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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 1st 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 22nd 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 5th 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 m³), 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 mm²) 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 Ω 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 μ 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 μm) 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 g/mm^2), 3.22 (0.152 mm; 0.166 g; 9.15 g/mm^2), 3.61 (0.178 mm;
261 0.407 g; 16.36 g/mm^2), and 3.84 (0.203 mm; 0.692 g; 21.39 g/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/mm²) 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^2). 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^2 (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 μ 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 μ 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 ± 0.22
388 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 ± 0.05
389 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⁻ rats). The cortical area activated by nail
395 displacement in HGn⁻ 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⁻ ($0.74 \pm 0.08 \text{ mm}^2$) 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⁻ 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⁻ ($1.08 \pm 0.13 \text{ mm}^2$) 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⁻ 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 ± 0.48 %) than in NG rats
439 (3.58 ± 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⁻ (5.35 ± 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 ± 2.15 %; HG: 5.98 ± 1.03 %; HGn⁻: 6.77 ± 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 ± 5.95 g, NGp: 43.01 ± 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 ± 0.11
459 and NGp rats: 1.26 ± 0.21) appeared to be similar to the ratio of HG to NG glabrous RF areas
460 measured in mm^2 (1.27 ± 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 ± 0.15
485 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 3rd 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 19th 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 2nd and the 8th
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 3rd week until the 19th 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 11th 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 1st week
538 of housing in NG environment (HG: 5.14 ± 0.48 %, HG1wS: 3.76 ± 0.30 ; $P<0.001$), thus
539 becoming similar to that recorded in NG (3.58 ± 0.57 %, $P=0.79$, ns) or NG1ws (3.72 ± 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 ± 7.82 g) and that of age-matched NGp rats (61.09 ± 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 ± 8.86 g) whose
550 weight was similar to that of age-matched NGp rats (93.32 ± 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 1st and the 2nd 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 ± 5.09 mm², NGp: 34.84 ± 5.93 mm²; $P=0.82$, ns)
558 or 2 weeks (HGp: 41.40 ± 3.91 mm², NGp: 41.73 ± 5.95 mm²; $P=0.89$, 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 ± 0.049 g, NG16wE: 0.427 ± 0.068 g,
606 $P = 0.16$, ns), in contrast to the significant increase in rats reared in HG (HG16wS: 0.343 ± 0.025
607 g, HG16wE: 0.459 ± 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 ± 0.31 %, NG16wE: 2.48 ± 0.24 %, $P < 0.001$), but
615 not in those reared in HG (HG16wS: 3.40 ± 0.32 %, HG16wE: 3.04 ± 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 ω 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° , 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 ω vector. The CA value is ± 0.838 m/s² (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 ω 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 18th 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 3rd 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 19th 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.

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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 1st 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 11th day after gestation to the
859 15th 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 2nd and the 8th
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.

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933

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- 1132
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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/mm²)
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 22nd 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 22nd 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 mm²;

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 1st 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.

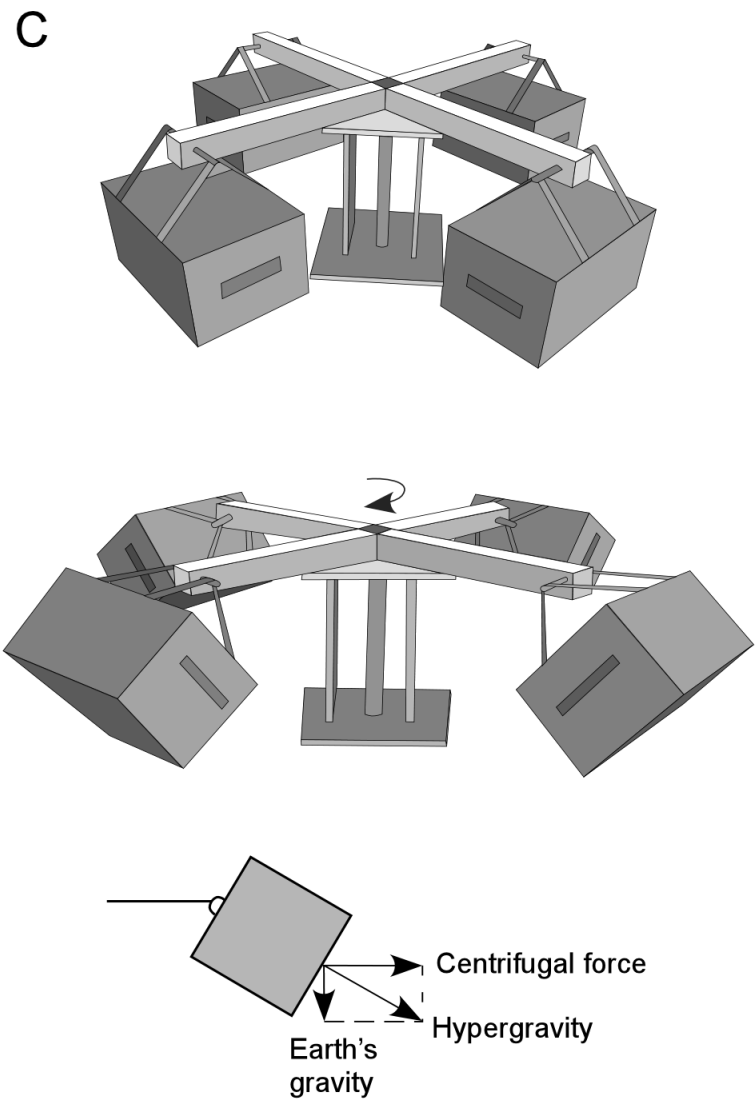
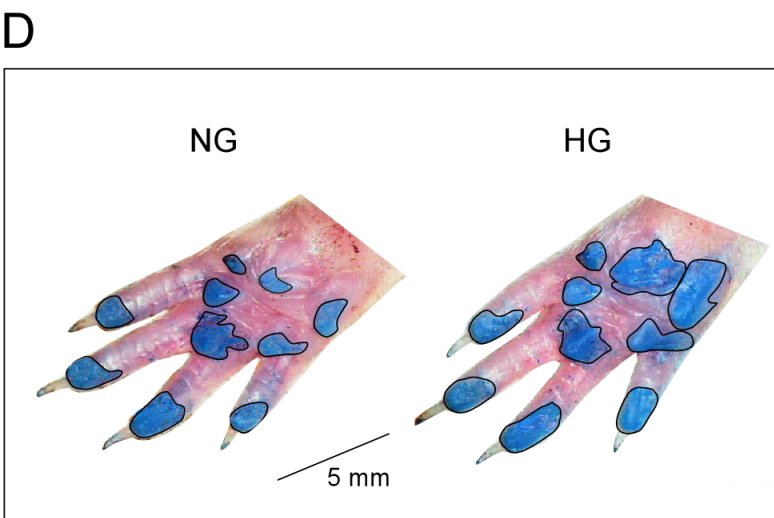
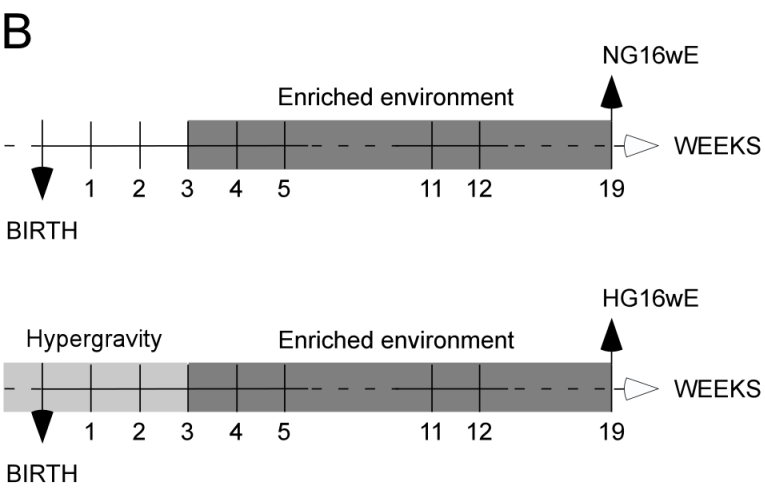
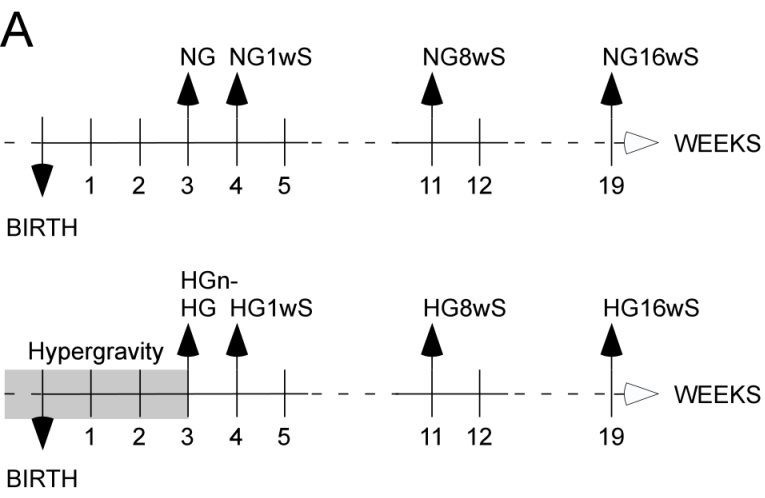
1233 **Fig. 10. Synopsis of findings on the development of the forepaw representation in the S1**
1234 **cortex and main effects of HG.**

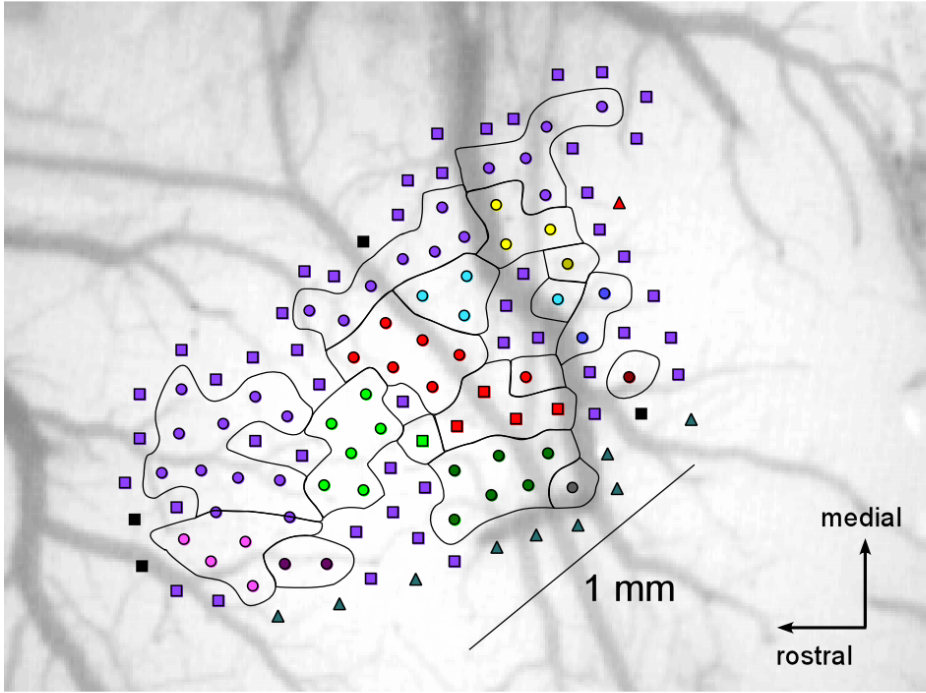
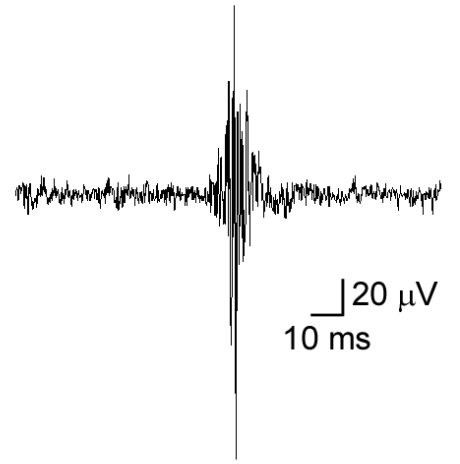
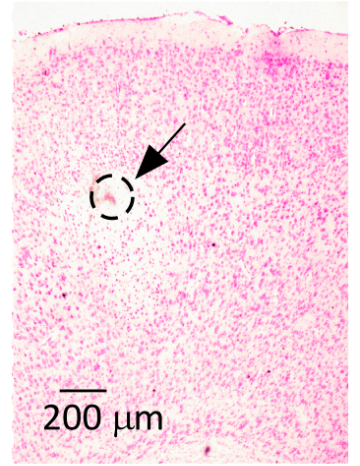
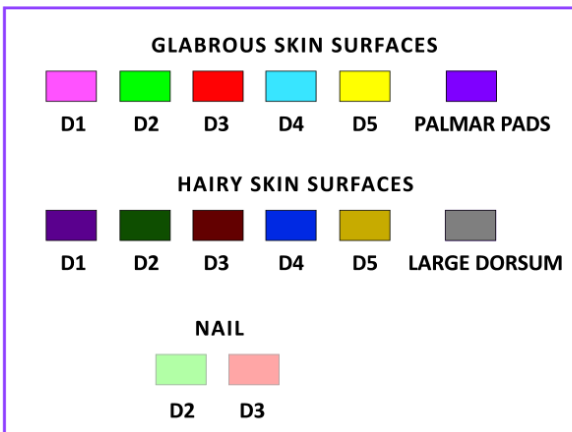
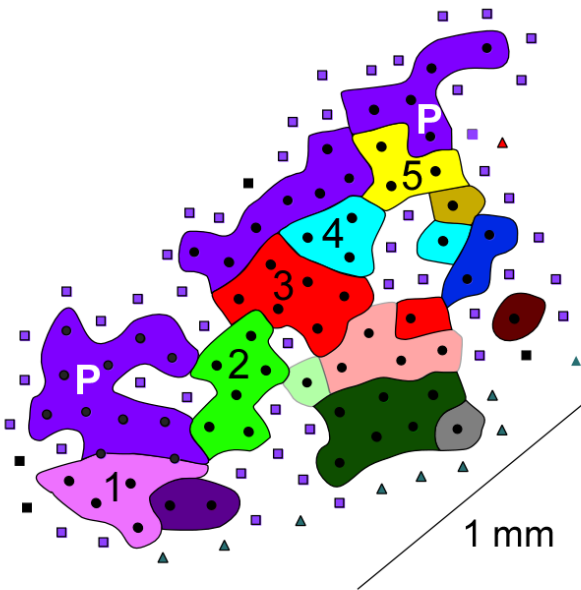
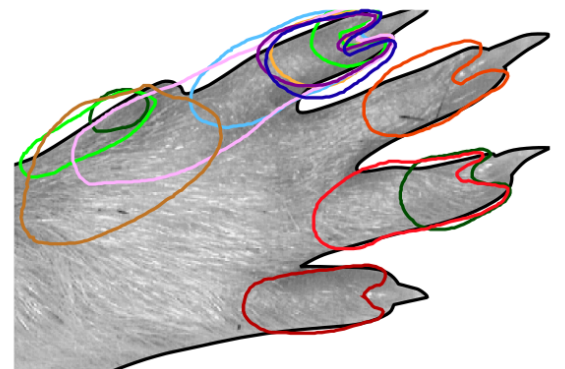
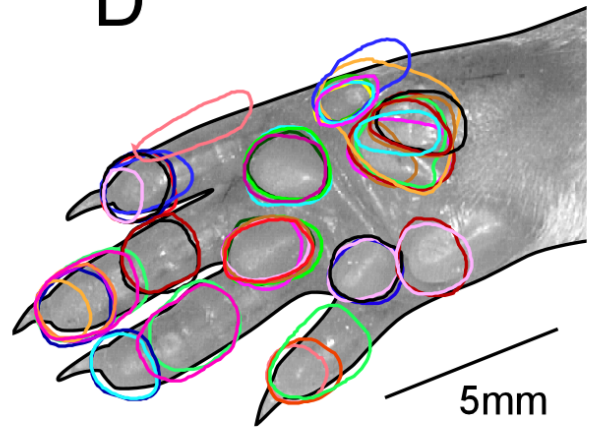
1235 **Table 1. Labels of the experimental groups.**

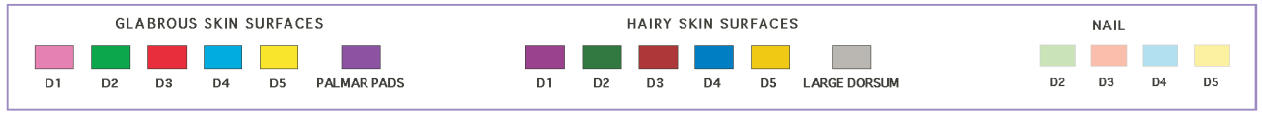
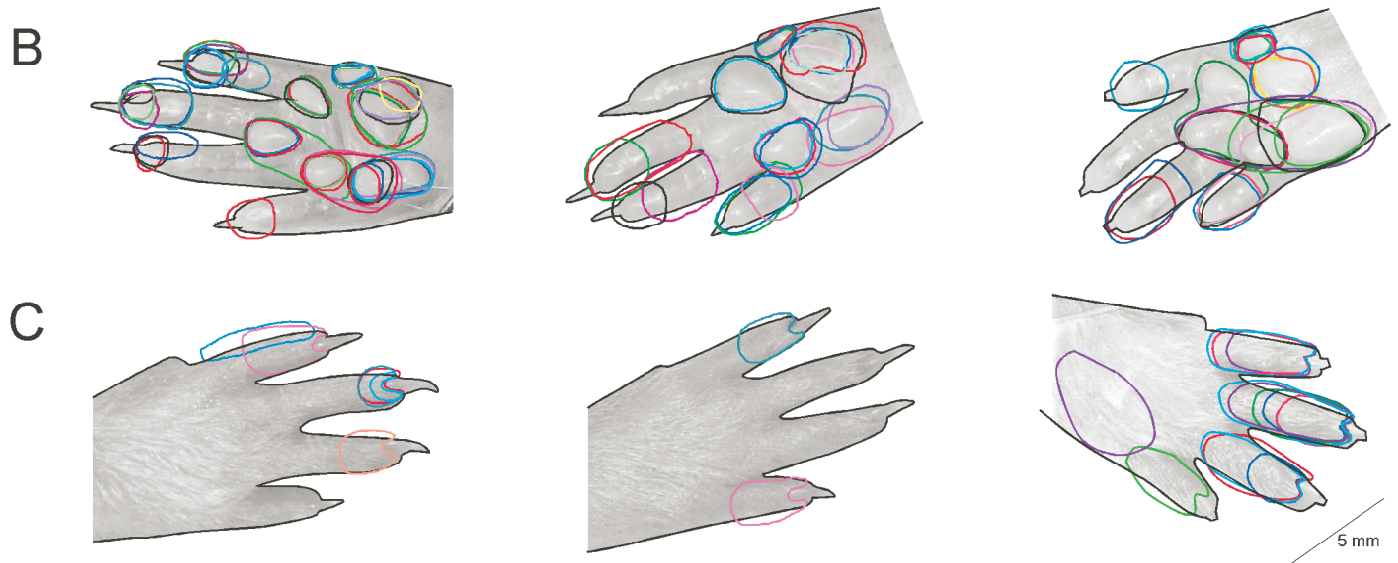
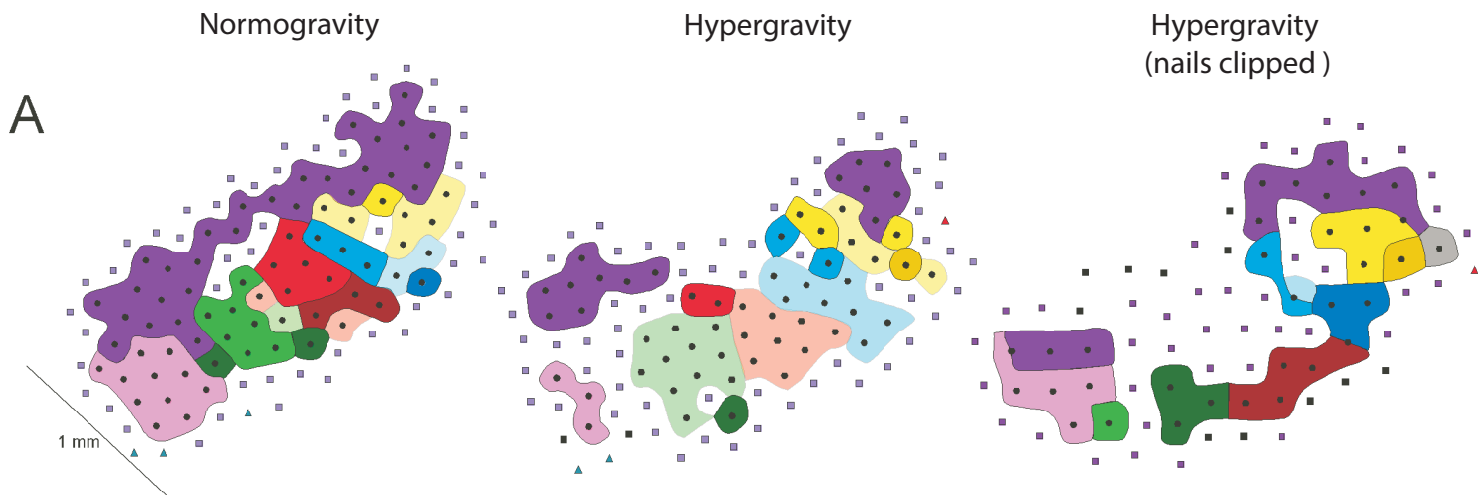
1236 **Table 2. Directional sensitivity of cortical neurons to nail displacement.** Mean percentages
1237 and number of neuronal responses to upward (U), downward (D) or both U and D nail
1238 displacement obtained in rats reared in NG from birth (NG, NG1wS, NG8wS, NG16wS) and in
1239 aged-matched rats 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.

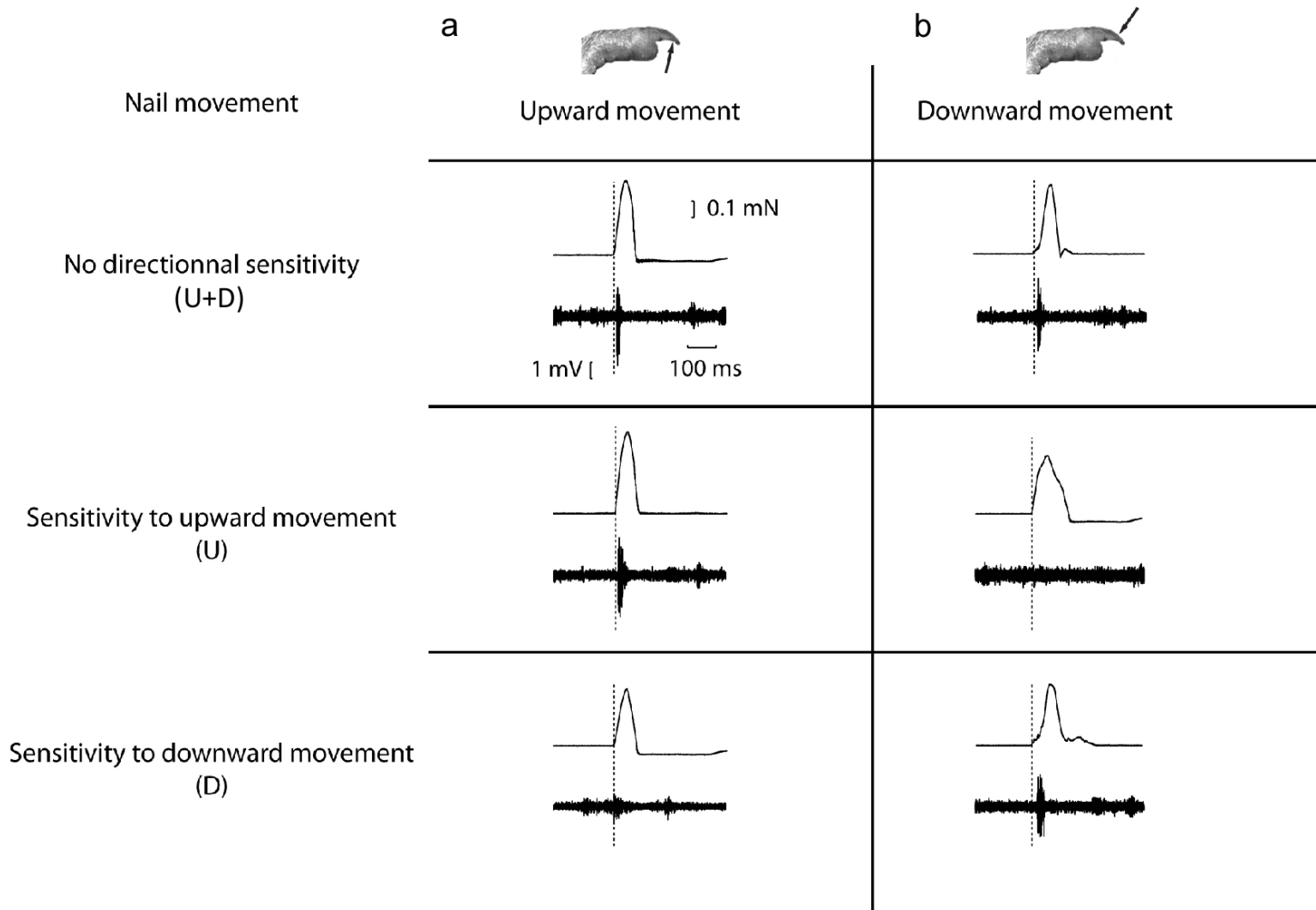
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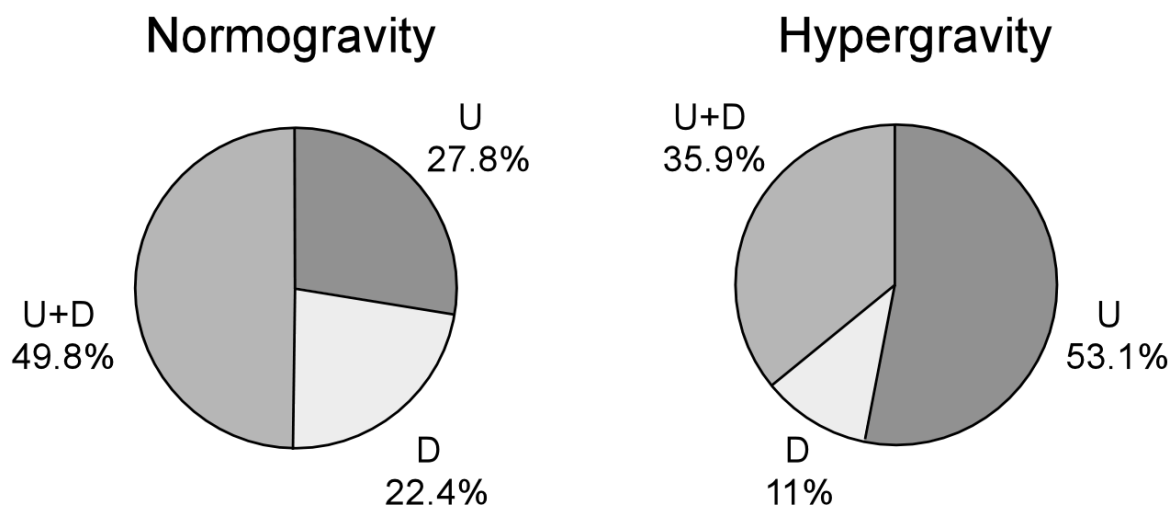
A**B****C****D**



A

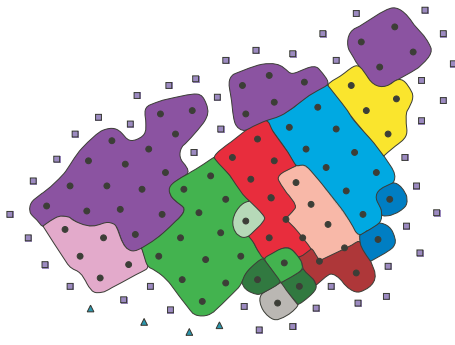


B



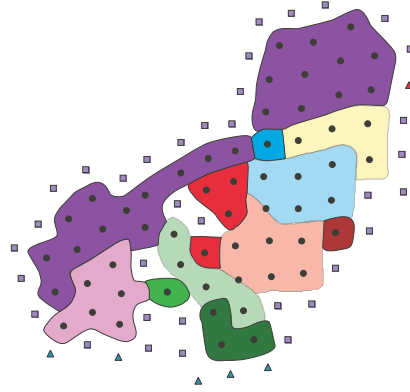
NG

NG: 3 w. old rats in control cages



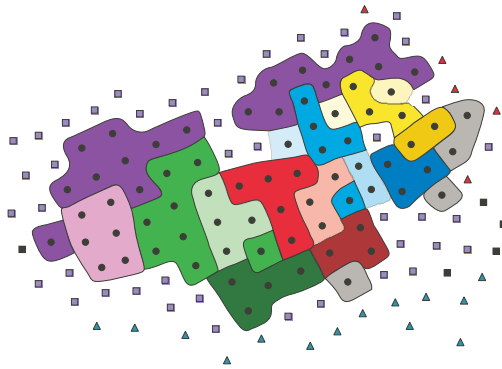
HG

HG: 3 w. old rats in centrifuge



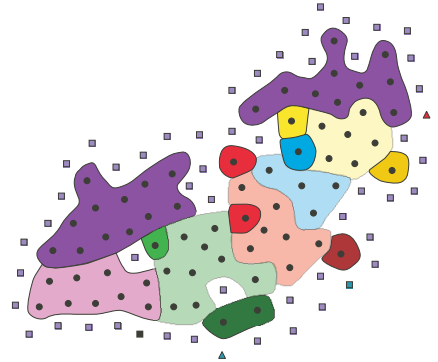
NG1wS

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



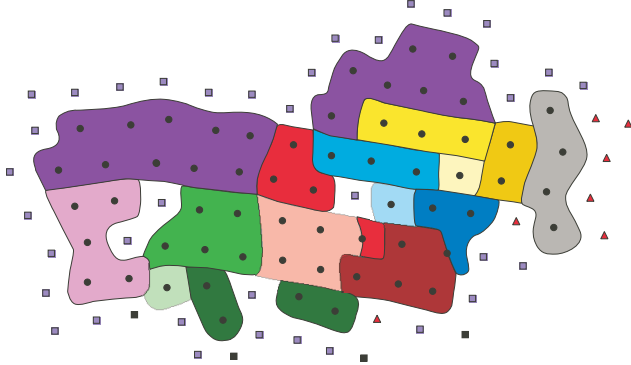
HG1wS

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



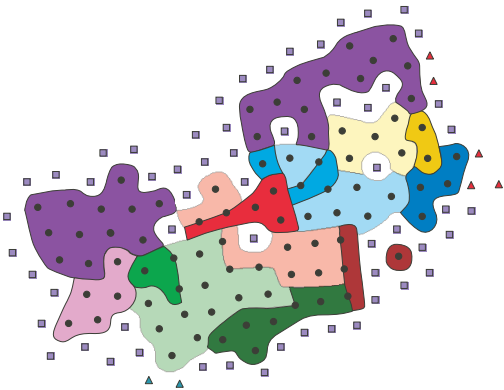
NG8wS

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



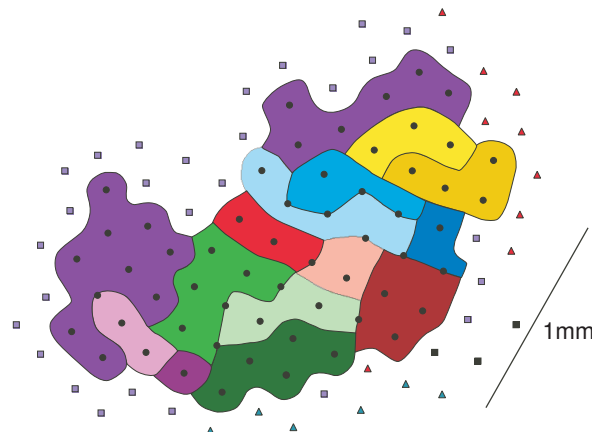
HG8wS

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



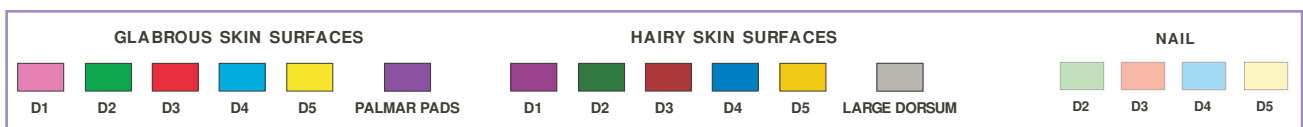
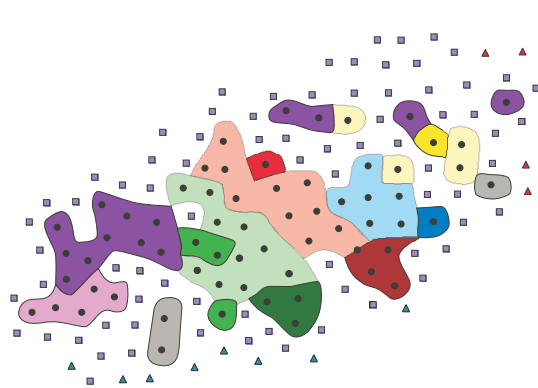
NG16wS

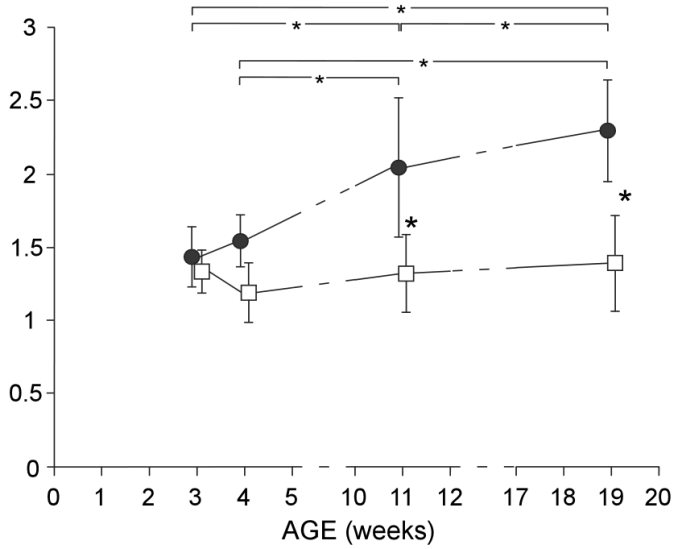
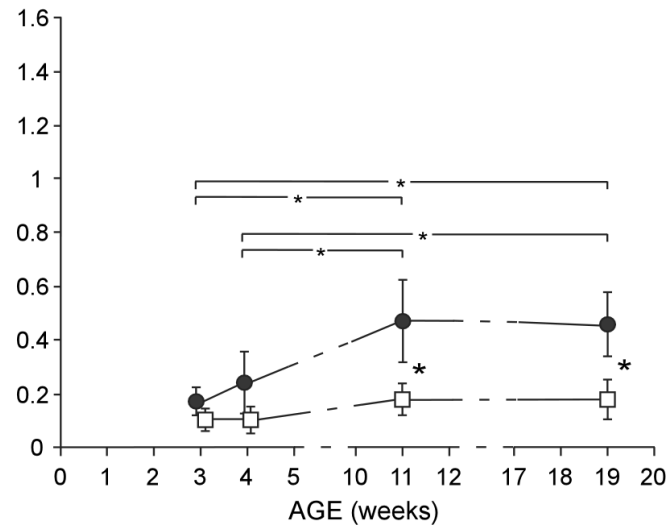
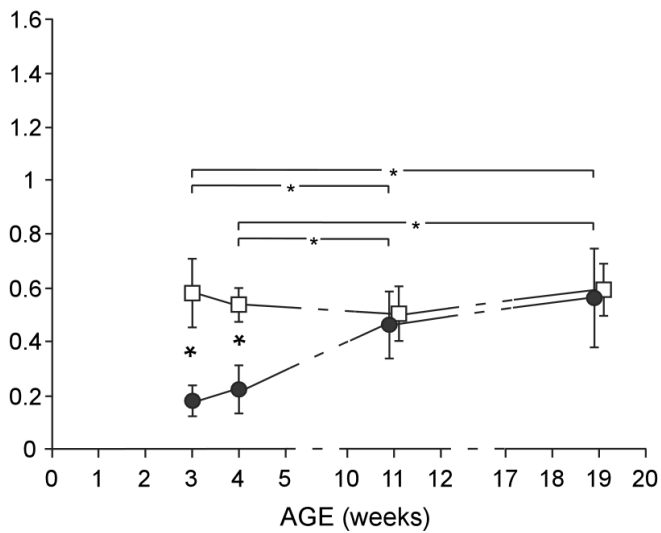
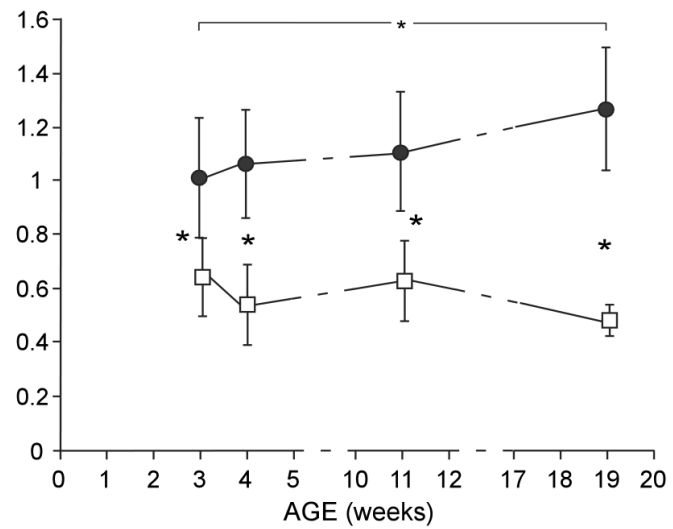
NG: 3 w. in control cages + 16 w. in standard environment



HG16wS

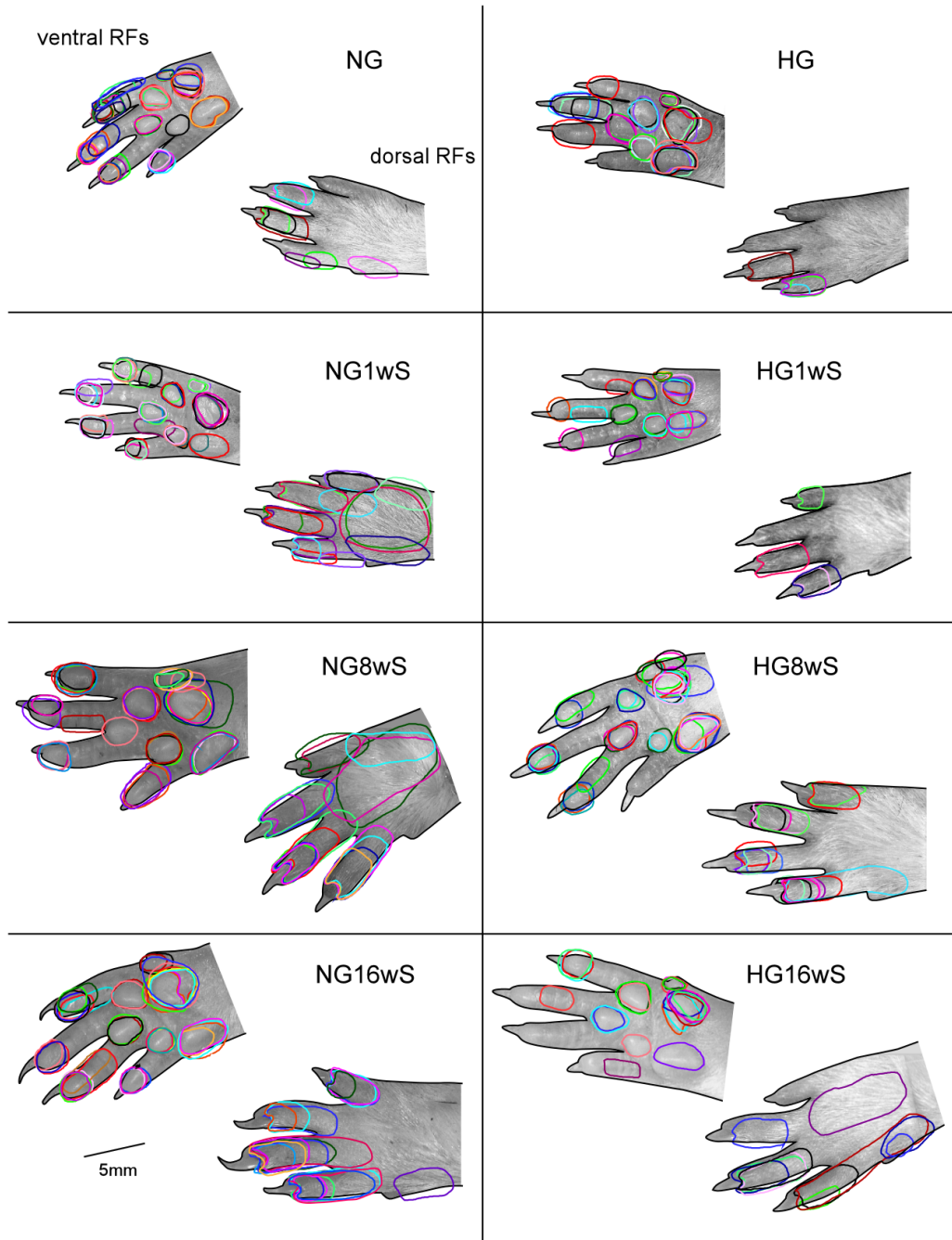
3 w. in HG + 16 w. in NG standard environment



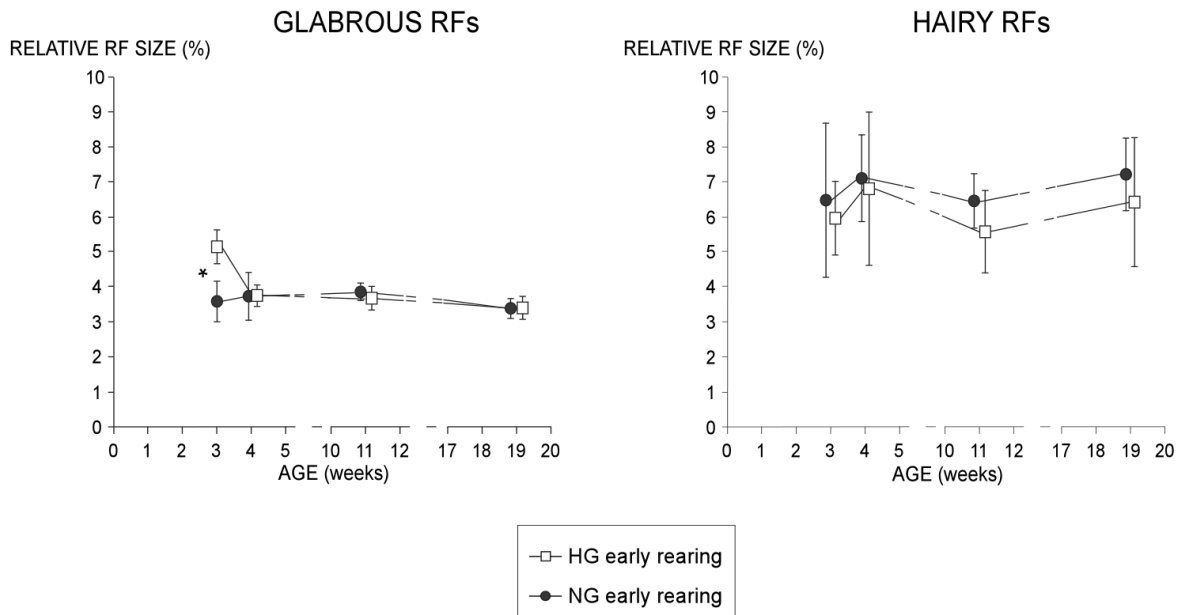
A**TOTAL FOREPAW MAP**CORTICAL AREA (mm²)**B****HAIRY SKIN**CORTICAL AREA (mm²)**C****NAIL**CORTICAL AREA (mm²)**D****GLABROUS SKIN**CORTICAL AREA (mm²)

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

A

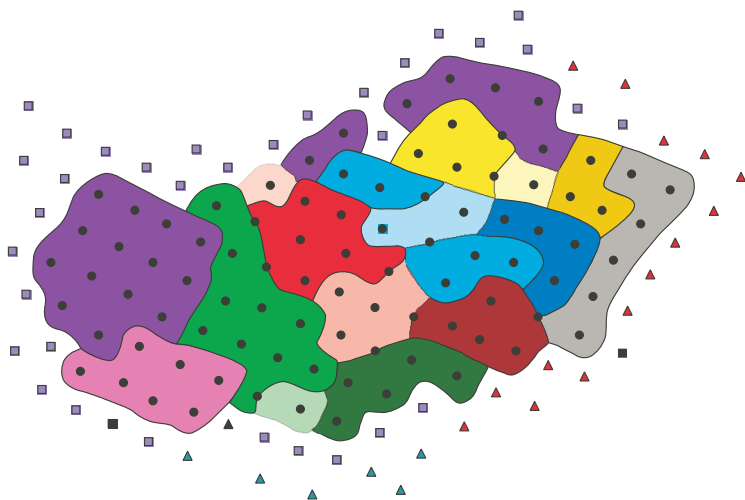


B



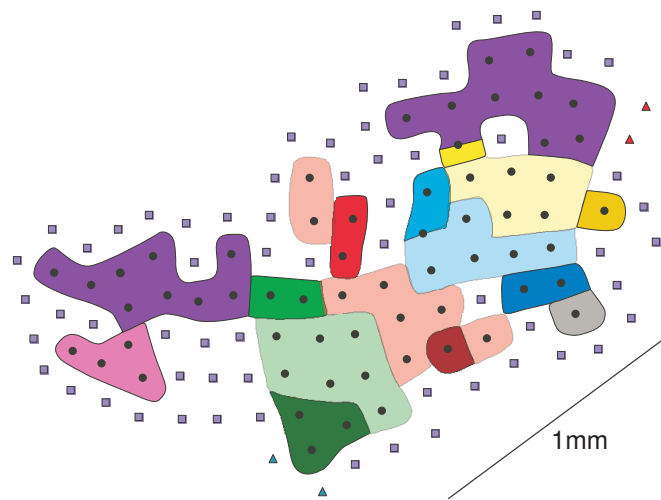
NG16wS

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



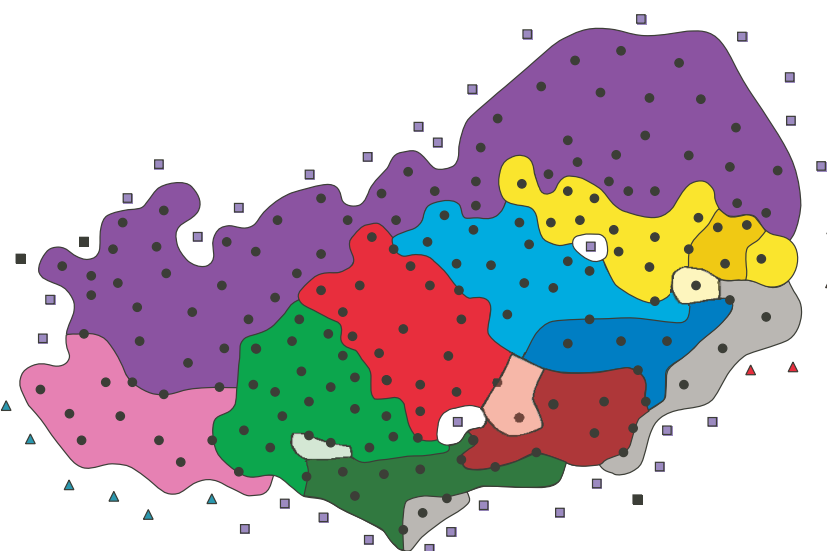
HG16wS

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



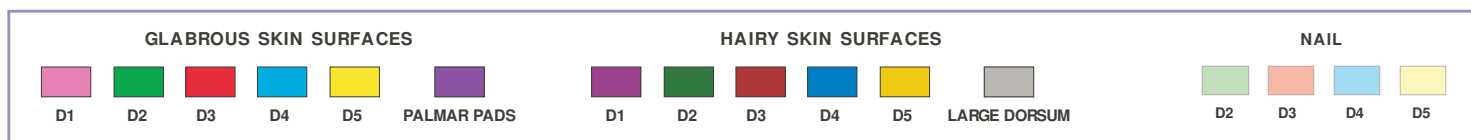
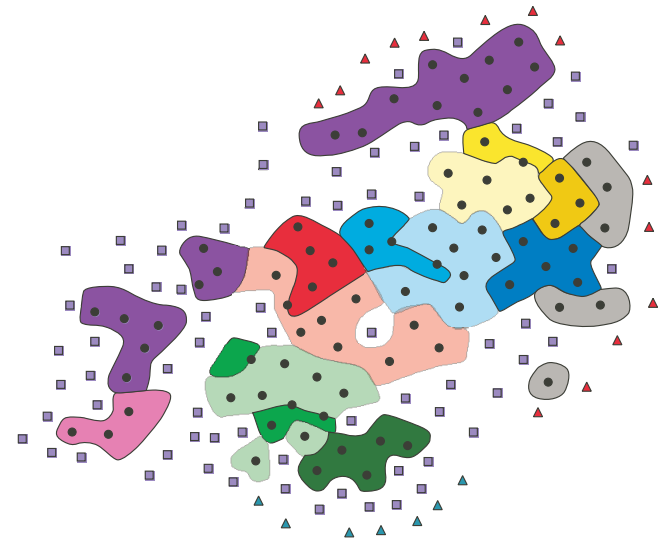
NG16wE

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



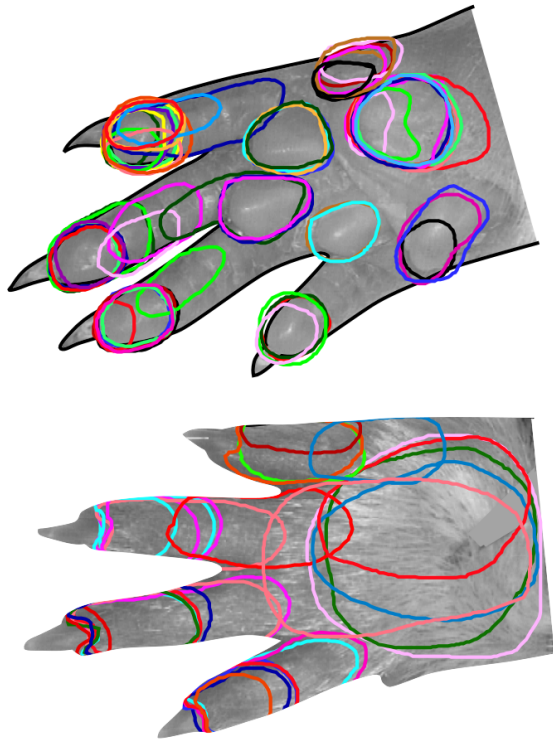
HG16wE

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



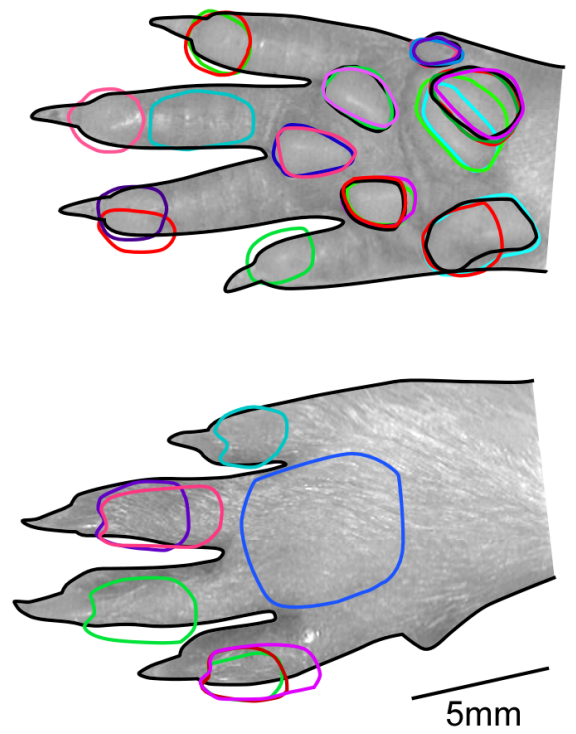
NG16wS

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



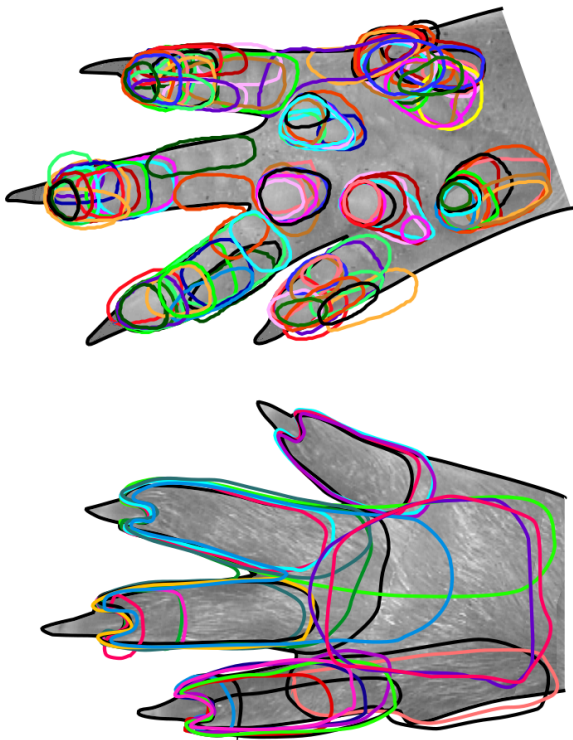
HG16wS

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



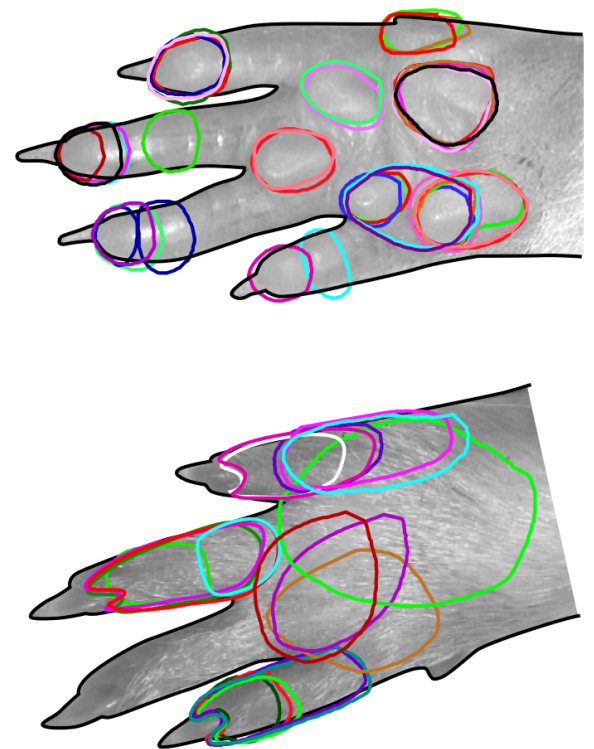
NG16wE

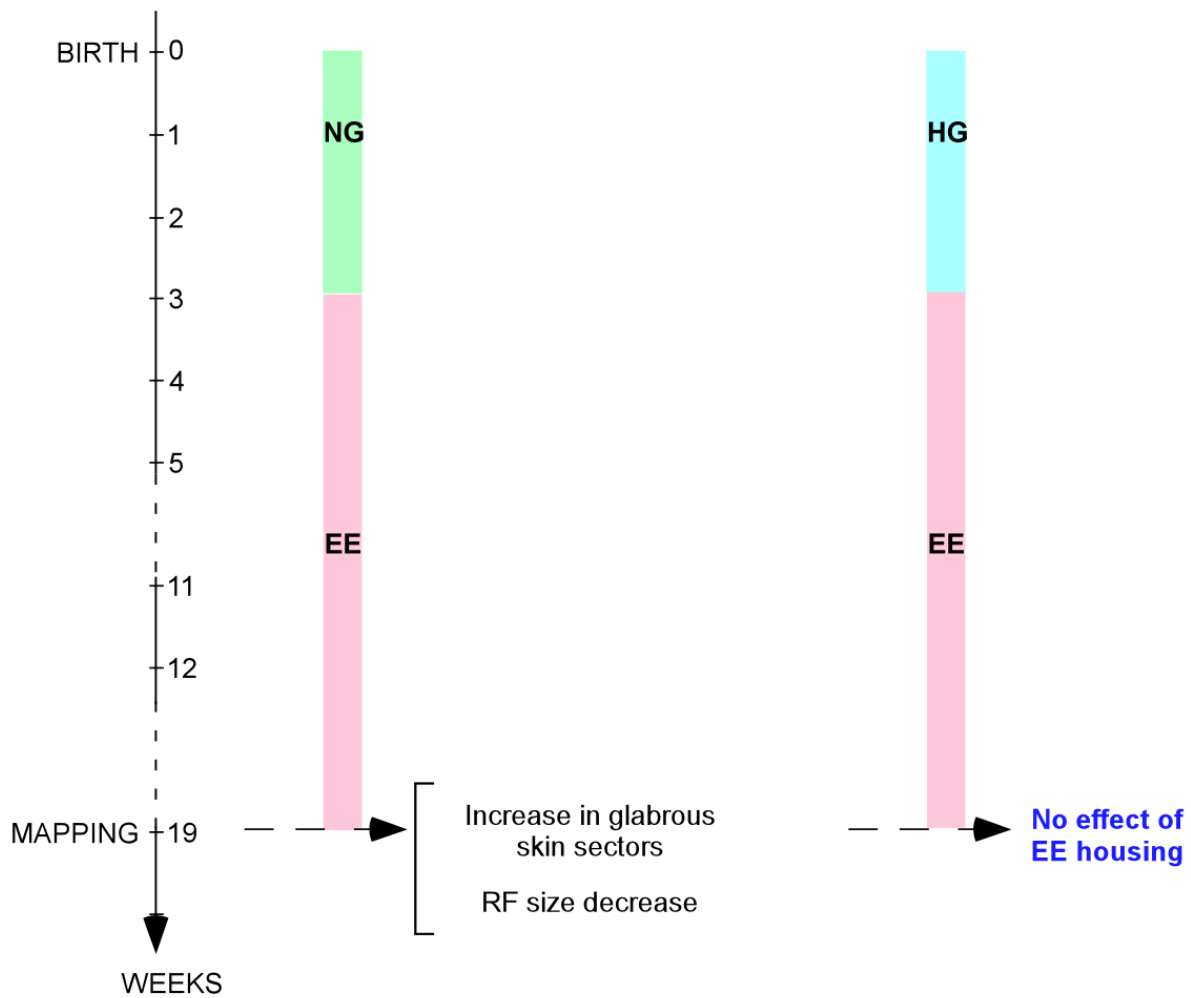
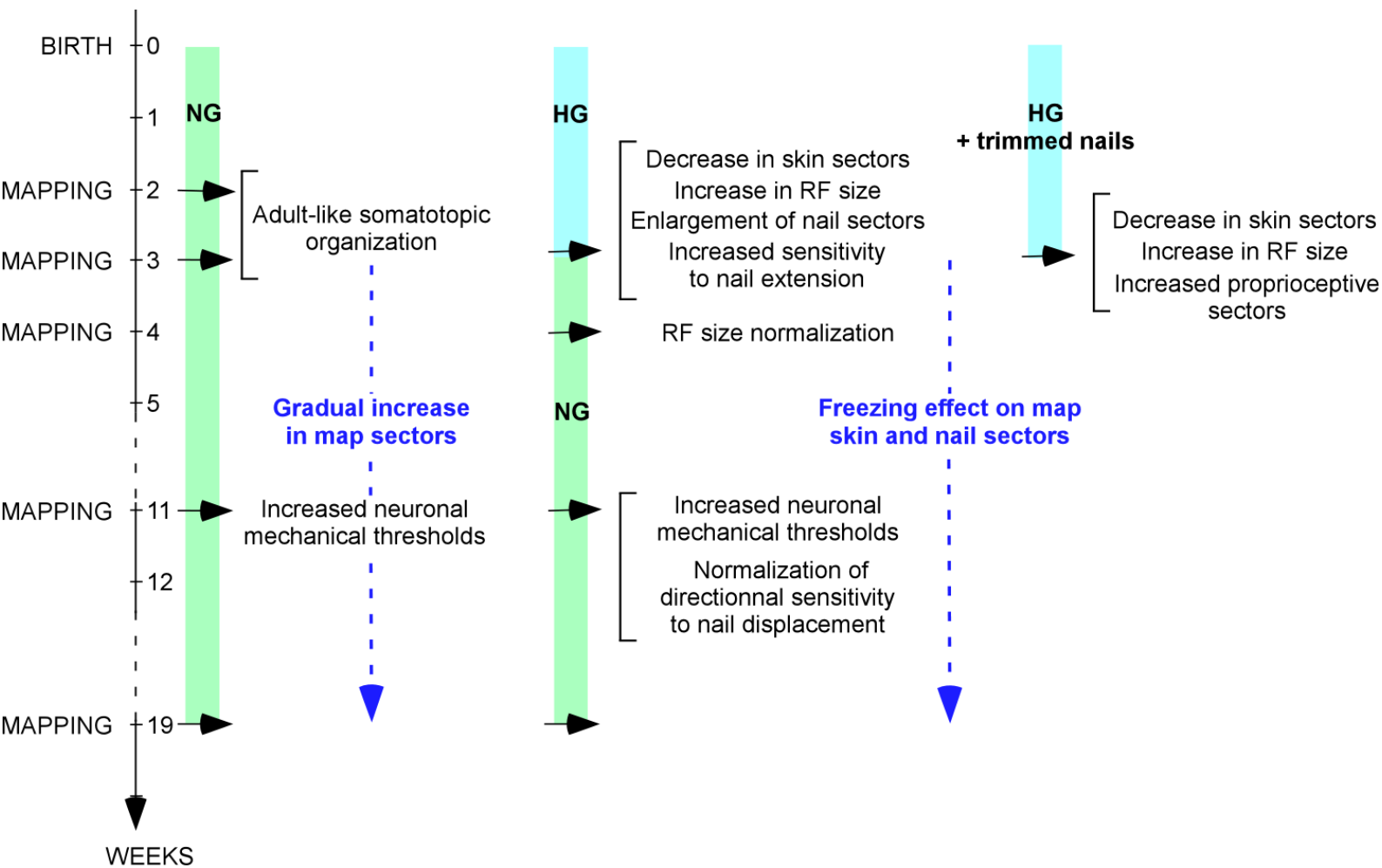
NG: 3 w. in control cages + 16 w. in enriched environment



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