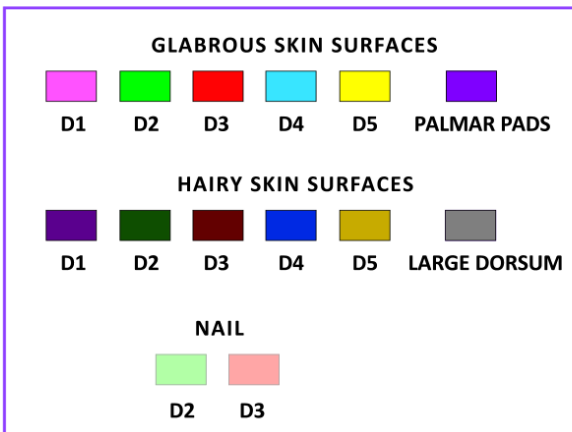
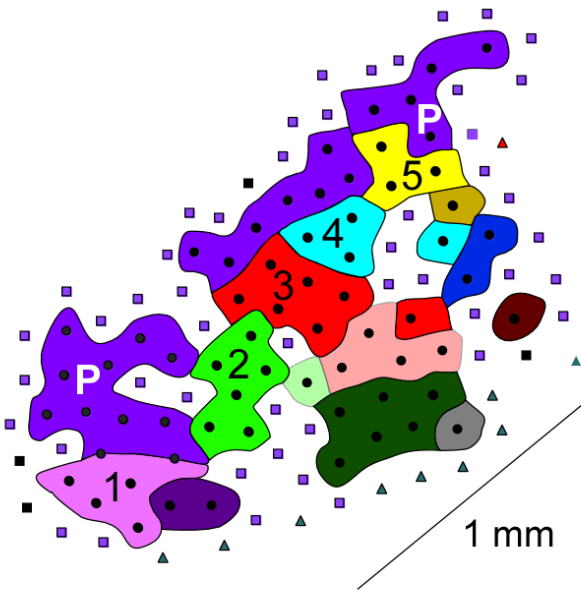
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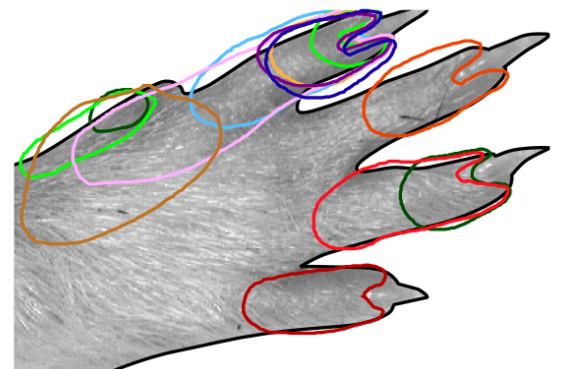
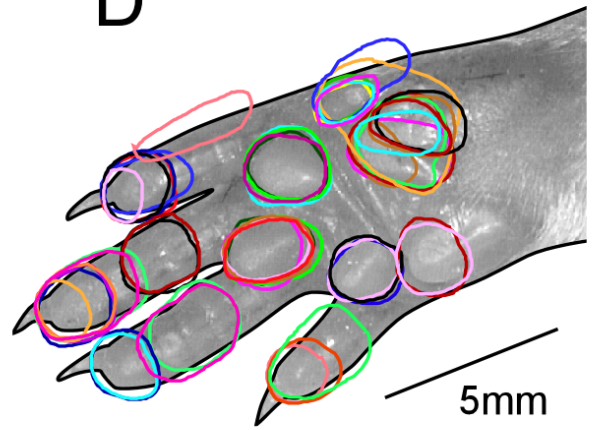
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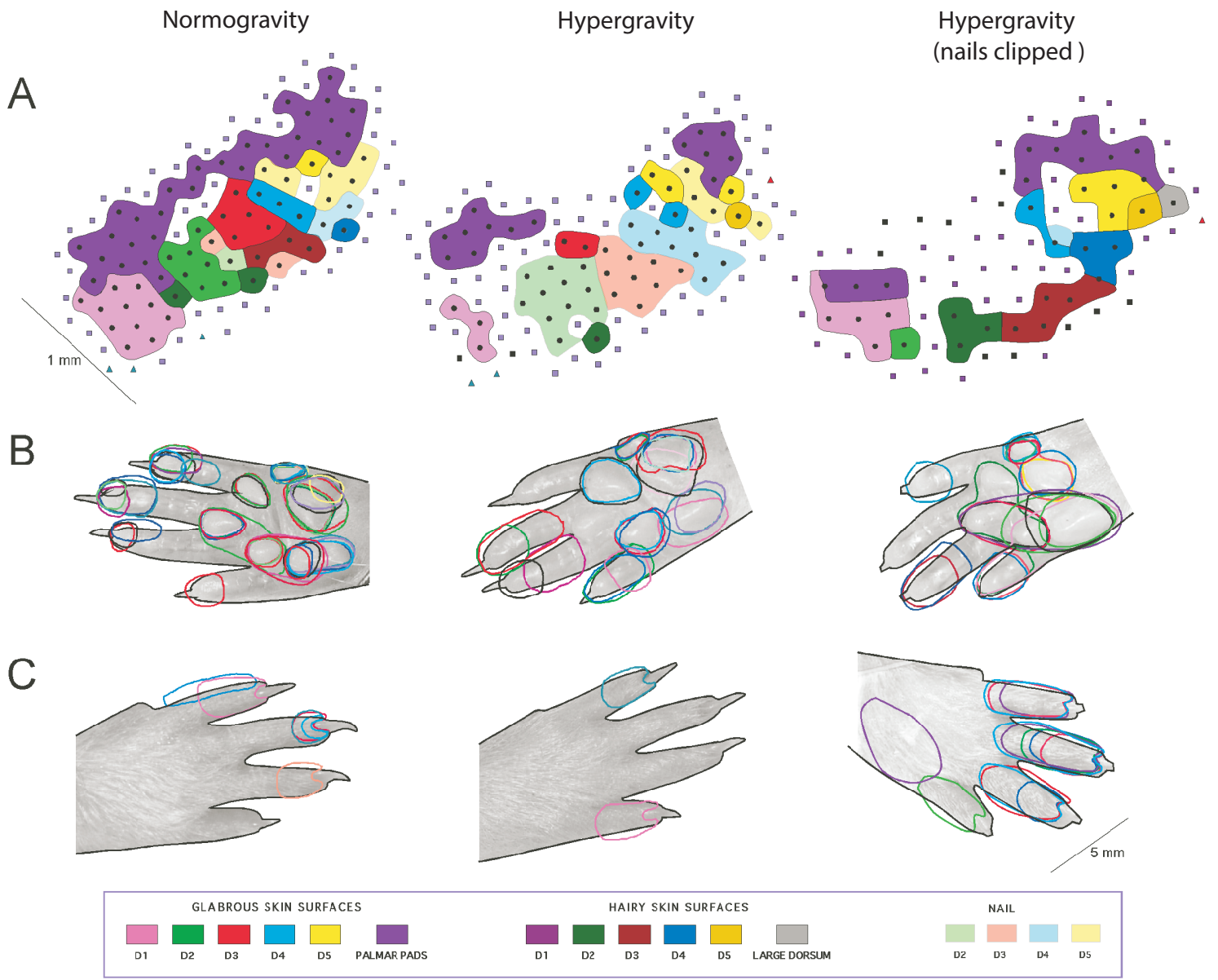


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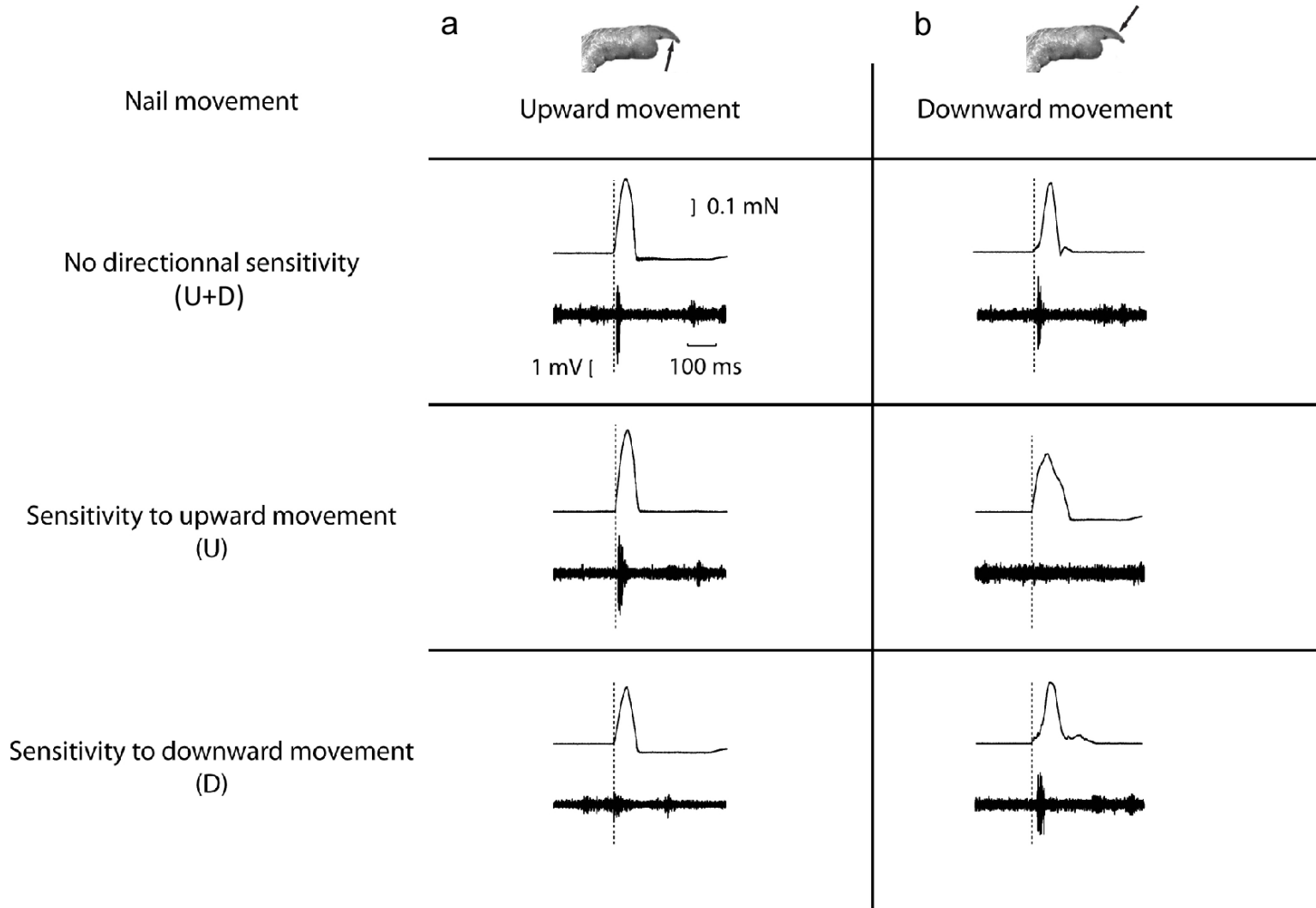


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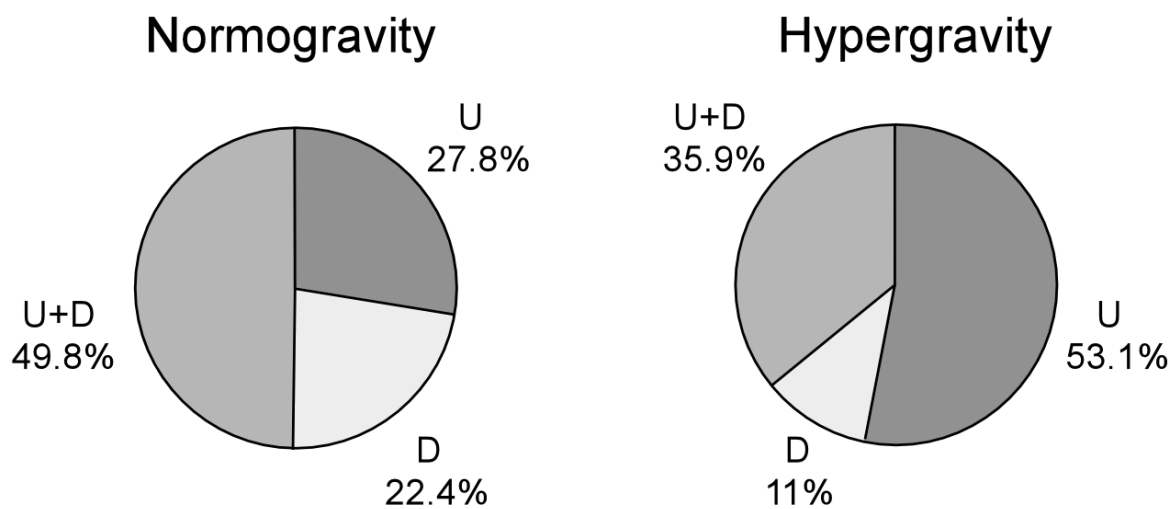




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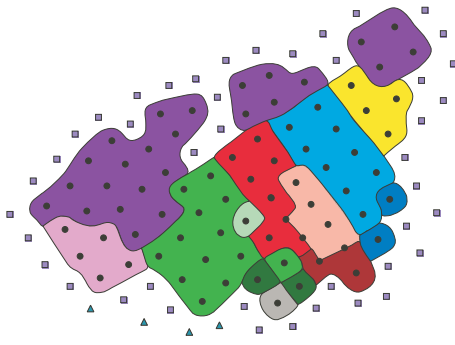
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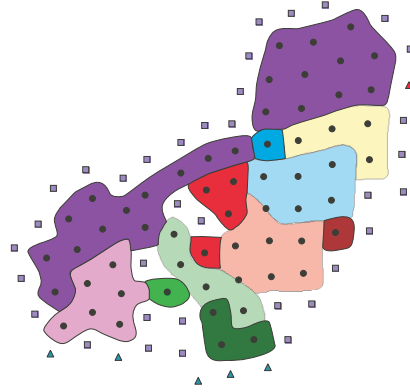
NG

NG: 3 w. old rats in control cages



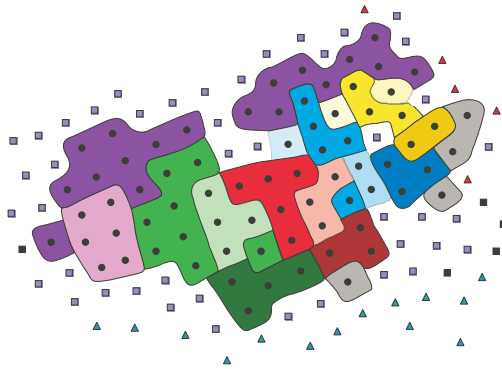
HG

HG: 3 w. old rats in centrifuge



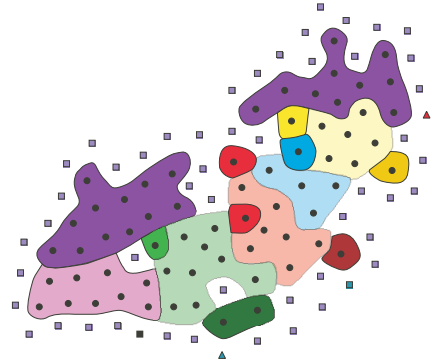
NG1wS

NG: 3 w. in control cages + 1 w. in standard environment



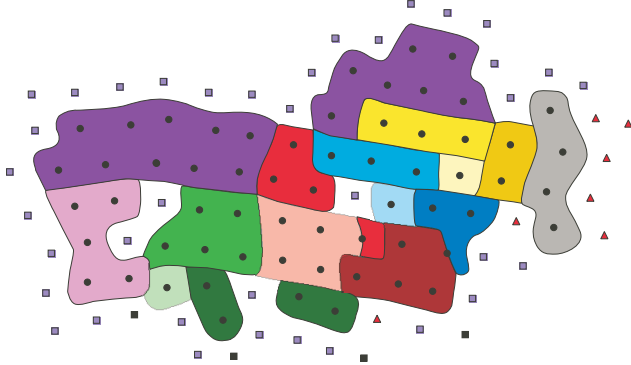
HG1wS

3 w. in HG + 1 w. in NG standard environment



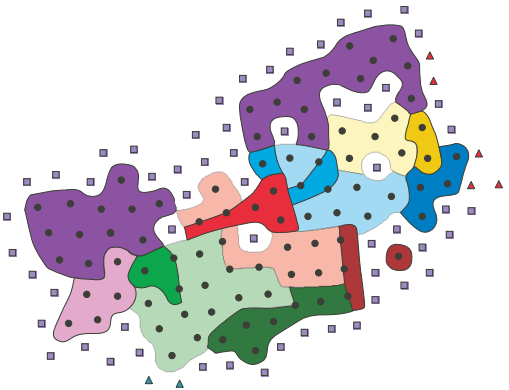
NG8wS

NG: 3 w. in control cages + 8 w. in standard environment



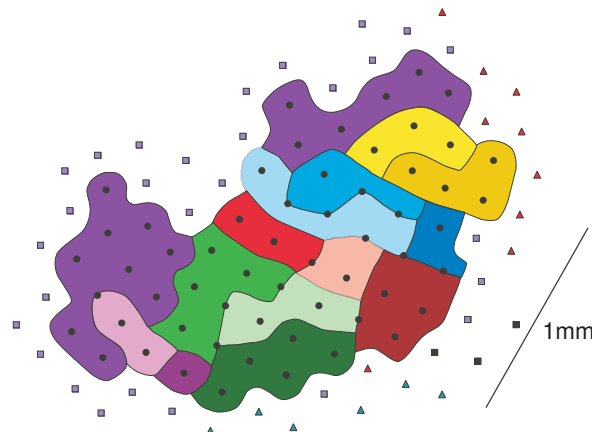
HG8wS

3 w. in HG + 8 w. in NG standard environment



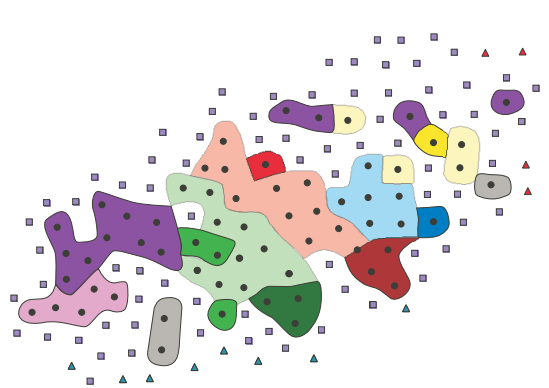
NG16wS

NG: 3 w. in control cages + 16 w. in standard environment



HG16wS

3 w. in HG + 16 w. in NG standard environment



GLABROUS SKIN SURFACES

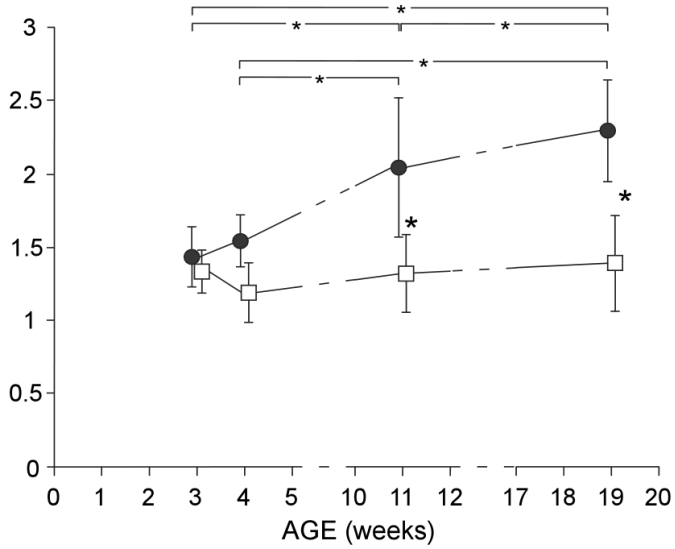
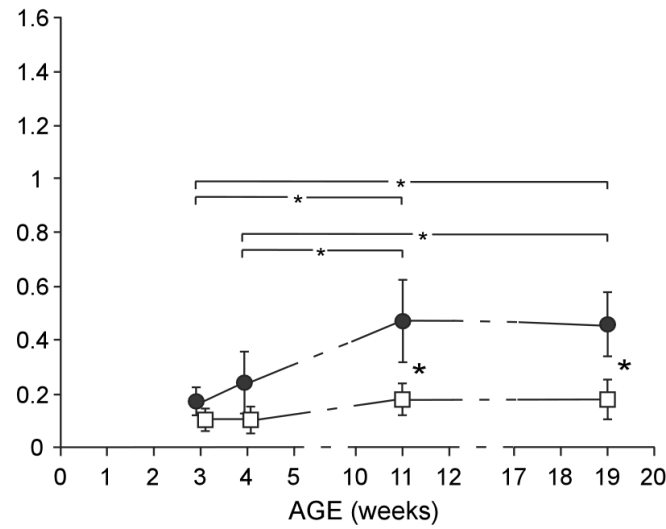
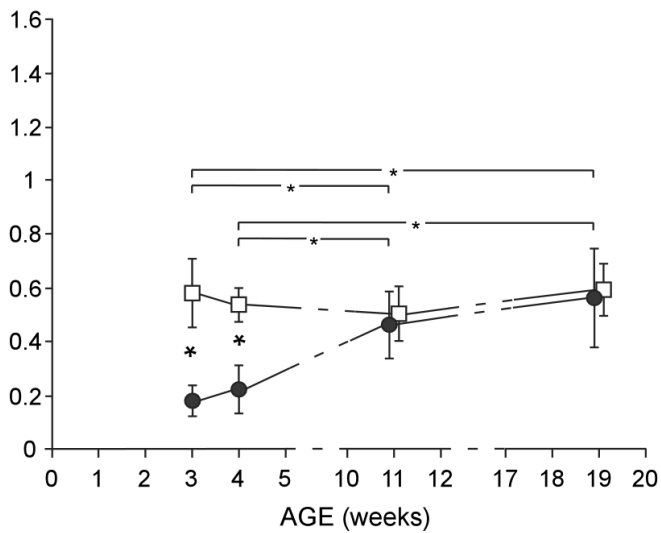
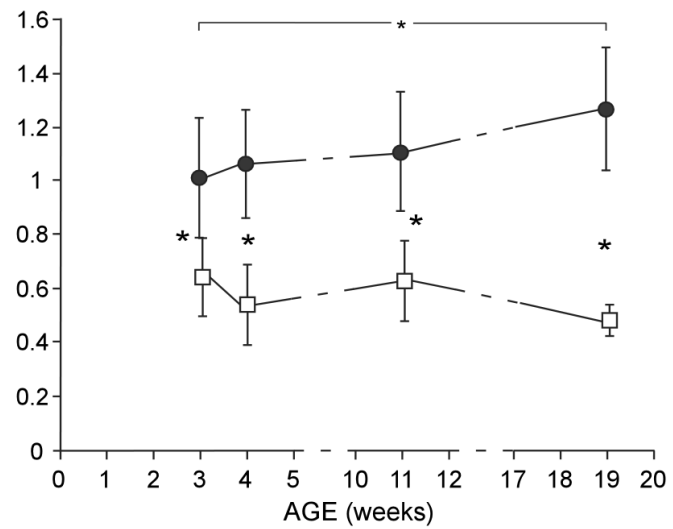


HAIRY SKIN SURFACES



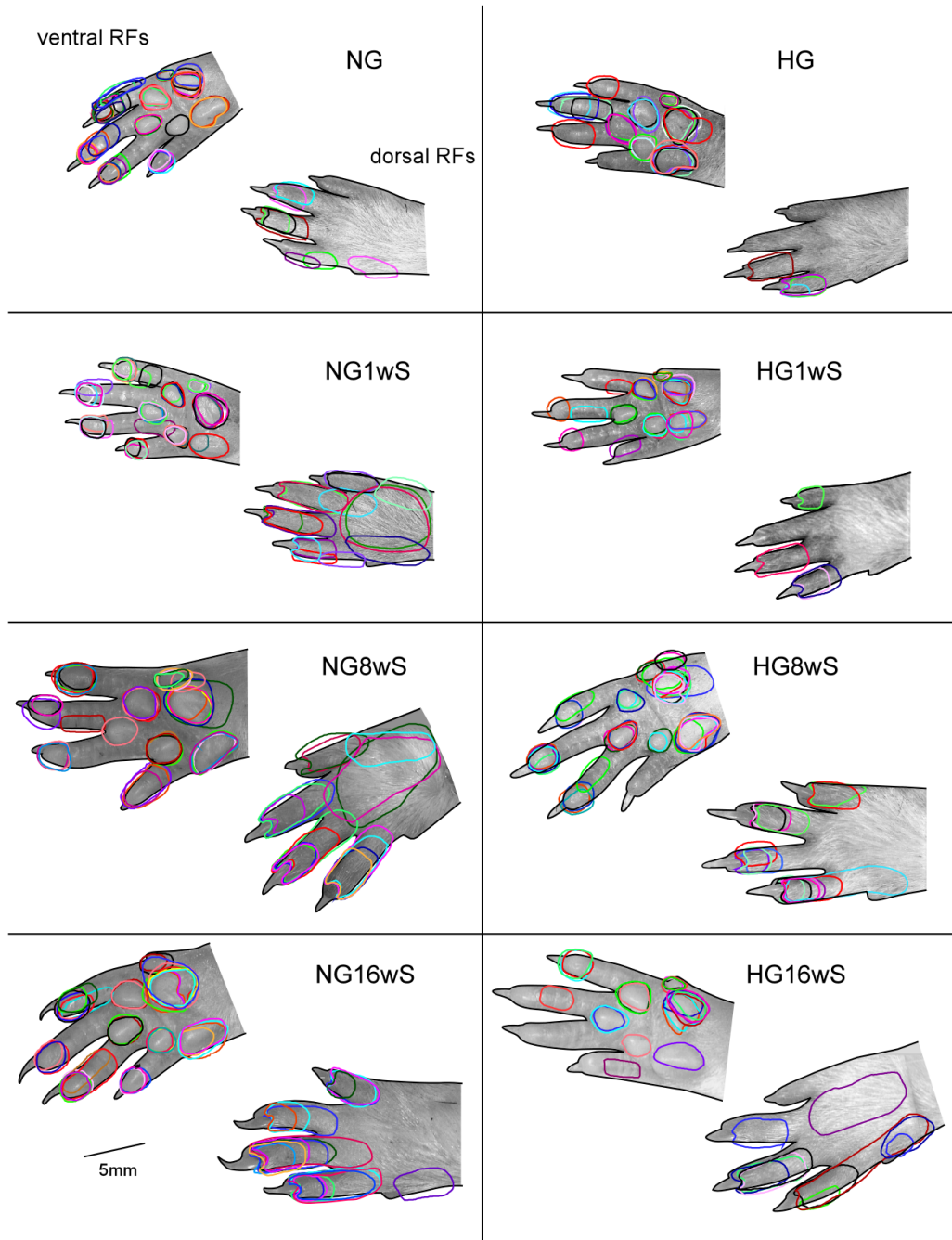
NAIL



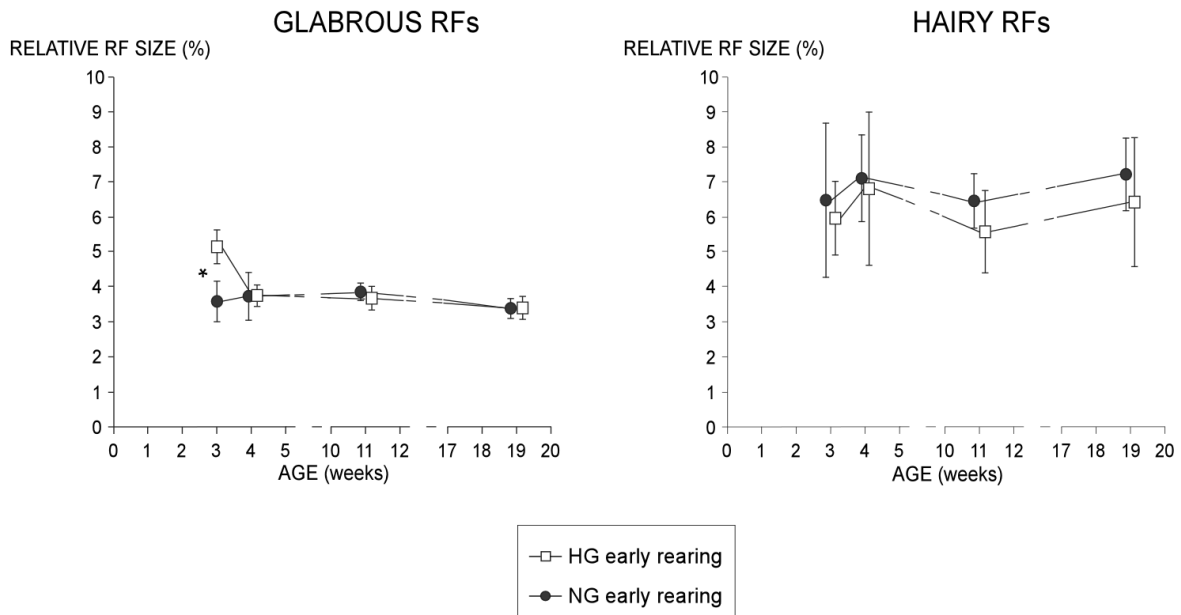
**A****TOTAL FOREPAW MAP**CORTICAL AREA (mm<sup>2</sup>)**B****HAIRY SKIN**CORTICAL AREA (mm<sup>2</sup>)**C****NAIL**CORTICAL AREA (mm<sup>2</sup>)**D****GLABROUS SKIN**CORTICAL AREA (mm<sup>2</sup>)

—□— HG early rearing  
 —●— NG early rearing

A

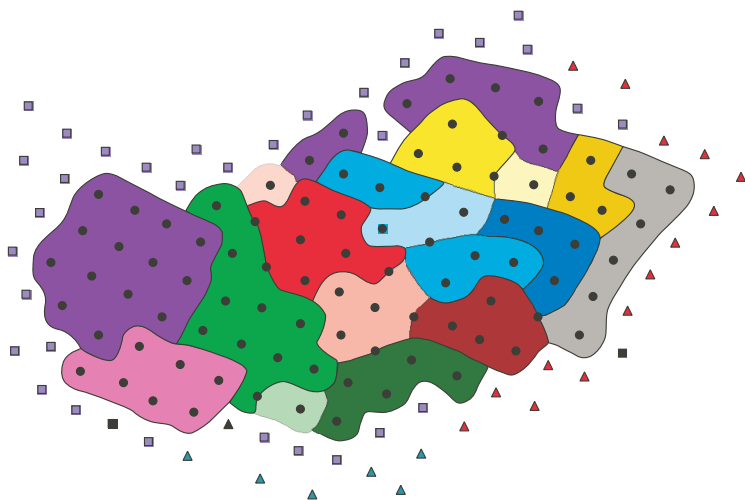


B



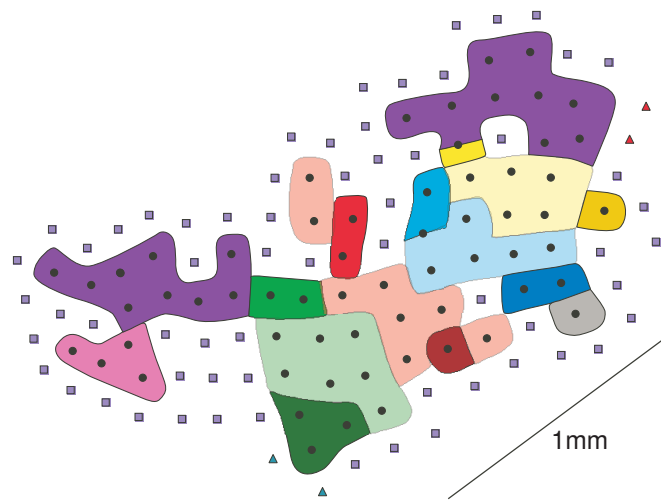
### NG16wS

NG: 3 w. in control cages + 16 w. in standard environment



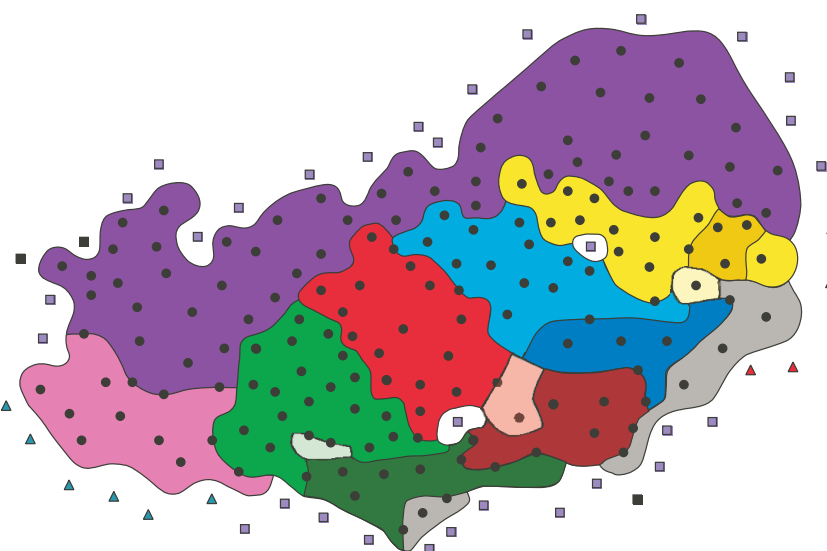
### HG16wS

3 w. in HG + 16 w. in NG standard environment



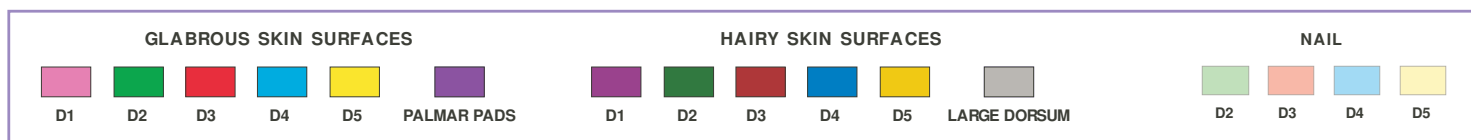
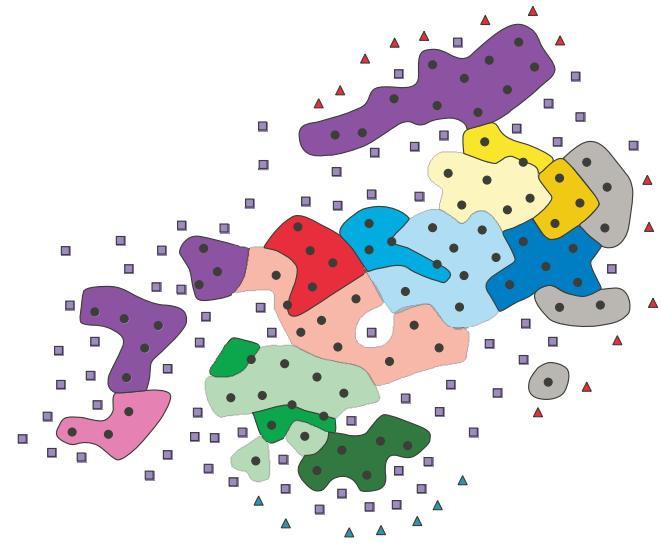
### NG16wE

NG: 3 w. in control cages + 16 w. in enriched environment



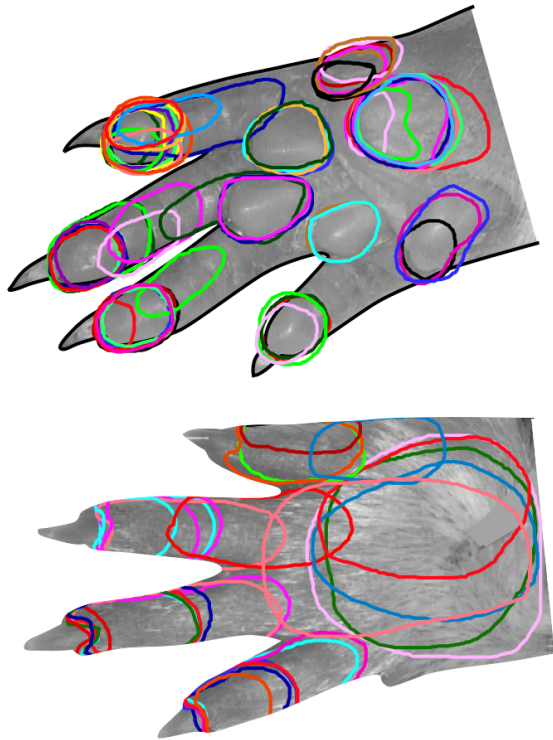
### HG16wE

3 w. in HG + 16 w. in NG enriched environment



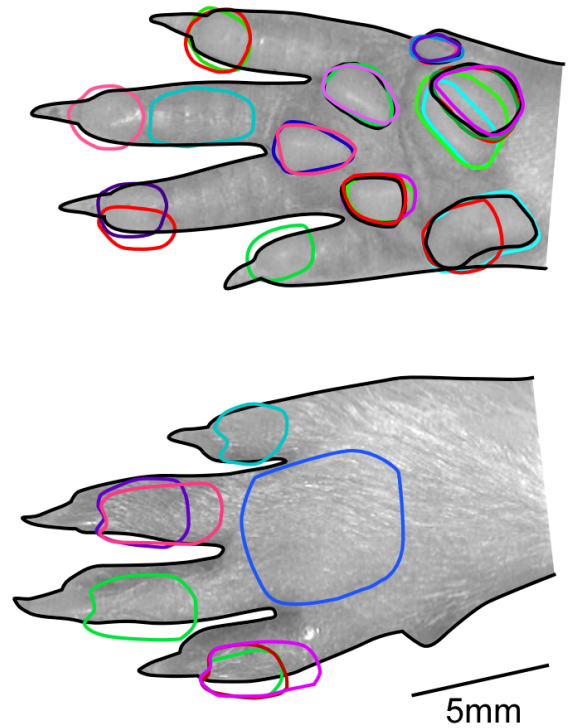
## NG16wS

NG: 3 w. in control cages + 16 w. in standard environment



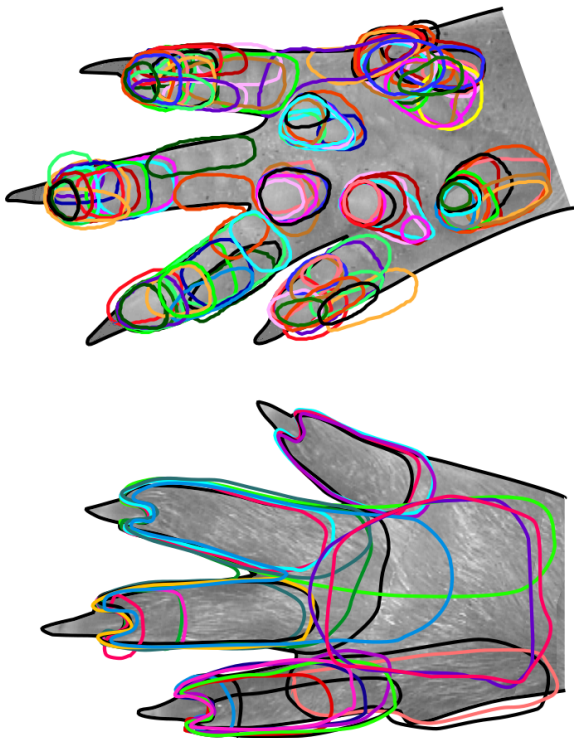
## HG16wS

3 w. in HG + 16 w. in NG standard environment



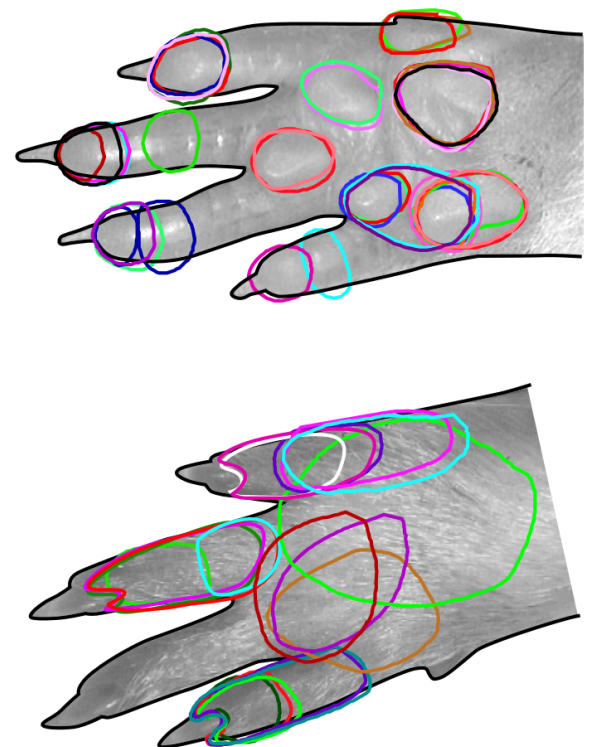
## NG16wE

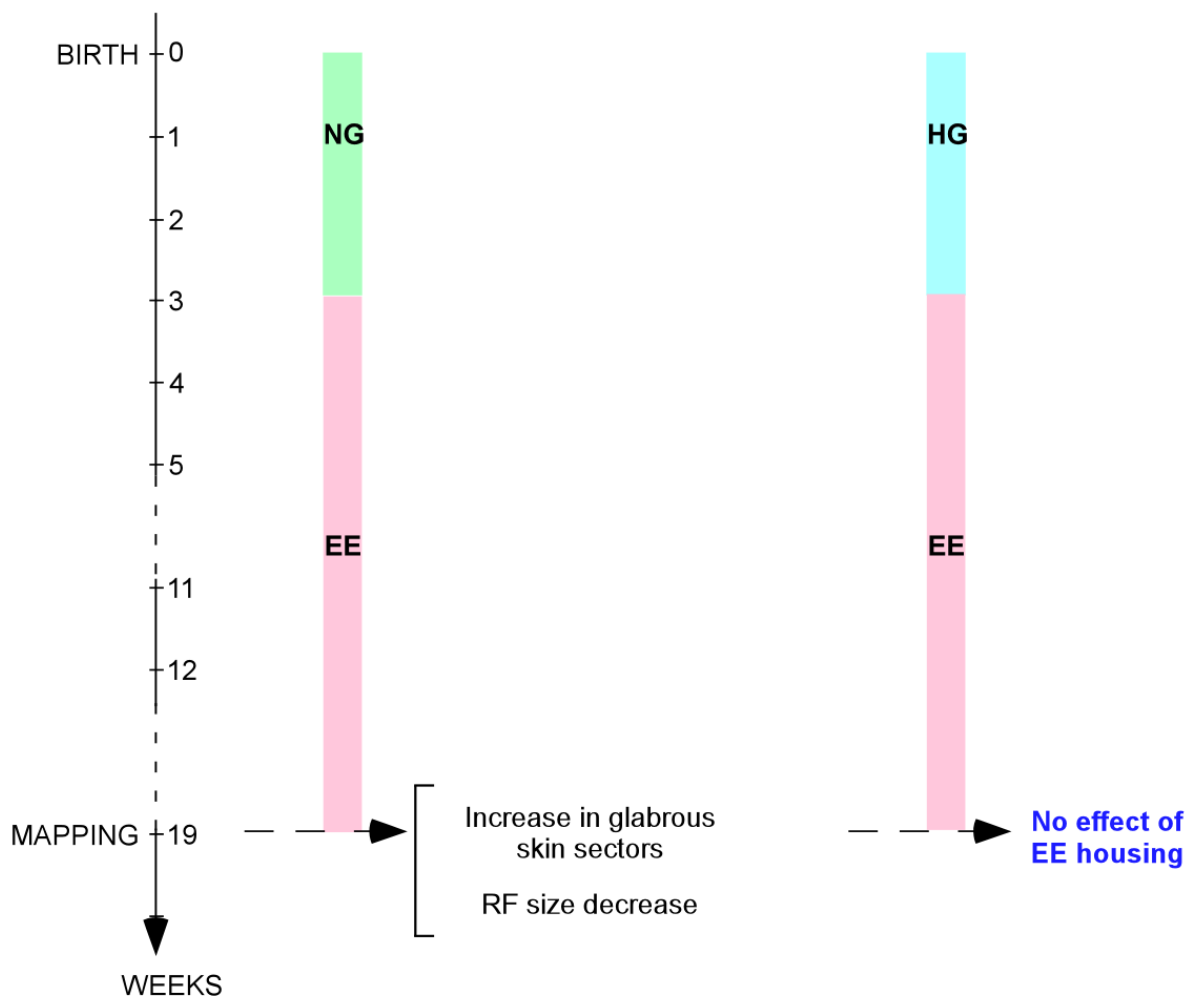
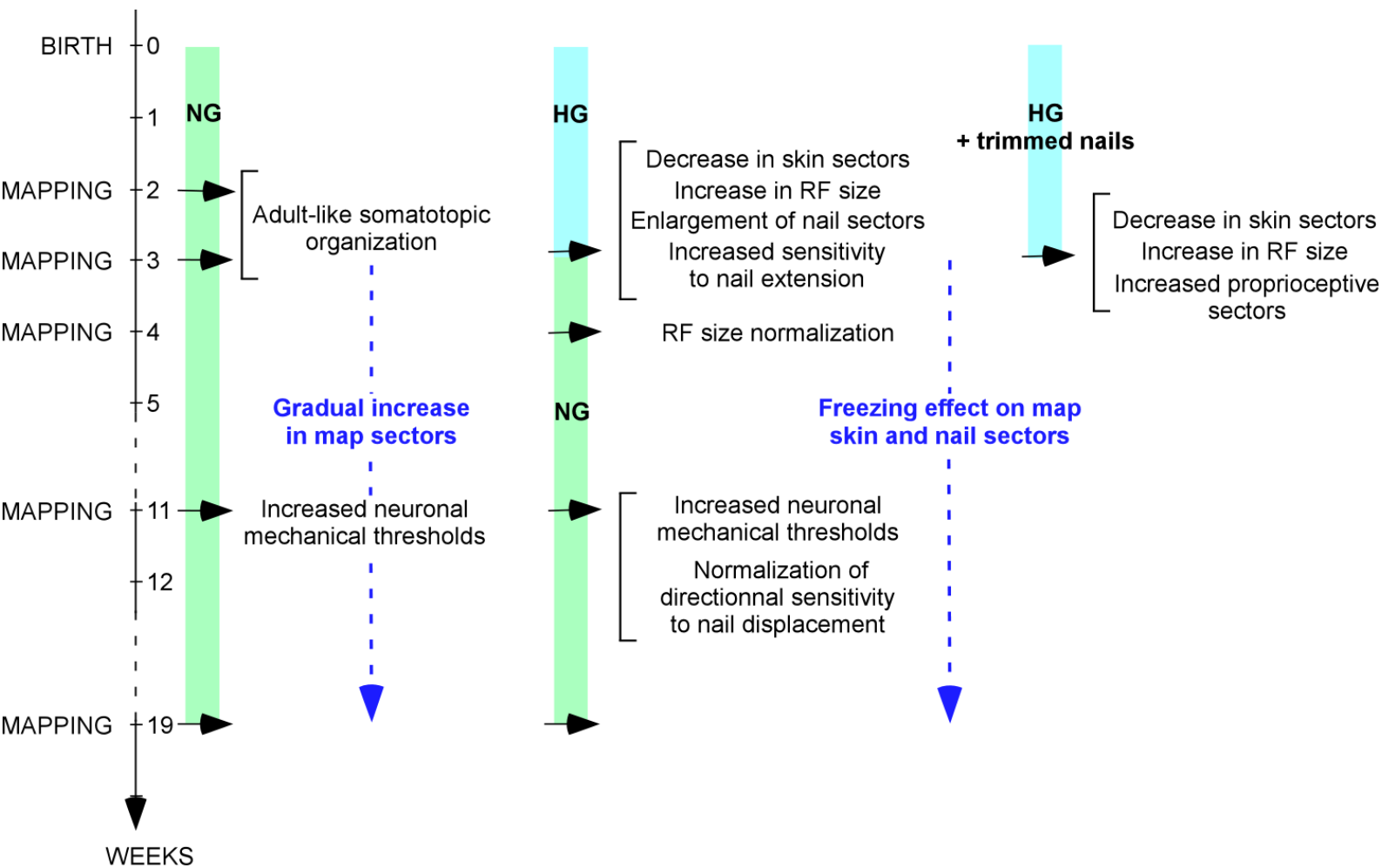
NG: 3 w. in control cages + 16 w. in enriched environment



## HG16wE

3 w. in HG + 16 w. in NG enriched environment





**Rats reared in normal gravity (NG, control cages) until postnatal day 21 (PN21) included.**

- NG Rats reared in NG until PN21 with their mother.
- NG1wS Rats reared in NG until PN21 and then housed in standard environment for 1 week.
- NG8wS Rats reared in NG until PN21 and then housed in standard environment for 8 weeks.
- NG16wS Rats reared in NG until PN21 and then housed in standard environment for 16 weeks.
- NG16wE Rats reared in NG until PN21 and then housed in enriched environment for 16 weeks.
  
- NGp Rats reared in NG until PN21, used for forepaw print measurements in HG or NG.

**Rats reared in hypergravity (HG, 2G) until PN21 included.**

- HG Rats reared in HG until PN21 with their mother.
- HGn- Rats reared in HG until PN21 with trimmed forepaw nails.
- HG1wS Rats reared in HG until PN21 and then housed in standard environment for 1 week.
- HG8wS Rats reared in HG until PN21 and then housed in standard environment for 8 weeks.
- HG16wS Rats reared in HG until PN21 and then housed in standard environment for 16 weeks.
- HG16wE Rats reared in HG until PN21 and then housed in enriched environment for 16 weeks.
  
- HGp Rats reared in HG until PN21, used for forepaw print measurements in HG or NG.

**Table 1**

Groups	NG	NG1wS	NG8wS	NG16wS
Upward nail displacement (U)	27.8±9.6 % (26)	20.6±2.1 % (22)	22.3±13.3 % (26)	19.4±9.5 % (29)
Downward nail displacement (D)	22.4±12.1 % (18)	13.3±3.5 % (14)	10.3±6.3 % (12)	9.5±8.1 % (13)
U+D	49.8±18.3 % (42)	66.1±10.9 % (79)	67.4±10.9 % (82)	71.1±10.2 % (108)
Groups	HG	HG1wS	HG8wS	HG16wS
Upward nail displacement (U)	53.1±5.6 % (146)	50.6±16.2 % (135)	22.7±7.9 % (70)	20.8±10.8 % (69)
Downward nail displacement (D)	11.0±8.7 % (29)	8.6±7.3 % (24)	6.5±6.1 % (19)	8.8±7.6 % (27)
U+D	35.9±9.0 % (115)	40.8±21.2 % (111)	70.8±12.5 % (216)	70.4±14.6 % (234)

Table 2



Groups	Mechanical thresholds
NG	0.203±0.027 g
HG	0.233±0.064 g
NG1wS	0.247±0.058 g
HG1wS	0.231±0.062 g
NG8wS	0.411±0.057 g
HG8wS	0.365±0.067 g
NG16wS	0.393±0.049 g
HG16wS	0.343±0.025 g
NG16wE	0.459±0.061 g
HG16wE	0.427±0.068 g

Table 3