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

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Y.Z.A and C.X. conceived and designed research, performed experiments, analyzed data and interpreted results, prepared figures and wrote manuscript. N.C. analyzed data and prepared figures.

Running title: HG influence on maturation of somatosensory maps
Abstract

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

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

Somatosensory cortical maps reflect a dynamic competition between proprioceptive and cutaneous inputs converging onto cortical networks (Jenkins et al., 1990; Recanzone et al., 1992; Xerri et al., 1994, Coq and Xerri, 1998). Gravity is a fundamental element in the external environment, stimulating vestibular receptors and exerting mechanical constraints on the musculo-skeletal system, while providing a reference frame for posture and
movements. We reasoned that changes in gravity modify the relative weight of proprioceptive and cutaneous inputs from the limbs converging on the S1 cortex and thus provide a unique opportunity to investigate the developmental emergence of somatosensory maps in an environment modifying inputs from the limbs, without sensory deprivation.

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

Our first aim was to examine both short- and long-term effects of early exposure to hypergravity (HG) on rat forepaw representation. HG has been shown to affect the development of the vestibular (Ross et Tomko, 1998) and motor systems (Brocard et al., 2003), and their functions such as air righting during free fall, locomotion, postural control and limb movement when the HG animals were subsequently exposed to normogravity (Bojados et al., 2013; Bouet et al., 2003, 2004). The enduring motor changes found in rats that grew up in altered gravity stress the key role of gravity during the motor development and suggest the existence of critical periods (Walton et al., 1992). However, it is still unknown whether HG has an impact on the emergence of somatosensory maps. During the developmental period, major morphological and biochemical changes take place, as behavior progresses through different stages of locomotion. The rat motor system matures slowly over the first postnatal weeks and the pups do not walk spontaneously before the end of the second week of life (Altman and Sudarshan, 1975) and clear topographical organization of forelimbs begins to appear by P15 in S1 (Seelke et al., 2012). Changes in
gravity induce alteration in joint and muscle proprioception (McCall et al., 2003). Consistently, HG during early development has been shown to modify postural control and locomotion in rodents (Bojados et al., 2013). Therefore, we hypothesized that HG, by modifying the pressure exerted on the ventral surfaces of the paws and muscle spindle stimulation, thereby changing the pattern of cutaneous and proprioceptive inputs to the S1 cortex compared to normogravity, would shape the topographical organization of the forepaw maps in a distinctive way.

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

Animals

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

To investigate the effects of an hypergravity exposure during the developmental period on forepaw representation in the S1 cortex, three experimental groups of male Long-Evans rats were used in which cortical mapping was carried out at the 22nd postnatal day (P22): rats reared in normogravity (NG group), rats which were fertilized, born and housed in a hypergravity (HG: 2G) environment generated by a centrifuge (see below) until the age of 3 weeks (P21; HG group), and rats reared in HG with trimmed forepaw nails (H Gn-group) (Fig. 1A). To constitute HG and HGn- groups, couples of rats were placed in the centrifuge generating the HG environment. After 1 week of HG exposure, adult males were taken out. After birth, the newborn rats remained with their mother and the pups were nursed normally until P22. The centrifuge was stopped once a week during half an hour for food renewal, water supply and home cage cleaning. In the HGn- group, the centrifuge was stopped every 2 days, from the 5th postnatal day until P22, and the animals were removed from the cages for about 10 min for trimming of forepaw nails. The NG animals were reared in gondolas similar to those equipping the centrifuge, with identical bedding (sawdust), in the same room and under the same conditions of room temperature, dark-light cycle and ambient noise, but without centrifugation. A fan was used to generate air flow through
openings in the front door of these static gondolas in order to replicate the rotation-induced airflow experienced by the HG rats.

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

The influence of early HG rearing on the use-dependent plasticity of the adult forepaw S1 map was analyzed by comparing the effect of an enriched environment (EE) in rats raised in NG versus in HG during the first three postnatal weeks. In the EE, rats were housed in a group of 12 in two large cages (76 cm wide X 76 cm long X 40 cm high) connected by two tunnels and containing objects of different shapes, sizes, and textures to promote tactile experience; these objects were renewed daily to stimulate exploratory behavior in the rats. Animals housed in standard cages were in groups of three in Plexiglas cages (26.5 x 42.5 x 18) without objects. The rats had food and water ad libitum, and were housed on a 12-h light-dark cycle (see Coq and Xerri, 1998). Rats born and raised either in HG or NG during the first 21 postnatal days and then maintained for 16 weeks in an EE (HG16wE and NG16wE, respectively) were compared to those born and raised either in HG or NG during the first 21 postnatal days and housed in a standard environment for 16 weeks (HG16wS and NG16wS, respectively) (Fig. 1B, E).

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

Hypergravity environment

The apparatus generating the HG environment (see Bouët et al., 2003, 2004) consisted of a centrifuge made of four free-swinging gondolas fixed to the four extremities of two horizontal cross-arms driven at a constant rotation speed by a DC motor (3.5kW) located in the vertical axis of the centrifuge. The gondolas (0.55/0.38/0.30 m³), located 76.5 cm from the axis of rotation, were equipped with standard home cages for rats, including an aeration and lighting system providing a 12h light–12h dark cycle. Gondolas were equipped with a video system composed of a wide-angle camera connected to a monitor and used to establish the birth date of the litters. The gravito-inertial vector of 2G inside the gondola was produced with an angular velocity of 3.81rad/s, which resulted in a constant tilt angle of the gondolas of 60° from the horizontal axis. The resultant force was measured using a force sensor located in the center of the floor of the gondola. During centrifugation, rats were therefore subjected to a vector perpendicular to the gondola’s floor, i.e. similar to that experienced in normal gravity (i.e. corresponding to the dorso-ventral axis of the animal) (Fig. 1C). The rats were totally free-moving in the home cage. Accordingly, they were additionally exposed to variable Coriolis forces depending on the speed and direction of the animal’s motion within the home cage. Video monitoring of the nursing rats and their pups in both NG and HG groups was performed 3 times per week during 15 min observation periods.
In the HG rats exposed to normogravity, postural and locomotor behavior was observed twice a day for 15 min, during the first week.

**Electrophysiological mapping**

**Surgical procedure**

Anesthesia was induced with halothane and an initial injection of sodium pentobarbital (30 mg/kg, i.p.). Rats were maintained at an areflexive level of anesthesia throughout the experiment by supplemental doses of sodium pentobarbital (3 mg/kg, i.p.). The core temperature was continuously monitored by a rectal thermistor probe and maintained between 37 °C and 38 °C by a heating pad. The head was placed in a stereotaxic frame. To prevent cerebral oedema, cerebrospinal fluid was first drained through an opening in the dura covering the foramen magnum after resection of posterior neck muscles. Then, a craniotomy (about 16 mm²) was made to expose the S1 cortex to be mapped. The dura protecting the exposed part of the cortex was incised and resected. The cortical surface was bathed in a thin layer of warm silicone fluid to prevent drying. At the end of the mapping session, rats received a lethal dose of sodium pentobarbital (150 mg/kg, i.p.) and the brain was prepared for histological processing.

**Electrophysiological mapping procedure**

The mapping procedure used in the present study has been described in detail in previous papers (Xerri and Zennou-Azogui, 2003). Magnified images of the exposed parietal cortex, and the ventral and dorsal surfaces of the forepaw contralateral to the cerebral hemisphere to be mapped, were digitized by using a high-resolution camera mounted on an operating microscope. Cutaneous receptive fields (RFs) were drawn on the digitized images of the forepaw and placements of microelectrode penetrations were recorded on the
digitized image of the cortex using Map 0.925 software (Peterson and Merzenich, 1995).

Conventional multiunit recording and receptive field mapping techniques were used to
reconstruct the forepaw representation. Sites of electrode penetration were identified
relative to the vasculature of the cortical surface. Unit clusters were recorded with parylene-
coated tungsten microelectrodes (about 1 MΩ at 1 kHz; WPI, UK). The electrode was moved
perpendicular to the cortical surface in Cartesian coordinates by a 3D stepping
micromanipulator (Märzhauser, FST; Canada). Using the recording artifact generated by the
microelectrode contact with the cortex surface as a zero level, we advanced the electrode to
a depth of about 650-700 microns to record responses from clusters of 2 to 4 neurons in
layer IV (Waters et al., 1995; Coq and Xerri, 1998). The inter electrode penetration distance
was close to 70-80 microns in all groups of rats. The amplitude of the background noise
usually ranged from 15 to 20 μV with a signal-to-noise ratio ranging from 4 to 6. The
multiunit signal was amplified, filtered (bandwidth: 0.5 - 5 kHz), and displayed on an
oscilloscope. This signal was also rectified and passed through a discriminator with an output
signal proportional to the part of the input signal that was higher than an adjustable
threshold set just above the background noise. The output of the discriminator was then
delivered to an audio monitor. At each recording site, bursts of activity elicited by natural
stimulation allowed us to classify neuronal responses as cutaneous or non-cutaneous. The
cutaneous receptive fields (RFs) of small clusters of neurons were defined at each recording
site as the areas of skin where just-visible skin indentation (about 100 - 150 μm) elicited
reliable changes in multiple-unit discharge. This stimulation, which is within the dynamic
range of cutaneous receptors (Gardner and Palmer, 1989), was produced with a fine-tipped,
hand-held glass probe and monitored using magnifying glasses (x4). The curvature of fingers
and pads was taken into account while drawing the RFs. The ridges running along the
glabrous skin of the digits and palm were used as reliable landmarks to delineate the RFs. The RF areas were transferred to the digital image of the forepaw and were subsequently measured offline using Map 0.925 software. Responses elicited by upward and downward nail displacements were examined while the tips of the digits were firmly maintained to minimize joint movement and skin deformation. As a general rule, light pressure on the nails elicited a burst of activity facilitating the assessment of neurons’ directional sensitivity, i.e. the direction of nail displacement (upward: U, downward D or both U+D) eliciting the most prominent neuronal responses. These responses were categorized as cutaneous and referred to as ‘nail’ responses. In our classification, high-threshold responses identified by taps and pressure on tendons, intrinsic muscles or joint manipulations, while no cutaneous response was found, were classified as proprioceptive (Jenkins et al. 1990, Recanzone et al., 1992; Xerri et al., 1998, Coq and Xerri., 2000).

The mechanical thresholds of neuronal responses to cutaneous glabrous stimulation were determined using von Frey monofilaments (Stoelting, Semmes-Weinstein aesthesiometer) that apply indenting stimuli at a relatively constant, predetermined force. The most commonly used filaments were 2.83 (diameter: 0.127 mm; bending force: 0.068 g; bending pressure: 5.37 g/mm²), 3.22 (0.152 mm; 0.166 g; 9.15 g/mm²), 3.61 (0.178 mm; 0.407 g; 16.36 g/mm²), and 3.84 (0.203 mm; 0.692 g; 21.39 g/mm²) (FiG 2B). Neuronal responses elicited by von Frey filaments above 3.84, starting from 4.08 (0.229 mm; 1.202 g; 29.20 g/mm²) were classified as noncutaneous presumably proprioceptive. For reproducible measurements, the filaments were used at a relatively constant room temperature (about 24 °C). The stimulation consisted in pressing a filament gently against the skin, perpendicular to its surface and at the center of the RF, until the filament began to bend. This procedure was done 5 to 10 times for each filament. We used stimulus series of increasing and
decreasing strengths to determine the mechanical threshold eliciting noticeable changes in neuronal discharge.

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

Experimental measurements

Cortical areas devoted to forepaw representation, i.e. excited by stimulation of glabrous or hairy skin areas, or by nail displacements, were calculated for each rat and described by their absolute areas (mm²). To assess relative representation of glabrous, hairy skin surface and nails as parts of the forepaw representation, the corresponding parts of the map were expressed as a percentage of the whole cutaneous map of the forepaw. Average values were computed for each group of rats. The absolute sizes of glabrous and hairy RFs were measured in mm² (Map 0.925 software), normalized relative to the ventral and dorsal
skin forepaw areas, respectively, and expressed as percentages. The relative RF areas measured in each rat were averaged and the mean size of RF was calculated for each group of rats. The stimulation thresholds obtained using the von Frey filaments were processed to calculate an average force threshold for each animal (Xerri and Zennou-Azogui, 2003, 2014). Every threshold value (0.068 g, 0.168 g, 0.407 g and 0.692 g) was multiplied by the number of recording sites displaying that threshold and the sum of these weighted values was divided by the total number of sites tested. A mean threshold was then calculated for each experimental group. To assess directional sensitivity of finger nails, their displacement was induced using a hand-held electronic Von-Frey device, designed in our laboratory, to apply single stimuli through a probe (0.8 mm in diameter) (Blanc and Coq, 2007). The force envelope randomly applied (mN) by hand to induce nail extension or flexion was converted by the electronic Von-Frey device into a potential variation (0.1 mN corresponded to 10 mV) which was recorded at 1 kHz with a multichannel acquisition system (Plexon, Dallas). Stimuli and neuronal responses were synchronized using a TTL signal emitted by the Von-Frey at the onset of the nail contact. The force profile and duration varied across trials. Only relatively similar force patterns were retained to determine the units’ sensitivity to nail displacement. Neuronal activity was recorded in a 600 ms time window which started from the trial onset. We elicited 15 to 20 stimulus trials for each of the cortical locations recorded. The percentage of neuronal responses exhibiting clear directional sensitivity to either extension or flexion of nails, or no directional sensitivity, was calculated for each rat and averaged for each experimental group.

Histology

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

Paw print measurements

The animals stepped on an inkpad placed for 3 min in their rearing cage. Then, the
animals were immediately anesthetized with halothane, and pictures of the left and right
forepaws were taken using a CCD camera for subsequent measurement of inked paw
surfaces. In addition, after cleaning the stained skin surfaces and one hour after recovery
from initial anesthesia, NGp rats were tested in hypergravity, while HGp rats were tested in
normogravity. Possible long-term effects of hypergravity on forepaw prints were also
evaluated under normogravity conditions, 1 and 2 weeks after NG housing, in NGp and HGp
animals. Inked surfaces of the right and left forepaws were measured in square mm using
Canvas software (ACD Systems, Inc.). A mean forepaw print value was calculated for each rat
and then for each group of rats.

Statistical Analysis

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

RESULTS

Methological consideration: coriolis force in HG

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

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

where CA stands for the Coriolis acceleration, v is the rat's velocity and \(\omega\) is the angular
velocity of the centrifuge and x the vectorial product.

Knowing that in our experimental conditions, \(\omega = 3.81\) rad/s and the cage deviation
from the horizontal was 60°, the maximum rat velocity in their cage being estimated at
about 0.10 m/s, several cases have been computed, depending on the orientation of the
rat's displacement. Obviously, the highest value of the Coriolis acceleration was obtained
when the rat moved in the lengthwise direction of the cage, thus perpendicular to the
\(\omega\) vector. The CA value is \(\pm 0.838\) m/s² (the signs indicate the direction of the Coriolis
acceleration). Thus, it appears that the value of the additional force on the rat's paws (and
nails) corresponded to an acceleration of 0.085 G (0.025 N for a rat weighing 30 g) i.e., a small fraction of the 2G “vertical” acceleration. In the case of transversal direction of displacement in the cage, knowing that the speed vector was at 30° from the \( \omega \) vector, the value is lower \( (\pm 0.419 \text{ m/s}^2) \) and corresponds to an acceleration of 0.043 G. On the basis of these calculations, in our experimental conditions, the Coriolis forces were of little significance in relation to the hypergravity force. In addition, considering that the rat moves in various directions within the cage, the Coriolis acceleration acting on the forepaw has various orientations. Therefore, the mechanical effects of Coriolis forces on the forepaw will tend to cancel one another out.

**Behavioral observations**

Regular video monitoring of the rats’ behavior during the early postnatal period in both NG and HG rats did not reveal any behavioral modification in HG rats with respect to the control NG rats housed in gondolas. After the HG rats were exposed to NG, we did not detect any abnormalities in postural or locomotor behavior, other than an intensification of the exploratory behavior that was found for the first 2 days in NG. Importantly, no behavioral sign of stress (diminished grooming, exploratory activity, lactation or nursing) was observed in HG rats, as compared to controls.

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

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

Short-term effects of early exposure to hypergravity

Organization of S1 forepaw maps

This study represents the first investigation of the experience-dependent developmental plasticity of forepaw representations in the S1 cortex using high-density electrophysiological mapping. A conspicuous feature of somatosensory maps derived from HG rats was a threefold enlargement of cortical sectors driven by nail displacements (0.58±0.12 mm²) as compared to those of NG rats (0.19±0.05 mm²) (F₁,₁₈ = 81.12; P<0.001). In the former, nail representation was found to take over the cortical zones serving the skin
surfaces (Fig. 3A). Skin representations in HG rats (0.76±0.14 mm²) were significantly smaller than those of NG rats (1.20±0.21 mm²) (F1, 18 = 35.45; P<0.001). This HG-induced decrease was found for both the glabrous (mean decrease: 36 %; HG: 0.65±0.15 mm²; NG: 1.02±0.22 mm²) (F1, 18 = 20.05; P<0.001) and hairy skin areas (39%; HG: 0.11±0.04 mm²; NG: 0.18±0.05 mm²) (F1, 18 = 11.77; P<0.01). No significant change in the overall area of the forepaw cutaneous map (including skin and nail representational regions) was observed in HG rats as compared to NG rats (HG: 1.33±0.14 mm²; NG: 1.43±0.21 mm²; F1, 18 = 1.45; P=0.24, ns).

This finding led us to hypothesize that hypergravity increased sensory inputs generated by nail displacements. Therefore, we assessed the forepaw map reorganization in animals whose nails were regularly clipped (HGn− rats). The cortical area activated by nail displacement in HGn− rats (0.07±0.06 mm²) was strongly reduced, compared with that of HG (0.58±0.12 mm²) (F2, 27 = 98.06; P<0.001) and NG rats (0.19±0.05 mm²) (P<0.01). Interestingly, the glabrous skin sectors were similarly reduced in the HG (0.65±0.15 mm²) and HGn− (0.74±0.08 mm²) groups (F2, 27 = 14.87; P=0.19, ns). This reduction was found to be only partially compensated for by an increased hairy skin representation in the HGn− rats (0.27±0.09 mm²), which was greater than that of either NG (0.18±0.05 mm²) (F2, 27 = 16.34; P<0.01) or HG rats (HG: 0.11±0.04 mm²) (P<0.001). Therefore, the total surface area of cutaneous map (skin + nail regions) was smaller in the HGn− (1.08±0.13 mm²) than in the HG (1.33±0.14 mm²) (F2, 27 = 12.06; post-hoc: P<0.01) or NG groups (1.43±0.21 mm²) (P<0.001). Proprioceptive zones were found to colonize the cutaneous territory in these HGn− rats (Fig. 3A). Overall, these findings show that early exposure to HG strongly modified the relative representation of competing cutaneous (glabrous or hairy skin, nail) and proprioceptive inputs converging onto layer IV cortical neurons.
Neuronal responsiveness

We reasoned that if early rearing in HG strongly modulates sensory inputs generated by nail-movement during locomotion and changes in posture, this environment may bias the directional sensitivity of cortical neurons to upward (U) versus downward (D) nail displacement. Repeated measures ANOVA showed a main effect of directional sensitivity, i.e., the percentage of responses to U, D or both U and D displacement (U+D; \( F_{2, 36} = 22.21, P<0.001 \)), no main effect of rearing environment (\( F_{1, 18} = 0.00001; P=0.99, \text{ ns} \)) and an interaction between these two factors (\( F_{2, 36} = 12.79, P<0.001 \)). Post-hoc tests indicated that NG rats exhibited a majority of neuronal responses elicited by both U and D nail displacement when compared to those elicited by U or D movement (\( P<0.01 \) or \( P<0.001 \), respectively) which were found in similar proportions (\( P=0.39, \text{ ns} \)) (Table 2). By contrast, HG rats displayed a larger proportion of neurons sensitive to U nail displacement than to D (\( P<0.001 \)) or U+D (\( P<0.03 \)). In HG rats, the neuronal population sensitive to U nail displacement increased (\( P<0.001 \)) as compared to those populations recorded in NG rats. No difference in D nail response proportion was shown between NG and HG rats (\( P=0.07, \text{ ns} \)) (Fig. 4). These results suggest that the HG increase in the population of U responses resulted from an effect on U+D neurons.

To assess neuronal responsiveness to glabrous skin stimulation, a weighted mean was calculated on the basis of the mechanical thresholds obtained using the von Frey filaments (see Methods). The mechanical thresholds to cutaneous stimulation recorded within the reduced glabrous skin cortical sectors in the HG rats were similar to those recorded in the NG rats (\( F_{1, 18} = 1.87; P=0.19, \text{ ns} \)) (Table 3).

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

**Paw print evaluation**

Forepaw print measurements, taken as an estimation of the ventral skin areas in contact with the floor during stance and locomotion, were obtained in HGp and NGp rats of matching body weights (HGp: 44.49±5.95 g, NGp: 43.01±6.18 g; P=0.59, ns) under both NG and HG conditions. Repeated measures ANOVA yielded a main effect of gravity condition (F_{1, 18} = 76.28, P<0.001), no effect of rearing environment when the NG and HG rats were tested under the same gravity conditions (F_{1, 18} = 0.085; P=0.77, ns) and no significant interaction between these two factors (F_{1, 18} = 4.32, P=0.06, ns). Mean HGp and NGp rat forepaw prints were similar when obtained under HG testing conditions (HGp: 40.67±9.41 mm², NGp: 43.52±3.85 mm²; P=0.06, ns) or under NG testing conditions (HGp: 33.87±5.65 mm², NGp: 32.47±2.99 mm²; P=0.34, ns). As expected, rats from either rearing environment exhibited larger forepaw prints when tested in HG than in NG (HGp rats: P<0.0003; NGp rats: P<0.001). Interestingly, the ratios of HG to NG print areas recorded in each rat (HGp rats: 1.19±0.11
and NGp rats: 1.26±0.21) appeared to be similar to the ratio of HG to NG glabrous RF areas measured in mm² (1.27±0.21; P=0.35 and P=0.33, respectively).

**Enduring effect of HG rearing**

**Organization of S1 forepaw maps**

In order to properly describe possible enduring effects of early exposure to HG, we compared the map data recorded in 2 groups of age-matched animals (Fig. 5, 6). In the first group, rats were reared in HG for 3 weeks and then housed in standard environment in NG until the end of postnatal week 19 (HG, HG1wS, HG8wS, HG16wS rats; see Methods). In the second group, rats were reared in standard environment in NG from birth until postnatal week 19 (NG, NG1wS, NG8wS, NG16wS rats). For the total surface area of the forepaw map (Fig. 6A), the ANOVA disclosed main effects of both early rearing environment (F₁, 72 = 86.63; P<0.001) and subsequent NG housing duration conditions (F₃, 72 = 12.55; P<0.001), as well as an interaction between these two factors (F₃, 72 = 11.93; P<0.001). A gradual increase in the total area of the forepaw map was found over the 16 weeks examined in the rats reared in NG from birth (NG: 1.43±0.21 mm², NG16wS: 2.30±0.35 mm², P< 0.001) (Fig. 5 and 6). This increase was found for the cortical territories allocated to hairy skin (NG: 0.18±0.05 mm², NG16wS: 0.47±0.12 mm², P<0.001) and nail (NG: 0.19±0.05 mm², NG16wS: 0.56±0.18 mm², P<0.001) representations (Fig. 5 and 6BC), as well as for the glabrous skin representations, although to a lesser extent (NG: 1.02±0.22 mm², NG16wS: 1.27±0.23 mm², P<0.01) (Fig. 6D). By contrast, no expansion of the forepaw map was recorded in rats reared in HG for the first 3 postnatal weeks and housed in NG over the following weeks (HG: 1.33±0.14 mm², HG16wS: 1.39±0.33 mm², P=0.84, ns) (Fig. 5, 6). We examined whether this apparent “freezing effect” of the HG rearing affected similarly the different sectors of the forepaw
cutaneous map. This HG-induced effect was observed for glabrous skin areas (HG: 0.65±0.15 mm$^2$, HG16wS: 0.49±0.06 mm$^2$, P=0.21, ns): ANOVA yielded no effect of duration of NG housing (F$_3$, $\bar{y}_2$ = 0.74; P=0.53 ns), but a main effect of early rearing environment (F$_1$, $\bar{y}_2$ = 177.70, P<0.001) and an interaction between these two factors (F$_3$, $\bar{y}_2$ = 4.69, P<0.01) (Fig. 5 and 6D). As for the hairy skin representation (HG: 0.11±0.04 mm$^2$, HG16wS: 0.19±0.07 mm$^2$, P=0.26, ns), ANOVA showed main effects of duration of NG housing (F$_3$, $\bar{y}_2$ = 22.77, P<0.001) and early rearing environment condition (F$_1$, $\bar{y}_2$ = 90.35; P<0.001) as well as an interaction between these two factors (F$_3$, $\bar{y}_2$ = 7.17, P<0.001) (Fig. 5 and 6B). Similar effects were found for the nail representation (HG: 0.58±0.12 mm$^2$, HG16wS: 0.59±0.09 mm$^2$, P=0.77, ns) with ANOVA showing main effects of NG housing duration (F$_3$, $\bar{y}_2$ = 14.49, P<0.001) and early rearing environment (F$_1$, $\bar{y}_2$ = 59.93, P<0.001) and an interaction between these two factors (F$_3$, $\bar{y}_2$ = 13.98, P<0.001) (Fig. 5, 6) (Fig. 5 and 6C). Overall, our findings indicate that early exposure to HG prevented the expansion of forepaw cutaneous representational zones normally taking place in NG over the first postnatal months, despite exposure to NG from the end of the 3rd postnatal week.

**Neuronal responsiveness**

Directional sensitivity of cortical neurons to upward versus downward nail displacement was analyzed in rats reared in HG for 3 weeks and then housed in NG environment until the end of the 19th postnatal week (HG, HG1wS, HG8wS, HG16wS rats). Repeated measures ANOVA showed no main effect of duration of NG housing (F$_3$, $\bar{y}_6$ = 0.67; P=0.58, ns), but a main effect of directional sensitivity (F$_2$, $\bar{y}_2$ = 106.88, P<0.001) as well as an interaction between these two factors (F$_6$, $\bar{y}_2$ = 16.48, P<0.001). The HG-induced changes in directional sensitivity of cortical neurons characterized by an increased proportion of U nail displacement responses were still observed when rats reared in HG were housed for one
week in NG. HG1wS rats displayed similar proportions of neurons within each category of response as compared to HG rats (U: P=0.68; D: P=0.92 and U+D: P=0.45) (Table 2). This long lasting effect of the HG early rearing was shown to dissipate between the 2nd and the 8th week spent under normal gravity conditions: HG8wS rats displayed a majority of neuronal responses elicited by U+D nail displacement (P<0.001, when U+D is compared to U or D responses), while the rest of the neuronal populations exhibited either U or D unidirectional sensitivity in proportions that are not statistically different (P=0.12). The proportions recorded in HG8wS rats are similar to those recorded in aged-matched NG8wS rats (U: 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, when compared to U, D, U+D proportions found in HG8wS).

In the rats housed in NG from birth and in HG rats exposed to NG from the end of the 3rd week until the 19th week, the mechanical thresholds of neuronal responses assessed with the von Frey filaments were found to be stable until the end of the first month (NG vs NG1wS: P=0.22; HG vs HG1wS: P=0.96) and to increase thereafter, reaching a maximum at the end of the 11th postnatal week (Table 3). No difference between animals reared in NG or HG was observed at this time (P=0.12) and at the end of postnatal week 19 (NG16wS vs HG16wS: P=0.085, ns). ANOVA showed a significant effect of NG housing duration (F_3, 72 = 57.59; P<0.001) but no effect of early rearing condition (F_1, 72 = 3.09; P=0.08, ns) on neuronal responsiveness, and no interaction between these two factors (F_3, 72 = 2.56; P=0.06, ns). Therefore, these age-dependent profiles of neuronal response thresholds within the glabrous skin cortical sectors were not affected by the early rearing gravity conditions.

**Size of cutaneous RFs**

Regarding the size of glabrous skin RFs (Fig. 7A), ANOVA performed between our experimental groups disclosed a significant effect of both NG housing duration (F_3, 72 = 17.19;
P<0.001) and early rearing conditions (F_{1, 72} = 14.17; P<0.0001) as well as an interaction between these two factors (F_{3, 72} = 17.32; P<0.001). Further analysis revealed that the HG-induced increase in relative glabrous RF size was reversed as early as the end of the 1st week of housing in NG environment (HG: 5.14±0.48 %, HG1wS: 3.76±0.30; P<0.001), thus 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 groups as shown by ANOVA which yielded no effect of NG housing duration (F_{3, 72} = 1.78; P=0.16, ns) or early rearing condition (F_{1, 72} = 3.40; P=0.07, ns), as well as no interaction between these two factors (F_{3, 72} = 0.17; P=0.92, ns) (Fig. 7B).

Forepaw prints

The enduring effect of early HG rearing on forepaw prints was evaluated under NG conditions, 1 and 2 weeks after NG housing. The weight of HGp rats after 1 week of NG (67.50 ± 7.82 g) and that of age-matched NGp rats (61.09 ± 4.03 g) was similar (P=0.08, ns). The same observation applies for HGp rats exposed for 2 weeks in NG (97.55 ± 8.86 g) whose weight was similar to that of age-matched NGp rats (93.32 ± 10.19 g; P=0.25, ns). Repeated measured ANOVA performed on forepaw prints at 1 and 2 weeks after the HG rearing in HGp rats and in age-matched NGp rats disclosed no effect of early rearing environment (F_{1, 18} = 0.07; P=0.80, ns), but a main effect of subsequent NG housing duration (F_{1, 18} = 18.46; P<0.001), with no interaction between these two factors (F_{1, 18} = 0.004; P=0.95, ns). Post-hoc tests indicated that forepaw prints increased between the 1st and the 2nd week in both groups (HGp: P<0.02; NGp: P<0.02), and that they were similar in HGp rats and age-matched NGp animals when tested 1 week (HGp: 34.30±5.09 mm\(^2\), NGp: 34.84±5.93 mm\(^2\); P=0.82, ns) or 2 weeks (HGp: 41.40±3.91 mm\(^2\), NGp: 41.73±5.95 mm\(^2\); P=0.89, ns) after HG rearing.
Influence of developmental HG experience on adult use-dependent plasticity of cortical maps.

We sought to determine the influence of early developmental HG experience on later use-dependent plasticity. For this purpose, we used the environmental enrichment (EE) paradigm which has been shown to strongly modify the S1 forepaw map organization in adult rats (Coq and Xerri, 1998). Age-matched rats raised in NG or HG during the first 3 postnatal weeks were subsequently housed in EE for 16 weeks. ANOVA performed on the data recorded in the 4 experimental groups (NG16wS, NG16wE, HG16wS, HG16wE) yielded a main effect of early rearing ($F_{1, 36} = 163.25; P<0.001$), but no effect of adult housing conditions ($F_{1, 36} = 0.86; P=0.36$, ns) on the overall forepaw cutaneous representation, with an interaction between these two factors ($F_{1, 36} = 9.11; P<0.01$). Further post hoc analysis showed an EE-induced expansion in the rats reared in NG during the first 3 postnatal weeks compared to the rats housed in a standard environment during the same period (NG16wE: $2.66\pm0.29$ mm$^2$; NG16wS: $2.30\pm0.35$ mm$^2$; $P<0.01$). By contrast, no such increase of the cutaneous maps was found when rats were exposed to EE after rearing in HG (HG16wS: $1.39\pm0.33$ mm$^2$; HG16wE: $1.20\pm0.17$ mm$^2$; $P=0.15$, ns) (Fig. 8). ANOVA yielded a main effect of both early rearing and adult housing conditions on the glabrous skin representation ($F_{1, 36} = 362.13; P<0.001$; $F_{1, 36} = 16.35; P<0.001$, respectively), with an interaction between these two factors ($F_{1, 36} = 11.31; P<0.01$). The post-hoc tests showed an expansion of cortical zones representing the glabrous skin only in rats raised during the early postnatal period in NG (NG16wS: $1.27\pm0.23$ mm$^2$, NG16wE: $1.64\pm0.19$ mm$^2$, $P<0.001$) but not in HG (HG16wS: $0.49\pm0.06$ mm$^2$, HG16wE: $0.52\pm0.08$ mm$^2$, $P=0.64$, ns). ANOVA performed on hairy skin
sectors showed an effect of both early rearing \((F_{1, 36}=80.62; P<0.001)\) and adult environment conditions \((F_{1, 36} = 5.92; P<0.02)\), with no interaction between these two factors \((F_{1, 36} = 1.47; P=0.23, \text{ns})\). Further post hoc analysis indicated that hairy skin areas were not modified by EE in rats reared in HG \((\text{HG16wS}: 0.19\pm 0.07 \text{ mm}^2, \text{HG16wE}: 0.23\pm 0.07 \text{ mm}^2, P=0.40, \text{ns})\), while they increased in the rats reared in NG \((\text{NG16wS}: 0.47\pm 0.12 \text{ mm}^2, \text{NG16wE}: 0.60\pm 0.17 \text{ mm}^2, P<0.01)\) during the first 3 weeks postnatal period, though to a lesser extent than glabrous skin sectors. Moreover, ANOVA yielded no effect of early rearing \((F_{1, 36} = 0.25; P=0.62, \text{ns})\), but an effect of adult housing conditions \((F_{1, 36} = 10.38; P<0.01)\) on the surface area of the nail sectors, with no interaction between these two factors \((F_{1, 36} = 0.02; P=0.90, \text{ns})\). The post hoc tests showed no EE-induced changes in nail regions both in rats reared in NG \((\text{NG16wS}: 0.56\pm 0.18 \text{ mm}^2, \text{NG16wE}: 0.43\pm 0.16 \text{ mm}^2, P=0.09, \text{ns})\) or in HG \((\text{HG16wS}: 0.59\pm 0.09 \text{ mm}^2, \text{HG16wE}: 0.45\pm 0.07 \text{ mm}^2, P=0.06, \text{ns})\) during the first 3 weeks postnatal period. Taken together, these findings show that the EE increased skin sectors in rats reared in NG, but not in HG, during the first 3 post-natal weeks. In addition, the expansion of nail representation induced by early HG was not modified by a subsequent exposure to the EE.

**Neuronal responsiveness**

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

As for the RFs recorded within the glabrous skin cortical zones, ANOVA showed a significant effect of the adult housing conditions (F_{1,36} = 19.87; P<0.001) but no effect of the early 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 RF sizes in rats reared in NG (NG16wS: 3.39±0.31 %, NG16wE: 2.48±0.24 %, P<0.001), but not in those reared in HG (HG16wS: 3.40±0.32 %, HG16wE: 3.04±0.75 %, P=0.19, ns). Moreover, ANOVA yielded no significant effect of early rearing (F_{1,36} = 0.31; P=0.58, ns) and an effect of adult housing (F_{1,36} = 4.51; P<0.05) conditions on hairy skin RFs, as well as no interaction between these two factors (F_{1,36} = 0.72; P=0.40, ns). Post-hoc tests indicated that the hairy skin RFs were not modified by the EE either in rats reared in NG (NG16wS: 7.21±1.01 %, NG16wE: 7.93±2.05 %; P=0.37, ns) or in those reared in HG (HG16wS: 6.42±1.78 %, HG16wE: 8.10±2.08 %; P=0.17, ns) during the early postnatal period of development.

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

DISCUSSION

Summary of results

In this mapping study of the maturation of somatosensory maps (see Fig. 10), we provide evidence that the forepaw cortical representations in 2 and 3 week-old rats are adult-like in
their somatotopic organization, but with lower mechanical thresholds than in the adults. A continuous expansion of each of the constituent territories of the map was observed until the 19th postnatal week. Secondly, we report both short and long-term effects of early and transient HG exposure on the development of somatotopic forepaw cortical maps in rats subsequently exposed to NG. Despite preservation of the surface area of the forepaw map, HG was found to shape the somatotopic organization of the cortical forepaw map, by expanding the nail representation at the expense of the skin territories in which ventral skin cutaneous RFs were found to be enlarged. In addition, nail responses were biased toward an increased sensitivity to nail extension. The early imprint of HG on the map organization was not modified by a subsequent exposure to NG during the 4 month period examined. However, after 1 week of housing in NG, neuronal RFs recorded in HG rats displayed sizes very similar to those recorded in NG rats, while directional sensitivity to nail displacement developed within 8 weeks. Thirdly, we found that early and transient HG exposure prevented the use-dependent reorganizational changes induced by enriched environment in adult animals reared under NG conditions from birth.

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

This study reports new findings on the development of forepaw somatosensory maps based on high-density electrophysiological maps. The organization and the development of representation in the forepaw barrel subfield (FBS) in the S1 cortex of the rat has been documented in very few studies, in contrast to whisker representation which has been extensively investigated. Barrel-like structures in the FBS develop around P5 (McCandlish et al., 1989; Rhoades et al., 1990). Experiments carried out to detect the earliest time when the S1 cortex is driven by somatosensory inputs indicate that responses are elicited in the snout subfield as early as 12 hours after birth, in the forepaw area by the end of the 1st postnatal
day and in the hindpaw area by the middle of P2, thus following a lateral to medial gradient (McCandlish et al., 1993). However, increases in amplitude and complexity of wave shape and decreases in response latencies were described over subsequent postnatal days, until P14. Seelke et al. (2012) who examined the time course of emergence of the topography of entire body maps have shown that early in development, most of S1 is occupied by vibrissae/face representations. These authors observed that from P10, representations of body parts including the forelimbs emerge with a crude topography, whereas a clear topographical organization began to appear by P15, along with a reduction of RF sizes. From P20, body maps were found to be similar to those recorded in the adult. Our results are in accordance with these data: the maps recorded at P15 in two rats and at P22 in larger samples appeared to be adult-like in their topographical organization (see Coq and Xerri, 1998, 1999; Xerri and Zennou-Azogui, 2003).

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

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

The alteration of the organization of forepaw somatosensory maps resulting from early HG suggests that this environment strongly modifies the cortical integration of afferent signals from somatosensory receptors. It is worth noting that rat pups are nursed over the first 3 postnatal weeks, with a daily time devoted to lactating behavior that decreases from about 80%, during the first days, to 30% on the 18th day postnatal (Rosselet et al. 2006). The motor system matures slowly over the first postnatal weeks and the pups do not walk spontaneously before the end of the second week of life. Altman and Sudarshan (1975) reported that, in the rat, walking and running predominate by the end of the second postnatal week. Consistently, clear topographical organization of the forelimb cortical map appears by P15 in S1 (Seelke et al., 2012). These findings suggest that the impact of HG on the forces exerted on the forepaws during the locomotor behavior was predominant during the 3rd postnatal week.

Previous studies have shown that changes in gravity during development influenced motor development in rodents. Bojados et al (2013) found that exposure to hypergravity before P10, i.e. the acquisition of locomotion, induces postural changes in mice, particularly a more extended ankle joint during locomotion (extension bias). As a mirror situation, rats exposed to microgravity from P14 to P30 exhibited changes in flexor muscle responses (Walton et al., 2005a). In rats born and reared during 100 days in hypergravity, the contractile properties and phenotype of hindlimb extensor muscle fibers were found to be modified (Picquet et al., 2002). Muscle characteristics were reinforced as the soleus, an antigravity muscle, became slower (100% expression of myosin heavy chain slow isoform I), whereas its agonist, the plantaris muscle, presented faster contractile behavior. Collectively, these data sustain the view that joint and muscle proprioception is altered by gravity
constraints both during development and in the adult, and that this alteration which
presumably reflects an adaptation to these constraints is very likely to induce enduring
changes in the processing of somatosensory information within S1. However, it is worth
mentionning that in our rats born and reared in hypergravity for 3 weeks, video monitoring
did not reveal impaired behavior, consistently with observations reported by Bouët et al.
(2004) who used a similar experimental device to ours.

It is widely recognized that early sensory experience plays a critical instructive role in
the structural and functional maturation of the sensory systems (Hensch, 2004; Fox and
Wong, 2005). According to this view, a process of competition for cortical space between
sensory inputs shapes and refines the organization of the neural circuits underlying the
visual, auditory and somatosensory cortical maps (Hensch, 2004). Patterns of activity elicited
by sensory experience are translated into networking of synaptic connections (Katz and
Shatz, 1996). Our results confirm that the topographically organized somatosensory forepaw
maps are sculpted by early sensory experience. They also reveal an experience-driven
competition between somatosensory sub-modalities that critically shapes cortical maps
during the early sensory events of postnatal life. We propose that HG, by increasing the
pressure exerted on the ventral surfaces of the paws and enhancing the antigravity muscle
activity, modifies the pattern of somatosensory afferent signals, thereby leading to the
emergence of a distinctive forepaw somatosensory map. The balance between cutaneous
submodalities was modified, as evidenced by the contraction of the map territories allocated
to skin surfaces that was paralleled by an expansion of cortical sectors serving nail areas.
This expansion of nail representation can be attributed to a relative increase in nail
stimulation in HG, as also suggested by a change in directional sensitivity reflected by the
greater percentage of responses to upward deflection. Interestingly, drastic reduction of
tactile input resulting from nail clipping in HGN- rats resulted in a prominent shrinkage of
cortical sectors serving the nails that unexpectedly was compensated for, not by an increase in skin surface representations, but an expansion of proprioceptive sectors. This finding suggests that somatosensory submodalities converging on cortical neuronal targets do not have equivalent effectiveness in competing for cortical space. The propensity of proprioceptive representation for “invading” cutaneous territories of the somatosensory cortical maps has been repeatedly documented in our previous studies on adult experience-dependent plasticity after tactile impoverishment or sensorimotor restriction (Coq and Xerri, 1999), as well as during aging (Coq and Xerri, 2000). An additional argument in favor of competition between cutaneous and proprioceptive inputs comes from the finding that in humans, a rapid increase in cortical proprioceptive activity elicited by stimulation of muscle afferents from the first dorsal interosseus occurs after transient cutaneous deafferentation of the cutaneous territory overlying the corresponding muscle, following anesthesia of the radial nerve (Tinazzi et al., 2003). In the present study, the emergence of proprioceptive sectors could be confused with an increase in the threshold of cutaneous responses. Accordingly, a decreased response sensitivity would be expected to occur in the spared cutaneous territories of the HG maps. However, our data show a lack of change in the mechanical thresholds recorded in the shrunken glabrous skin representational zones. The reduced glabrous skin representation observed in both HG and HGN- rats more likely reveals a redistribution of effective inputs within these cortical sectors of the forepaw maps due to the HG environment. The dynamic balance between competing inputs distributed within thalamocortical and intracortical networks is underpinned by the convergence of cutaneous and proprioceptive inputs onto single layer IV neurons of the S1 cortex (Lamour and Jobert, 1982; Chapin and Lin, 1984; Sievert and Neafsey, 1986; Gioanni, 1987). This convergence enables an experience-dependent, behaviorally relevant allocation and refinement of somatosensory cortex representational territories.
In this study, the spatial resolution of the contracted forepaw cutaneous maps was found to have deteriorated after early rearing in HG, as indicated by the increase in the sizes of the glabrous RFs, whereas hairy RF sizes were not affected. Previous studies on adult experience-dependent plasticity of somatosensory maps have described RF sharpening or expansion in response to specific task learning or changes in subject-environment interaction (see Xerri et al., 2008 for review). These studies have underscored the influence of the temporal patterning, i.e. the degree of local synchrony of concurrent sensory inputs on the representational segregation of cortical somatosensory maps through receptive field remodelling (Allard et al., 1991; Recanzone et al., 1992; Diamond et al., 1993; Armstrong-James et al., 1994; Wang et al., 1995; Godde et al., 1996; Byl et al., 1996; Rosselet et al., 2008). A consistent finding in all these studies was that temporally synchronized stimulation of large skin territories induced RF enlargement, whereas temporally distributed skin stimulation resulted in RF decreasing. Along the same lines, in an earlier study, we performed severe sensorimotor deprivation by using single-forelimb casting in rats. This procedure led to an over-reliance on the non-constrained forelimb for postural balance and increased mechanical pressure on the ventral skin surfaces of the uncast forepaw (Coq and Xerri, 1999). Although the forced use of the non-cast forelimb did not affect the area of the corresponding cutaneous map, it led to enlargement of glabrous skin RFs, probably as a result of concomitant stimulation of larger than usual glabrous skin territories. In the present study, the 2G environment induced broader simultaneous contact of forepaw ventral skin surfaces with the cage floor during stance and locomotion, as confirmed by the increased paw prints. Accordingly, the glabrous skin RF enlargement recorded in HG young rats can be attributed to the spatial aggregation of formerly segregated thalamo-cortical inputs through unmasking/strengthening of formerly subthreshold synapses. The cutaneous RF enlargement found in HG rats is consistent with the decrease in GABA immunoreactivity
recorded in the limb representation of the rats S1 cortex after 14 days of exposure to hypergravity (D’amelio et al., 1998). Furthermore, an expansion of cutaneous RFs in the S1 cortex was found when GABA-mediated local inhibition was antagonized (Dykes et al., 1984; Alloway et al., 1989; Tremere et al., 2001; Chowdhury and Rasmusson, 2002).

Enduring effects of early exposure to hypergravity

Here, we show that postnatal changes in somatosensory map organization examined after 1 week, 8 weeks and 16 weeks in NG was impacted by neonatal exposure to HG for 3 weeks. Gradual increase in the area of the constituent regions of the forepaw maps was found to be typical of rats reared in NG. Interestingly, the HG-induced contraction of skin representation and concomitant increase in nail areas found after HG rearing for 3 weeks were maintained over the following 16 weeks of housing in NG. Therefore, this early experience impeded normal development of somatosensory maps after exposure to NG, thereby indicating a lack of adaptation of the S1 cortex to new somatosensory experience occurring in NG.

These enduring effects of HG rearing on somatosensory maps could be attributed to an impaired development of posture and locomotion that would tend to maintain changes in the patterns of somatosensory signals conveyed to the S1 cortex. Consistently, previous studies on the effects of altered gravity on the development of motor functions have shown enduring changes in locomotion (Walton 1992; Bojados et al., 2013), swimming behavior (Walton et al., 2005a) and development of surface righting (Walton et al., 2005b), and thus suggested the existence of a critical period in motor development. Persisting extension bias was observed by Bojados et al. (2013) in 2 month-old mice centrifuged from conception to P10 or P30. In addition, rats born and reared until the age of 3 months in HG were found to exhibit a lower body position, an enlarged support surface and an exaggerated foot
dorsiflexion (Bouet et al., 2003, 2004). These rats also showed alterations in locomotor pattern, when exposed to normogravity. They exhibited a faster locomotor rhythm (increased number of steps/sec) (Bouët et al., 2003, 2004). However, all these alterations disappeared within 3 weeks, reflecting a gradual adaptation to normogravity. It is important to underline that in all these studies the HG-induced alterations to the rodents’ posture and locomotion were reported after a longer exposure to HG than in the present study, during a period of adaptation to NG. In our study, we did not observe the postural changes reported by Bouët et al. (2003, 2004) and we found only a transient increase in exploratory behavior. Also of relevance for potential environmental stress, no changes in corticosterone levels were measured in rats exposed to 2G (Moran et al., 2001). Therefore, the available evidence suggests that the long-term cortical changes induced by the 3-week early exposure to HG were not accounted for by enduring behavioral alterations.

Regarding descending pathways, Gimenez y Ribotta et al. (1998) showed a delayed and modified development of serotonergic projections to the spinal cord of rats exposed to HG from the 11th day after gestation to the 15th day postnatal and which appeared to be smaller than controls. The organization and ultrastucture of these projections were durably affected, even after 8 months in normogravity. However, no behavioral correlates were provided in this study, leaving open the question of the functional consequences of alterations in the organization of the serotonergic projections. As previously mentioned, Picquet et al. (2002) showed that soleus antigravity muscle of rats conceived, born, and reared in HG until 100 days expressed 100 % of slow myosin phenotype. In a subsequent study, the same authors showed that in these animals, normogravity from 100 days to 115 or 220 days did not transform the muscle phenotype, suggesting the existence of a critical period in muscle phenotype determination (Picquet et al., 2005). One may hypothesize that these persisting changes in descending pathways and muscle properties during subsequent
exposure to NG were likely to lastingly influence the cortical integration of proprioceptive inputs and thus the corresponding representations in the S1 map. By contrast, the paw print measurements of rats exposed to NG after HG rearing were identical to those of rats reared in NG, suggesting that the flows of cutaneous information to the S1 cortex were similar in NG and in HG rats subjected to normogravity. This hypothesis is supported by the normalization of the sizes of ventral skin RFs in HG rats as early as the end of the first week of housing in NG and that of the directional sensitivity of the responses to nail displacement between the 2nd and the 8th week of normogravity. However, such normalization of neuronal responses within cortical sectors serving cutaneous inputs does not preclude the enduring imprint of early HG environment on the cortical integration of somatosensory afferents. This is suggested by persistent alterations of the forepaw map organization such as the decreased area of ventral skin sectors and the increased nail sectors, as well as the lack of age-related expansion of hairy skin zones of the forepaw maps. Furthermore, it is noteworthy that the HG-induced freezing effects lasted at least 16 weeks after exposure to normal gravity. Collectively, these findings sustain the view that the first 3 postnatal weeks represent a time window that is critical for the developmental segregation of somatotopic maps. Importantly, forelimb motor maps were first detectable at the end of the second postnatal week (Young et al., 2012). Moreover, during rat locomotion development, pivoting predominates during the second half of the first week, crawling during most of the second week and walking or running by the end of the second week (Altman and Sudarshan, 1975). Therefore, the behaviorally induced influence of HG on the forepaw map formation reported in the present study was predominant during or even confined to the third postnatal week. The present findings support the view that the maturation of somatosensory maps is strongly influenced by use-dependent changes taking place within a narrow critical postnatal time window.
The long-lasting effects of HG reported herein may reflect enduring changes in neuronal network connectivity during early development. The enduring effects of early HG rearing are at variance with the reversibility of alteration found in adult somatosensory maps following various manipulations (see for reviews Buonomano and Merzenich, 1998; Xerri, 2008; Barnes and Finnerty, 2010), such as experimental syndactyly (Clark et al., 1988), limb immobilization (Coq and Xerri, 1999) or natural episodic behavior (nursing; Rosselet et al., 2006). These HG-induced sustained alterations provide an additional argument in favor of a critical period in the maturation of somatosensory maps, previously shown for the development of the whisker-barrel system (see Erzurumlu and Gaspar, 2012, for review).

Herein, we report the persistent effect of early experience on the shape and area of somatosensory map territories. By contrast, other response characteristics, namely the spatial selectivity, i.e. the size of glabrous skin RFs and the directional sensitivity to nail displacement, were found to display an adaptation to NG. This finding is in accordance with an earlier study in adult rats in which we showed that the spatial selectivity of S1 neurons resulting from ongoing segregation/desegregation of inputs converging onto neuronal targets was reshaped on a time scale closely dependent upon behaviorally driven changes in somatosensory inputs (Rosselet et al., 2006). The reversibility versus retention of different neuronal response properties, after the animals were exposed to NG, shows that the use-dependent synaptic mechanisms leading to masking/unmasking of less/more effective inputs or to synaptogenesis at maturity are expressed within limits of representational malleability stabilized by somatosensory experience during a critical developmental period. This view is substantiated by our findings that the potential for experience-dependent plasticity in the adult was constrained by somatosensory experience during development. In the present study, HG rearing was found to impede the use-dependent map remodeling induced by enriched environment in adult animals reared in normogravity, originally
described by Coq and Xerri (1998). It seems that early HG experience shaped the neural network underpinning the forepaw cortical maps in such a way that adult experience-dependent plasticity was limited. Interestingly, the influence of tactile interactions between parents and offspring during early life on intrinsic connections within S1 and callosal connections has been highlighted by a recent study in rodents (Seelke et al., 2016). Therefore, one cannot rule out the possibility that subtle HG-induced changes in nursing behavior that were not detected using video monitoring could have induced alteration in cortical connectivity.

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

Acknowledgments

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References


Chowdhury SA, Rasmusson DD. Comparison of receptive field expansion produced by GABA(B) and GABA(A) receptor antagonists in raccoon primary somatosensory cortex. *Exp Brain Res* 144: 114–121, 2002.


Gimenez y Ribotta MG, Sandillon F, Privat A. Influence of hypergravity on the development of monoaminergic systems in the rat spinal cord. *Developmental brain research* 111: 147–


Peterson BE, Merzenich MM. MAP: a Macintosh program for generating categorical maps


**Shoykhet M, Land PW, Simons DJ.** Whisker trimming begun at birth or on postnatal day 12


**Captions**

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

**Fig. 2. Electrophysiological map of cutaneous surfaces obtained in a 2-week old rat.** Map elaboration is based on the response characteristics (somatosensory submodality, location and size) of the receptive fields (RFs) of neurons recorded within layer IV of the S1 cortex. A. Magnified image of the forepaw area of the cortex with a superimposed drawing of the boundaries encompassing cortical sites the RFs of which were restricted to a common...
forepaw subdivision. Within these boundaries, colored dots indicate cortical sites where cutaneous hairy and glabrous skin responses were elicited while colored squares show cortical sites where neuronal responses were elicited by nail displacements. Green and red triangles mark cortical sites excited by lower lip and wrist stimulation, respectively. Violet squares refer to cortical sites classified as “proprioceptive” (responses to strong pressure on skin and tendons and/or joint movement). Black squares mark cortical sites with no stimulus-evoked discharges. B. Photomicrograph of Nissl-stained section from the forepaw area of cortex with an electrolytic lesion in layer IV (arrow) and recording trace illustrating a typical multiunit discharge elicited by a von Frey filament (3.22: 0.166 g; 9.15 g/mm²) applied at the center of a RF on digit 3. C. Colored sectors of the cutaneous map with black dots indicating cutaneous (skin or nail sensitive) cortical sites. Maps recorded at PN15 appeared to be adult-like in their topographical organization. D. Samples of cutaneous RFs on glabrous and hairy skin surfaces corresponding to the cortical sites illustrated in A and C. 1 to 5: digit 1 to 5; P: pads.

Fig. 3. Effect of early exposure to hypergravity on the development of the somatotopic cutaneous maps of the forepaw. Representative maps obtained on the 22nd postnatal day in a rat born and housed in normogravity (NG) or hypergravity (HG) and in a rat reared in HG with forepaw nails trimmed until the age of 3 weeks (HGn-) (A). Glabrous (B) and hairy skin (C) RFs of which the locations on the forepaw were used to reconstruct the cutaneous maps illustrated in A. Note the HG-induced increase in the size of glabrous RFs recorded in HG and HGn- rats. Note in HG and HGn- rats the HG-induced decrease in the cortical area serving skin surfaces of the forepaw. Nail representation was shown to take over the skin zones in the HG rat, while in the HGn- rat, proprioceptive zones colonized the cortical territory compensating for the decrease in nail and skin areas. Same conventions as in Fig.2.
Fig. 4. Influence of hypergravity on neurons’ directional sensitivity to nail displacement. A. Classification of neuronal responses according to their sensitivity to nail upward (a) or downward (b) displacement induced by an electronic von Frey device generating force profiles. B. Percentages of neuronal responses elicited by upward (U), downward (D) or both upward and downward (U+D) movement of the nails obtained on the 22nd postnatal day in rats born and housed in normogravity (NG) or hypergravity (HG) (see Table 2 for mean percentages of neuronal responses within experimental groups). Note that hypergravity resulted in a higher percentage of neurons responding to nail upward displacement.

Fig. 5. Enduring effect of early hypergravity rearing on cutaneous forepaw maps. Forepaw representations obtained in a rat representative of the group of animals reared in NG from birth (NG, NG1wS, NG8wS, NG16wS groups) (left) and an age-matched rat representative of those born and reared in hypergravity during the first 3 postnatal weeks at P22 (HG group) and after 1 week (HG1wS group), 8 weeks (HG8wS group) or 16 weeks (HG16wS group) of housing in normogravity (NG) (right). Note the “freezing effect” of early HG rearing which prevented the expansion of forepaw skin sectors, while nail expansion was maintained, despite exposure to NG for 16 weeks.

Fig. 6. Effect of early hypergravity rearing on age-dependent changes in the representational organization of forepaw cutaneous cortical maps. Cortical areas (in mm²; mean ± SD) of the total cutaneous maps (A) and different sectors of the maps (B-D) as a function of age (in weeks) in rats born and reared in hypergravity until the age of 3 weeks, in rats housed for 1, 8 and 16 weeks in normogravity (empty squares) after the hypergravity rearing, and in age-matched rats reared in normogravity from birth (black dots). * indicates
statistically significant differences (P<0.05) between NG and HG rats at the same time points.

Horizontal bars applies to rats reared in normogravity and indicate statistically significant
differences between time points. Note that none of the map representational zones in the
HG rats increased as a function of age, in contrast with those recorded in NG rats.

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

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

Fig. 9. Influence of early HG rearing on use-dependent plasticity of glabrous skin RFs in
young adults. Examples of glabrous and hairy skin RFs corresponding to the cutaneous maps
shown in Fig. 8. HG during the first 3 postnatal weeks prevented the EE-induced decrease in
glabrous RF size found in rats reared in NG.
Fig. 10. Synopsis of findings on the development of the forepaw representation in the S1 cortex and main effects of HG.

Table 1. Labels of the experimental groups.

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

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

Hypergravity

Hypergravity (nails clipped)

A

B

C

GLABROUS SKIN SURFACES

HAIRY SKIN SURFACES

NAIL
A

Nail movement

<table>
<thead>
<tr>
<th>No directional sensitivity (U+D)</th>
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<tbody>
<tr>
<td>Sensitivity to upward movement (U)</td>
</tr>
<tr>
<td>Sensitivity to downward movement (D)</td>
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</tbody>
</table>

Upward movement

Downward movement

1 mV | 0.1 mN | 100 ms

B

Normogravity

Hypergravity

<table>
<thead>
<tr>
<th>Normogravity</th>
<th>Hypergravity</th>
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<tbody>
<tr>
<td>U</td>
<td>U+D</td>
</tr>
<tr>
<td>27.8%</td>
<td>35.9%</td>
</tr>
<tr>
<td>49.8%</td>
<td>11%</td>
</tr>
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</table>
NG
NG: 3 w. old rats in control cages

NG1wS
NG: 3 w. in control cages + 1 w. in standard environment

NG8wS
NG: 3 w. in control cages + 8 w. in standard environment

NG16wS
NG: 3 w. in control cages + 16 w. in standard environment

HG
HG: 3 w. old rats in centrifuge

HG1wS
3 w. in HG + 1 w. in NG standard environment

HG8wS
3 w. in HG + 8 w. in NG standard environment

HG16wS
3 w. in HG + 16 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

NG16wE
NG: 3 w. in control cages + 16 w. in enriched environment

HG16wE
3 w. in HG + 16 w. in NG enriched environment

GLABROUS SKIN SURFACES
- D1
- D2
- D3
- D4
- D5
- PALMAR PADS

HAIRY SKIN SURFACES
- D1
- D2
- D3
- D4
- D5
- LARGE DORSUM

NAIL
NG16wS
NG: 3 w. in control cages + 16 w. in standard environment
3 w. in HG + 16 w. in NG standard environment

HG16wS

NG16wE
NG: 3 w. in control cages + 16 w. in enriched environment
3 w. in HG + 16 w. in NG enriched environment

HG16wE

5mm
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
<table>
<thead>
<tr>
<th>Groups</th>
<th>NG</th>
<th>NG1wS</th>
<th>NG8wS</th>
<th>NG16wS</th>
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<tr>
<td>Upward nail displacement (U)</td>
<td>27.8±9.6 % (26)</td>
<td>20.6±2.1 % (22)</td>
<td>22.3±13.3 % (26)</td>
<td>19.4±9.5 % (29)</td>
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<tr>
<td>Downward nail displacement (D)</td>
<td>22.4±12.1 % (18)</td>
<td>13.3±3.5 % (14)</td>
<td>10.3±6.3 % (12)</td>
<td>9.5±8.1 % (13)</td>
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<td>U+D</td>
<td>49.8±18.3 % (42)</td>
<td>66.1±10.9 % (79)</td>
<td>67.4±10.9 % (82)</td>
<td>71.1±10.2 % (108)</td>
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<table>
<thead>
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<th>Groups</th>
<th>HG</th>
<th>HG1wS</th>
<th>HG8wS</th>
<th>HG16wS</th>
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<tbody>
<tr>
<td>Upward nail displacement (U)</td>
<td>53.1±5.6 % (146)</td>
<td>50.6±16.2 % (135)</td>
<td>22.7±7.9 % (70)</td>
<td>20.8±10.8 % (69)</td>
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<tr>
<td>Downward nail displacement (D)</td>
<td>11.0±8.7 % (29)</td>
<td>8.6±7.3 % (24)</td>
<td>6.5±6.1 % (19)</td>
<td>8.8±7.6 % (27)</td>
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<td>U+D</td>
<td>35.9±9.0 % (115)</td>
<td>40.8±21.2 % (111)</td>
<td>70.8±12.5 % (216)</td>
<td>70.4±14.6 % (234)</td>
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Table 2
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<th>Groups</th>
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<td>NG</td>
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</tr>
<tr>
<td>HG</td>
<td>0.233±0.064 g</td>
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<tr>
<td>NG1wS</td>
<td>0.247±0.058 g</td>
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<tr>
<td>HG1wS</td>
<td>0.231±0.062 g</td>
</tr>
<tr>
<td>NG8wS</td>
<td>0.411±0.057 g</td>
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<tr>
<td>HG8wS</td>
<td>0.365±0.067 g</td>
</tr>
<tr>
<td>NG16wS</td>
<td>0.393±0.049 g</td>
</tr>
<tr>
<td>HG16wS</td>
<td>0.343±0.025 g</td>
</tr>
<tr>
<td>NG16wE</td>
<td>0.459±0.061 g</td>
</tr>
<tr>
<td>HG16wE</td>
<td>0.427±0.068 g</td>
</tr>
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</table>

Table 3