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# EFFECTS OF HYPERGRAVITY EXPOSURE ON CD-1 MOUSE CENTRAL NERVOUS SYSTEM DEVELOPMENT: ASSESSING FOR SHORT-, MEDIUM- AND LONG-TERM BEHAVIOURAL AND NEUROBIOLOGICAL RESPONSES

"Effetti dell'esposizione a ipergravità sullo sviluppo del sistema nervoso centrale: valutazione delle risposte comportamentale e neurobiologica di breve-, medio- e lungo-termine nel topo CD-1"

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# Sintesi

Lo studio oggetto della presente tesi di Dottorato è stato svolto nell'ambito del progetto di ricerca "*Neurobehavioural effects of gravitational environment in developing mice*", convenzione tra Istituto Superiore di Sanità e Agenzia Spaziale Italiana (ASI; Sezione Scienze della Vita), in collaborazione con l'Istituto di Neurobiologia e Medicina Molecolare del CNR/EBRI di Roma. L'obiettivo principale di tale progetto è la caratterizzazione e la validazione di un modello animale di riferimento per la valutazione degli effetti indotti dall'esposizione ad alterazioni nell'ambiente gravitazionale, potenzialmente trasferibile nell'ambito degli esperimenti previsti a bordo della Stazione Spaziale Internazionale.

Con l'assemblaggio della Stazione Spaziale Internazionale (ISS) orbitante intorno alla Terra, le missioni spaziali sono diventate più frequenti e la permanenza degli astronauti nello spazio più lunga. Inoltre, sebbene si sia ancora nella fase iniziale dell'esplorazione robotica di Marte, non è improbabile che l'uomo arrivi a colonizzare questo pianeta nel giro di alcune generazioni. Si prospetta pertanto una realtà che in pochi decenni vedrà la presenza permanente dell'uomo nello spazio, su Marte, o impegnato in missioni spaziali di lunga durata. Per il raggiungimento di tali obiettivi è tuttavia necessario approfondire le ricerche scientifiche sui possibili rischi associati a esposizioni all'ambiente spaziale (quali per esempio l'esposizione ai raggi cosmici o a prolungati periodi di microgravità), al fine di mettere a punto adeguate contromisure che salvaguardino la salute degli astronauti durante le missioni spaziali.

La mole crescente di dati provenienti da ricerche condotte nell'ambito della biologia spaziale ha evidenziato come la forza di gravità svolga un ruolo cruciale in molti processi biologici. Tra i vari sistemi biologici, il sistema nervoso – il cui corretto sviluppo e funzionamento sono prioritari sin dalle primissime fasi di vita per la successiva sopravvivenza dell'animale – è noto per essere particolarmente sensibile ad alterazioni dell'ambiente di sviluppo. La possibilità che alterazioni nell'ambiente gravitazionale, o più in generale che esposizioni all'ambiente spaziale,

possano interferire con i "normali" percorsi ontogenetici del sistema nervoso centrale e periferico è di particolare rilevanza nello studio della neurobiologia dello sviluppo. La valutazione degli effetti dell'esposizione all'ambiente spaziale sul sistema nervoso e sul comportamento, oltre a essere necessaria per permettere la permanenza dell'uomo e di altre specie animali nello spazio, rappresenta un'opportunità unica di approfondire la comprensione dei fenomeni alla base dello sviluppo del sistema nervoso e della sua plasticità, in termini di risposta adattativa a cambiamenti nell'ambiente esterno. L'approfondimento di tali conoscenze potrebbe inoltre rivelarsi assai utile per la prevenzione e il trattamento di psicopatologie e malattie neurodegenerative, comprese quelle forme reversibili di patologie associate alla "senescenza accelerata", sperimentata dagli astronauti durante le missioni spaziali.

In tale contesto, gli studi a terra, che utilizzano il paradigma dell'esposizione a ipergravità acuta su modelli animali, rappresentano un prerequisito essenziale per il successo degli esperimenti condotti in ambiente spaziale. Il progetto sviluppato nel presente studio si inserisce nell'ambito di tali attività di ricerca. In particolare, è noto che variazioni nell'ambiente gravitazionale possono indurre nei mammiferi - uomo compreso - la sindrome da *motion sickness* (MS; nausea, vomito, spossatezza e senso di malessere generalizzato), innescata come conseguenza di un "conflitto sensoriale" che coinvolge il sistema vestibolare. Tale sindrome si manifesta quando le informazioni sensoriali che riguardano la posizione del corpo nello spazio sono contraddittorie o differenti da quelle predette sulla base dell'esperienza. Durante le missioni spaziali, gli astronauti possono sperimentare un disturbo analogo, che talvolta può debilitare al punto da compromettere l'esito della missione. Sebbene la MS sia stata ben caratterizzata nell'uomo e nei roditori quali ratto e topo, i dati di letteratura riguardanti differenze di sesso sulla vulnerabilità a tale sindrome sono piuttosto scarsi e spesso contrastanti. Inoltre, la suscettibilità all'insorgenza della MS, e - più in generale - la vulnerabilità del sistema nervoso centrale a modificazioni nel campo gravitazionale, cambia durante lo sviluppo. Pertanto, ulteriori ricerche

potrebbero contribuire a caratterizzare fasi ontogenetiche di particolare vulnerabilità e/o resistenza a modificazioni del campo gravitazionale.

Scopo di questa tesi è stato quindi quello di caratterizzare gli effetti prodotti dell'esposizione a *2g* di ipergravità in esemplari maschi e femmine di topo CD-1, sia durante la fase adulta (4 mesi di età) sia durante le fasi ontogenetiche precoce (2-9 giorni di età) e tardiva (28, 42 e 60 giorni di età). In tali studi, l'ipergravità è stata prodotta sperimentalmente mediante rotazione degli animali in un'apposita *facility*: la centrifuga. In particolare, sono stati caratterizzati gli effetti dell'ipergravità sulla risposta comportamentale di breve-, medio- e lungo-termine del topo CD-1. Inoltre, sono stati misurati i livelli di neurotrofine *Nerve Growth Factor* (NGF) e *Brain Derived Neurotrophic Factor* (BDNF) in specifiche aree cerebrali, al fine di correlare eventuali modificazioni nel profilo comportamentale con cambiamenti nei livelli cerebrali di tali neurotrofine, il cui ruolo nei fenomeni di plasticità del sistema nervoso centrale (soprattutto durante lo sviluppo), che sottostanno alla risposta di adattamento allo stress, è stato ampiamente documentato.

I risultati di tali esperimenti evidenziano come l'esposizione a 2*g* di ipergravità inducano cambiamenti comportamentali e neurobiologici sottili ma rilevanti sia nella fase adulta sia nella fase dello sviluppo post-natale del topo CD-1. In generale, i risultati di questo studio confermano ed estendono i dati disponibili in letteratura sulla risposta neurocomportamentale dei roditori all'esposizione a stimoli ipergravitazionali. In particolare, oltre alle alterazioni del campo gravitazionale, lo stress associato alla rotazione *per se*, così come pure l'adattamento e i cambiamenti plastici in specifiche aree cerebrali, potrebbero contribuire significativamente a modificare il profilo comportamentale del topo CD-1.

Pertanto, il topo rappresenta un modello particolarmente adatto per future ricerche di biologia spaziale, finalizzate alla comprensione delle conseguenze neurobiologiche e comportamentali dell'adattamento e il riadattamento a differenti ambienti gravitazionali.

# GENERAL INTRODUCTION

## 1.1 Space biology

Space exploration dates back officially to October 4, 1957, with the launch of the first artificial satellite orbiting around the Earth, the Soviet Sputnik 1. One month later (November 3, 1957), the first living creature was launched into the space aboard the Soviet Sputnik 2, the dog Laika. Six other dogs were sent into space between 1960-1961. This early series of animal flights demonstrated that short-term spaceflights were safe from a biological and medical perspective, and prepared the way for the first human space mission by Yuri Alekseyevich Gagarin, who was launched aboard Vostok-1 on April 12, 1961 (West, 2000; Morey-Holton et al., 2007).

Physiological measurements assessed during early animal and human spaceflights were relatively crude, mainly focused on survival, safety, and performance. Nevertheless, data collected from these early space biology's studies opened a new challenge: the moon exploration (Apollo Lunar Missions, December 1968-December 1972; Morey-Holton et al., 2007).

Over the past 30 years the technology and the degree of methodological sophistication have greatly progressed, culminating in the US Spacelab studies of the 1990s. The US Spacelab program has enabled high-caliber research in microgravity. Main goal of these Shuttle Spacelab studies has been gain insight into physiological adaptation to space environment. Eleven Spacelab missions were flown over a period of 15 years (from 1983 to 1998), and numerous experiments were performed involving research in different disciplines, such as cardiovascular/cardiopulmonary, musculoskeletal, endocrine, cell biology and regulatory physiology. In particular, the Neurolab (STS-90), launched on April 1998, was a dedicated 16-days Life Sciences mission that focused exclusively on neuroscience research. The Neurolab mission has represented a milestone in the space neurosciences history, which has allowed the collection of a great number of precious data concerning the effects of spaceflight exposure on nervous system.

With the assembly of the International Space Station (ISS) long-duration space travels have become more frequent, ISS being a permanent outpost orbiting around the Earth, where astronauts could reside for long periods of time. Indeed, since November 2000, the ISS is continuously inhabited by at least 2 astronauts, which are replaced by a new space-crew about every 6 months (see Figure 1).

**Figure 1:** The International Space Station (ISS) orbiting around the Earth

Today, we are at the threshold of manned exploration of planets in our solar system, beginning with Mars (Kalb and Solomon, 2007). Therefore, it is likely that within few decades we could assist to a permanent presence of human in the space, and the colonization of Mars represents a realistic prospect.

However, to achieve these goals, if on the one hand technologic advances in material sciences, robotics, and power generation will be essential; on the other hand, scientific researches on the risks to humans experiencing space environment (i.e. exposure to cosmic rays or to prolonged period of weightlessness) will be also increasingly important, to

setting up adequate countermeasures to protect human health during space missions.

A great number of experimental results have been accumulated during more than 40 years of research in space biology, thought only about 400 human beings have really experienced the space environment.

Indeed, from a biological perspective, long-duration spaceflights beyond low Earth orbit represents a unique challenge. The effects of spaceflight on biologic systems are profound (Figure 2), and increased research into the responses and adaptations of life to prolonged spaceflight is essential for the success of the future space explorations (Kalb and Solomon, 2007).

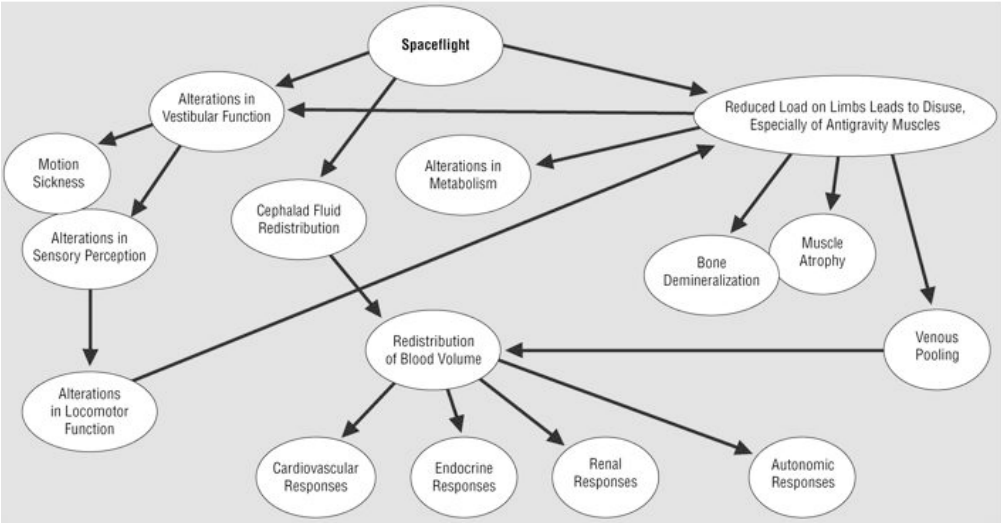


**Figure 2:** Overview of physiologic responses to spaceflight. From Kalb and Solomon, 2007

In particular, the effects of spaceflight on nervous system could have important implications for prolonged stay outside Earth's gravitational field, i.e. in the case of Mars exploration.

To this purpose, it is important to improve the knowledge on the influence of altered gravitational environments on central nervous system and behaviour. In this view, ground-based and space research using animal models represent useful tools to investigate the impact of gravity (hypergravity, microgravity and weightlessness) on nervous system. Data

coming from these studies could provide insight into basic biological phenomena underlying the plasticity of the nervous system and its adaptive responses to a changing environment.

Indeed, the nervous system is renowned to be extremely vulnerable to environmental changes, especially during its development, being characterised by a high degree of plasticity (see for example Andersen, 2003).

A number of data on the plasticity of the nervous system have been derived from experiments where subjects underwent sensorial deprivation during critical developmental periods (Hubel and Wiesel, 1970). The microgravity environment offers an important opportunity for studying developmental neurobiology representing both a deprivation (lack of gravitational attraction) as well as enrichment, animals free-floating in a three-dimensional space. This environment represents a unique situation to investigate some aspects of neurobehavioural development as well as offering a powerful model to study possible rearrangements, both at the nervous system and behavioural levels by selective alteration of environmental parameters.

In this context, numerous National Space Agencies are financing scientific projects (including both ground-based and space research) in view of the next spatial missions.

## 1.2 The importance of animal models in space biology studies

Animals in space have contributed significantly to our understanding of the effects of gravity on living system including humans. After the dog Laika, a great variety of both vertebrate (tadpoles, frogs, rats, mice, etc.) and invertebrate (flies, scorpions, protozoa, etc.) species have been launched into space (Gazenko and Ilyn, 1986; Snetkova et al., 1995; Ronca, 2003).

Mammal models represent a critical element for research in space biology. In particular, among rodents, the laboratory mouse is an eminently suitable animal model for research in space. Indeed, mouse is a smaller-

sized species than the rat; its biology is well known; and it is available in a variety of genetically modified strains. Moreover, mice reportedly tolerate chronic changes of gravity (i.e. hypergravity) better than rats (see for example Oyama and Platt, 1967; Figure 3).

**Figure 3:** Swiss CD-1 outbred mouse. The laboratory mouse is an eminently suitable animal model for research in space biology

The possibility that changes in gravitational field could interfere with nervous system ontogenesis is of particular relevance for studies in developmental neurobiology.

Indeed, as previously explained, studying the effects of exposure to altered gravitational environments on nervous system, besides acquisition of knowledge relevant for spaceflights and prolonged permanence of both humans and animals in space, offers a unique opportunity to gain insight in the developing nervous system, particularly, in the relation between stimulus and response, which can be plastically modified.

Further understanding of basic processes underlying the development of the nervous system, with a special emphasis on brain plasticity, might be relevant for the prevention and treatment of psychopathologies and neurodegenerative diseases. Data coming from these studies could result useful for the prevention and treatment of those reversible forms of mood disorders and neurodegenerative syndromes related to a rapid ageing process experienced by astronauts during space missions (Clément and Reschke, 1996; Wassersug, 1999; Strollo, 2000).

Indeed, changes in behavioural and neurobiological parameters assessed in animals exposed to hyper- or microgravity during different developing phases, could integrate data concerning the “accelerated ageing processes” and the “reversible ageing processes” displayed by astronauts

during and after spaceflight respectively (Clément and Reschke, 1996; Wassersug, 1999; Strollo, 2000).

## 1.3 Ground-based studies: the paradigm of hypergravity exposure by using the centrifuge device

Ground-based studies represent an essential prerequisite for the success of flight experiments, often offering also some advantages in comparison with research in space. Indeed, these kinds of studies can be conducted for a small fraction of the cost of research in space. They allow to setting up experimental protocols, also encouraging the production of dedicated (*ad hoc*) hardware and software systems to collect and transfer data at a distance.

The international space organizations are greatly encouraging ground-based experiments, also to promote a more efficacious collaboration and coordination between the research units involved in research aboard the ISS.

Reproducing environments that simulate on the Earth (Earth's gravitational field matches to 1*g*) microgravity or weightlessness condition is practically impossible. Thus, ground experiments employing animal models use paradigms significantly validated by scientific literature, such as the "tail suspension" paradigm, to mimic solicitations induced by exposure to microgravity or weightlessness, and the hypergravity exposure paradigm, hypergravity being rotationally-induced by the centrifuge device (Walton et al., 1997; Walton, 1998; Le Bourg, 1999).

The hypergravity rotationally-induced is the most common used paradigm to study the effects of exposure to changes in gravitational field on animal models (Le Bourg, 1999; see Figure 4 and 5). This kind of ground-based experiments use *g*-loads generated by centrifugation with the expectation that behavioural and physiological reactions to hypergravity help to explain reactions to the microgravity challenge faced by orbiting animals (Burton, 1999; Le Bourg, 1999).

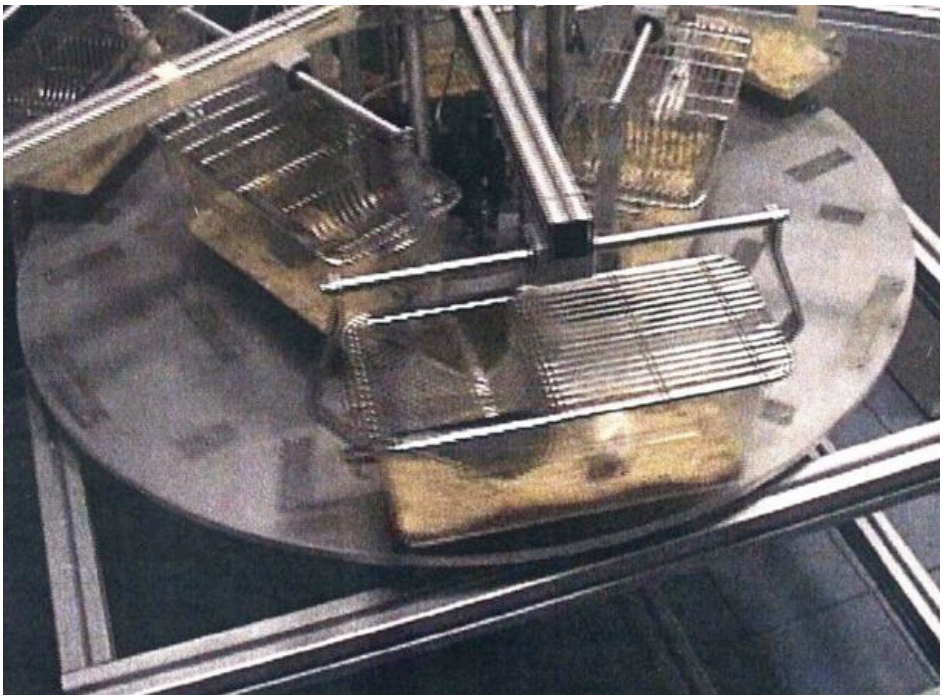

**Figure 4:** The centrifuge facility at the "Istituto Superiore di Sanità", Rome, I-00161, Italy (designed and manufactured by "Isolceram", Rocca Priora, Rome, I-00040, Italy)

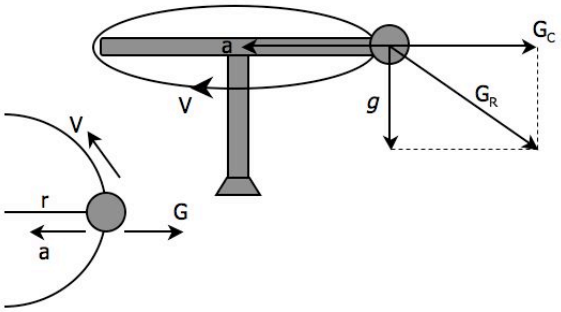


**Figure 5:** Diagram showing physics of acceleration. r, radius of rotation; V, rate of motion (velocity); a, acceleration (centripetal) force; $G_C$, inertial (centrifugal) force; *g*, Earth's gravitational constant; $G_R$, result G vector: $G_R = (G^2_C + g^2)^{1/2}$ (i.e. $g^2 = 1$). Modified from Burton, 1999

Indeed, a variety of studies indicate that researches using the hypergravity paradigm can be used to predict results of 0*g* (Burton, 1999). In particular, specific quantitative and qualitative relationships between the intensity of *g* field and biological processes have been described. Specifically, it is possible to develop continuous mathematical relationships between different levels of *g* (or gravity) and measured physiologic or anatomical changes. These relationships could be extrapolated mathematically to 0*g* thus providing a model that predicts the effects of weightlessness; i.e. separate the effect of body weight from body mass on physiologic processes and anatomic structures (Burton, 1999; Figure 6). Moreover, the theoretical "continuity principle" provides a physical basis for this concept. Indeed, the "continuity principle" states that acceleration *g* fields are continuous above and below Earth's gravity and that biological responses exhibit a similar continuity (Burton, 1999).

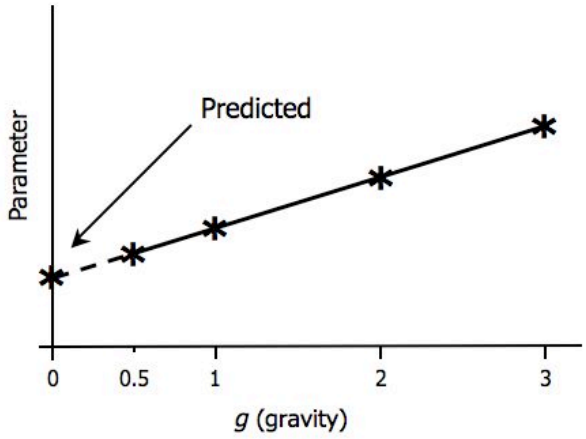


**Figure 6:** The parameter is a function of *g* (gravity). Parameter at 0*g* is only mass dependent in the absence of weight from gravity. Modified from Burton, 1999

## 1.4 Effects of changes in gravitational field on vestibular system: the motion sickness syndrome

With the advent of long-term interplanetary missions, involving increasing numbers of people, space biology is becoming an emerging area of research devoted to assess the effects of altered gravity conditions on human health. The necessity to develop reliable animal models to protect human health has led to a variety of animal studies concentrating on motion sickness (MS) syndrome. Indeed, during space flights, astronauts may experience the so-called "Space Adaptation Syndrome" (Reschke et al., 1986; Homick et al., 1997), a related disorder to MS, which may highly debilitate astronauts compromising the success of the missions.

Exposure to altered gravity has been extensively reported to cause MS in mammals. MS has been described as an illness triggered by a "sensory conflict" involving the vestibular system, occurring when sensory inputs regarding body position in space are contradictory or different from those predicted from experience (for reviews see Money, 1970; Yates et al., 1998; Figure 7).

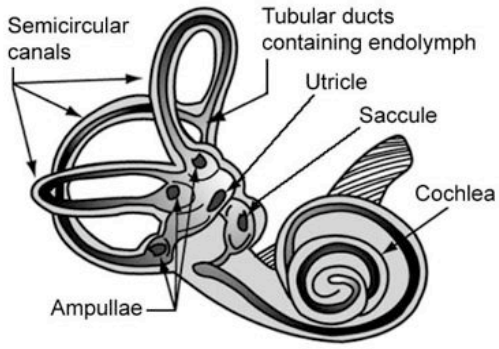


**Figure 7:** The vestibular system, which is key to our senses of balance, motion, and body position, is comprised of three *semicircular canals* connected to two membranous sacs called the *saccule* and *utricle*. The saccule and utricle are often referred to as the *otolith organs*. The otolith organs allow us to sense the direction and speed of linear acceleration and the position (tilt) of the head. The semicircular canals allow us to sense the direction and speed of angular acceleration. Fom NASA's *Educational Brief – The effects of space flight on the human vestibular system* (EB-2002-09-011-KSC), 2002

In a large variety of animal species MS is associated with the occurrence of an emetic response. However, many species, including rodents and rabbits, lack the ability to vomit and thus are unable to exhibit the most obvious indicator of MS (Borison, 1989; Yates et al., 1998). Mitchell and coworkers (1977a, b) demonstrated that rats engaged in anti-nausea pica behavior (the ingestion of significant amount of non-nutritive substances, such as kaolin, sawdust and wood) following exposure to hypergravity. They investigated the effects of rotation on postrotational consumption of food, water and kaolin (a hydrated aluminum silicate with a clay-like taste) and observed a significant increase from baseline in postrotational kaolin consumption. Since kaolin consumption in species incapable of emesis corresponded to several of the principle characteristics of MS exhibited by species capable of emesis, pica behaviour was validated as a quantitative index of MS.

MS has been recently characterized also in the mouse, a more suitable species for space missions. In this species acute exposure for 1h to rotationally-generated 2*g* induced pica behaviour and pronounced changes in specific behavioural endpoints (Santucci et al., 2000; Francia et al., 2004). Specifically, *open eyes resting* behaviour showed a characteristic profile over time: substantially absent before rotation, it appeared after 5 minutes of rotation and rapidly increased, lasting to very high levels throughout all hypergravity exposure and slowly decreasing only after rotation. These results indicate that in mice, in addition to pica behaviour, *open eyes resting* can be considered as a behavioural index of MS. Moreover, although both males and females experienced MS, males recovered faster than females (Santucci et al., 2000; Francia et al., 2004).

In this mouse model of MS, changes in mouse behavioural repertoire following hypergravity exposure appeared to parallel changes in brain levels of nerve growth factor (NGF; Santucci et al., 2000). Specifically, hypergravity exposure induced a markedly increase of NGF levels in both frontal cortex and hypothalamus, two brain area known to be related to MS syndrome (Santucci et al., 2000).

The few data available from the literature report sex differences in susceptibility to MS in Japanese house musk shrew (*Suncus murinus*) and humans (*Homo sapiens*), with females resulting, in general, more responsive than males to motion stimuli (Javid and Naylor, 1999; Dobie et al., 2001). Moreover, studies on humans indicate that although women show greater incidence in MS history, they did not differ from men in severity of symptoms (Jokerst et al., 1999; Park and Hu, 1999). A study on the role of women in military aviation underlines that although men are - on the average - larger, stronger, and more aerobically fit than women, there is great variability within each sex and a large overlap between the sexes. Gender differences disappear when allowance is made for size, strength, and fitness (Lyons, 1992). Overall, data regarding gender differences in vulnerability to MS are rather scarce and often also contrasting. Thus, differences in gender susceptibility to MS need to be further investigated.

Susceptibility to MS, and - more in general - the vulnerability of the vestibular system upon exposure to rotational stimuli, changes with age (Tyler and Bard, 1949; Money, 1970; McCaffrey et al, 1980; Sondag et al., 1997; Wubbels and de Jong, 2000; Bouët et al., 2003). In rats, effects of exposure to hypergravity result particularly marked just after birth and again at weaning. Recent data confirm a transient mild sickness associated with hypergravity, with a decrease in spontaneous activity in mice exposed to 2*g* on postnatal day 28 (Francia et al., 2004). Moreover, age and gender-specific changes in cerebellar structure as well as subtle alterations in vestibular functions have been reported both in adolescent and adult rats and mice early exposed to hypergravity (Oyama and Platt, 1967; Giménez y Ribotta et al., 1998; Baer et al., 2000; Bouët et al., 2004; Francia et al., 2004; Sajdel-Sulkowska et al., 2005; Sajdel-Sulkowska, 2007).

## 1.5 Effects of exposure to altered gravitational environments on central nervous system development

The developing nervous system is characterized by a marked degree of plasticity and environmental conditions during specific ontogenetic periods greatly influence its organization and function. The Earth's gravitational field represents a constant environmental force under which life has evolved so that its influence on the development of the nervous system results of crucial importance. Indeed, an increasing body of evidence both from ground-based and space research indicates that exposure to altered gravitational environment affects development of the vestibular system and its functions supporting the idea that changes in the gravitational environment, besides acquisition of knowledge relevant for spaceflight, may represent a tool to gain insight in the developing nervous system, particularly, in the relation between stimulus and response, which can be plastically modified.

In rodents, such as rats and hamsters, chronic prenatal or postnatal manipulations performed to reduce or increase gravity (micro or hypergravity, respectively) can induce deficiencies in water immersion righting performance, an impairment in surface righting and startle responses, difficulty to maintain the balance during swim and a decrease in both swimming and walking speed (Sondag et al., 1997; Fox et al., 1998; Walton, 1998; Ronca and Alberts, 2000; Wubbels and de Jong, 2000; Bouët et al., 2003; Bouët et al., 2004; Nguon et al., 2006).

Most of these motor deficits disappear after few days spent in normal gravity, while habituation phenomena in physiological parameters such as excreted corticosterone take place as early as few days of exposure (Ortiz et al., 1999). However, some anomalies in motor coordination and – in general – vestibular-induced behaviours persist into adulthood if animals are exposed to altered gravitational environments at "critical periods" of development. In particular, within chronic studies, Giménez y Ribotta and coworkers (1998) observed a substantial and persistent delay in the development of the cortical monaminergic projections to the spinal cord in

young rats exposed to hypergravity from day 11 of gestation to postnatal day 15 (PND 15). In rats, an altered cerebellar growth was observed following continuous exposure to hypergravity from gestational day 11 to PND 6 or PND 21 (Sajdel-Sulkowska et al., 2001), while a substantial impairment in motor coordination - accompanied by a marked decrease in cerebellar mass - was observed following exposure from PND 6 to 21 (Nguon et al., 2006; Sajdel-Sulkowska, 2007). However, discrete and sexually dimorphic windows of vulnerability of the rat's developing cerebellum were evidenced. Indeed, male rats were most dramatically affected by exposure to hypergravity during the second week of gestation (gestational day 8-15), when they showed a very poor motor coordination (Nguon et al., 2006; Sajdel-Sulkowska, 2007).

In addition, rats flown on the space shuttle (Neurolab) from PND 8 to PND 24 showed an abnormal development of extensor motor neurons (Inglis et al., 1999) and changes in the number and morphology of cortical synapses (DeFelipe et al., 2002), while appeared to use different search strategies in the early phases of a spatial learning task in the absence of clear deficits in the ability to use spatial information and to form memories of place (Temple et al., 2002). Changes in spatial learning as well as in exploratory behaviour have been also reported in periadolescent mice exposed to hypergravity (Francia et al., 2004), while spatial disorientation has been described in developing rat following hypergravity exposure or vestibular deprivation (Geisler et al., 1997; Bouët et al., 2003).

Thus, data here reviewed indicate the perinatal (both fetal and neonatal development) period as well as the periadolescent period as windows of heightened vulnerability of the developing central nervous system to changes in gravitational environment. Indeed, central nervous system is undergoing genetically programmed cell birth, proliferation, migration, synaptogenesis and cell apoptosis during the early phase of its development, and disturbances in any of these processes can affect its structure and functions (see for example Andersen, 2003). On the other hand, the late postnatal development corresponding to the adolescent phase overlaps with brain rearrangement spur, and although motor and sensory development at

this ages are almost concluded, modification in stressor-sensitive regions to environmental experience during adolescence might lead to substantial and long-lasting neurobehavioural alterations (Spear, 2000; Andersen and Theicher, 2008).

## 1.6 Neurotrophins as modulators of brain plasticity and coping response to stressful experience

Among the many modulators of the development of the nervous system nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) play a major biological role.

NGF and BDNF are well-studied neurotrophins involved in the neurogenesis, differentiation, growth and maintenance of selected peripheral and central populations of neuronal cells during development and at adulthood (Alleva and Francia, 2008; Cirulli et al., 2008a,b). In concert to hypothalamic-pituitary adrenal axis, NGF and BDNF play a key role in modulating brain plasticity and behavioural coping to stress, especially during ontogenetic critical periods, when developing brain is particularly sensitive to external stimulations. Indeed, it is now evident that these neurotrophins not only support neurogenesis, differentiation and survival of postmitotic neurons, but are important mediators of synaptic and morphological plasticity (Levi-Montalcini, 1987; Thoenen, 1995; Lewin and Barde, 1996). The expression of NGF and BDNF has been localised to the neocortex, hippocampus, and hypothalamus of both developing and adult central nervous system. Neocortex and hippocampus are brain regions related to cognitive functions, while hypothalamus is involved in the maintenance of the physiological homeostasis and in the adaptive response to stress (Mobley et al., 1986; Levi-Montalcini et al., 1990; Alleva et al., 1993). Stressful events can affects the expression of neurotrophins, which in turn act as transducers of the stressful stimuli on central nervous system mediating the behavioural coping response (Alleva et al., 1996). The expression of these neurotrophins and their receptors is developmentally

regulated with significant increases in their expression at times of maximal neuronal growth, differentiation and synaptogenesis (Castren et al., 1992; Thoenen, 1995; Smith 1996; Russo-Neustadt et al., 2001). Changes in the expression of these neurotrophins at critical times during development – as a consequence of a stressful experience - could promote a cascade of events interfering with maturations of specific brain area, setting the stage for altered responsiveness to stress at adulthood (Cirulli et al., 2003).

## 1.7 Aim of the thesis

Exposure to altered gravitational environments, especially during specific developmental periods, can enduringly affect central nervous system functions leading to stable changes in the behavioural response. In particular, previous studies indicate that adult mammals experience MS upon changes of gravitational field. Although MS has been characterized in both rat and mouse upon hypergravity exposure (Mitchell et al., 1977; Ossenkopp and Ossenkopp, 1985; Takeda et al., 1993; Santucci et al., 2000; Francia et al., 2004), data regarding gender differences in vulnerability to MS are rather scarce and often also contrasting. Thus, differences in gender susceptibility to MS need to be further investigated. Moreover, susceptibility to MS, and - more in general - the vulnerability of the central nervous system upon exposure to hypergravity stimuli, changes during development (Tyler and Bard, 1949; Money, 1970; McCaffrey et al, 1980; Sondag et al., 1997; Wubbels and de Jong, 2000; Bouët et al., 2003). Further investigations could help to better characterize developmental phases of vulnerability/resilience to altered gravity exposure (Oyama and Platt, 1967; Giménez y Ribotta et al., 1998; Baer et al., 2000; Bouët et al., 2004; Francia et al., 2004; Sajdel-Sulkowska et al., 2005; Sajdel-Sulkowska, 2007).

The present study attempted to refine a mouse model to study the short-, medium- and long-lasting effects of altered gravity exposure on behavioural and neurobiological parameters. The ultimate goal of this

project was the smooth transfer of this animal model from ground-based settings to weightlessness aboard of the ISS. To this purpose, behavioural and neurobiological studies on both adult and developing mouse were performed upon acute exposure to 2*g* rotationally-generated hypergravity, which generally mimics some effects of launch into the space (Le Bourg, 1999).

*Objective 1*: (Chapter 1) We provided a detailed characterization of the behavioural responses of adult CD-1 mice of both sexes exposed to acute 2*g* hypergravity. In particular, to evaluate sex differences in MS vulnerability, the expression of pica behaviour as well as general behavioural repertoire were assessed before, during and after 1h of exposure to 2*g* rotationally-generated hypergravity in both male and female mice. Moreover, in order to evaluate early onset of habituation phenomena, an additional group of mice was exposed for 2h to 2*g* hypergravity. The short-term effects of altered gravitational environment on mice explorative behaviour and emotional/anxiety responses were also investigated in the hole-board test and in a plus-maze apparatus, respectively 20min and 24h after the end of rotation.

*Objective 2*: (Chapter 2) Aim of the second part of the study was to investigate the short-, medium- and long-term consequences of repeated acute exposure to hypergravity during early postnatal development in mice. In this study the relevance of maternal experience on pups' neurobehavioural responses to altered gravitational stimuli were also assessed. To this purpose, CD-1 mouse pups of both sexes delivered by primiparous and biparous dams were exposed to 1h of 2*g* rotationally-induced hypergravity from PND2 to PND9 with their mothers. Sensorimotor responses and somatic growth were daily measured from PND2 to PND15. Ultrasonic vocalisations were recorded on PND2, 5 and 9, and homing behaviour was evaluated on PND12. In addition, spatial orientation ability was assessed in a T-maze on PND18, while mice exploratory behaviour and locomotor activity were evaluated in an open-

field test on PND21. Long-term effects of hypergravity exposure on both spatial learning (Morris water-maze test) and brain levels of NGF and BDNF were also investigated at adulthood.

*Objective 3*: (Chapter 3) Following the evaluation of the effects of early exposure to hypergravity, in this part of the research we aimed to establish age-related differences in the neurobehavioural response to hypergravity exposure during late postnatal development. Early adolescent (PND 28), adolescent (PND 42) and young-adult (PND 60) male and female CD-1 mice were exposed to acute 2*g* rotationally-generated hypergravity. MS index and behavioural performances before, during and after rotation were recorded, and long-lasting effects on exploratory behaviour (hole-board test) and emotional/anxiety-like responses (plus-maze test) were investigated. Furthermore, in order to correlate behavioural changes with alterations in central levels of neurotrophins, brain amounts of NGF and BDNF were also assessed on PND 90, following a re-exposure to hypergravity.

# CHAPTER 1

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# Behavioural responses to hypergravity in the CD-1 mouse

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**Abstract**

Male and female mice were subjected to rotationally-generated hypergravity of different duration (1 or 2 h) and linear acceleration (2 or 1.08 G). Pica behaviour and spontaneous activity were investigated before, during, and after rotation. Moreover, hole-board and plus-maze tests were performed 20 min and 24 h after the end of rotation. Pica behaviour arose in the post-rotation days and was more pronounced after 1 h of 2 G exposure. Spontaneous activity was almost or totally suppressed during rotation and failed to regain the pre-rotational levels in the group exposed to 2 G for 1 h. Exploratory behaviour in the hole-board was also impaired. A clear effect of hypergravity exposure emerged in the plus maze, with 2 G mice totalizing a minor number of arm entries than the other groups and also showing an altered emotional/anxiety profile. Generally, females were more susceptible than males to the changes in gravitational environment.


*Keywords:* Pica behaviour; Hypergravity; Mice; Spontaneous activity; Hole-board; Plus maze

## 1. Introduction

With the advent of long term interplanetary missions, involving increasing numbers of people, space biology is becoming an emerging area of research devoted to assess the effects of altered gravity conditions on human health. The necessity to develop reliable animal models to protect human health has led to a variety of animals studies concentrating on motion sickness (MS) syndrome (for reviews see [1,2]), which mimics a related disorder arising in astronauts during space missions (Space Adaptation Syndrome; [3–5]).

In laboratory rats and mice, species incapable of emetic response, pica behaviour (the consumption of non-nutritive substances such as kaolin), has been considered an appropriate index of MS [6–11].

The mouse, a much smaller-sized mammalian species than the rat, may be more suitable for space missions (standard habitat holding rack size in a shuttle or in the ISS is around 1000 $in^3$). In this species, besides inducing picaism, acute exposure for 1h to rotationally-generated 2 G hypergravity caused marked changes in the normal pattern of spontaneous activity [11] as well as a dramatic increase of neurotrophin levels in both frontal cortex and hypothalamus, two brain areas known to be involved in motion sickness syndrome [12].

The aim of the present study was to better characterise the behavioural responses of CD-1 mice after acute hypergravity exposure. MS was assessed both in male and female individuals since sex-dependent differences in mice pica behaviour has been previously found [11]. The short term effects of altered

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gravitational environment on mice explorative behaviour and emotional/anxiety responses were evaluated in the hole-board and in a plus-maze apparatus respectively 20 min and 24 h after the end of rotation.

Acute exposure experiments are generally aimed at gaining insight on the effects of short episodes of hypergravity that mimic what normally occurs at launch [13]. In this study, behavioural responses to hypergravity have been observed and used to have quantitative/qualitative measures of the response to the environmental challenge. Moreover, in order to evaluate early onset of habituation phenomena, an additional group of mice was exposed for 2 h to rotationally-generated hypergravity.

## 2. Materials and methods

### 2.1. Animals

Mice of an outbred Swiss-derived strain (CD-1) weighing 30–33 g (virgin males) or 28–30 g (virgin nulliparous females) were purchased from a commercial breeder (Charles River Italy, I-22050 Calco, Italy). Upon arrival at the laboratory, animals were kept in an air-conditioned room (temperature 21 ± 1 °C, relative humidity 60 ± 10%; lights on from 08:30 p.m. to 08:30 a.m.). Males and females were housed separately in groups of 5–7 in 42 × 27 × 14 cm Plexiglas boxes with sawdust as bedding. Pellet food and tap-water were continuously available. Ten days later, mice were randomly assigned to one of the following groups of 20 animals (10 males and 10 females): (1) stationary control (SC); (2) rotational control rotated at 1.08 G for 1 h (RC1); (3) rotational control rotated at 1.08 G for 2 h (RC2); (4) hypergravity rotated at 2 G for 1h (HG1); (5) hypergravity rotated at 2 G for 2 h (HG2).

### 2.2. Food and kaolin

Seven days before the rotation day (adaptation period) animals were singly housed in 33 × 13 × 14 cm in Plexiglas boxes with free access to pellet food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, I-20019, Italy) kaolin (Pharmaceutical grade kaolin hydrate aluminium silicate, Sigma, Milan, I-20151, Italy) and water. Kaolin pellets (prepared according to Mitchell et al. [7,8] and Santucci et al. [11]) were placed in the cages adjacent to food.

During the last 4 days of the adaptation period and for 5 days after rotation, food and kaolin were weighed daily at 11:30 a.m. to the nearest 0.1 g (to evaluate consumption rate) and refilled. Moreover, spilled food and kaolin pieces were collected, dried and weighed to obtain correct consumption values [6–8,14,15]. SC group for food and kaolin were placed close to the rotation apparatus during the rotation test (see below) where they were subjected to the noise and vibration of the turntable apparatus but not rotated.

### 2.3. Rotational device, procedures, and behavioural observations

The apparatus was a custom-made prototype designed and manufactured by Isolceram, Rocca Priora, I-00040, Italy, consisting of a turntable (radius = 50 cm) set in motion by a central rotor the number of turns of which could be adjusted by a digital switch. The turntable could hold up to six home cages and during the experiment it was rotated at the constant rate of 56 or 47.56 rpm, producing a resultant linear acceleration of 2 or 1.08 G, respectively.

After adaptation, HG and RC mice were rotated at 2 and 1.08 G, respectively for 1h (HG1 and RC1) or 2 h (HG2 and RC2), and their behaviours videorecorded both during, and for 1 h before and after rotation. After each rotational session, videotapes were scored by a highly trained observer using a dedicated ethological software [16]. The presence of the following behavioural items (see [11,17] for details) were recorded during 5 × 5 min-blocks (0–5, 10–15, 25–30, 40–45, 55–60) for each hour of videorecording.

*Bar holding*: grasping the metal top of cage holding itself above the level of the ground; *exploring*: moving around the cage, exploring the environment; *rearing*: standing on hind legs; *wall rearing*: standing on hind legs and placing forelimbs on the wall of the cage; *sniffing*: sniffing the environment; *resting* (either with *open* or *closed eyes*): no visible movements, eyes open or closed; *head moving*: moving the head up and down; *digging*: digging in the sawdust, pushing and kicking it around using the snout and/or both the forepaws and hindpaws, mostly moving around the cage and sometimes changing the whole arrangement of the substrate material; *grooming*: wiping, licking, combing any part of the body. Moreover, locomotor activity was evaluated: the cage was subdivided into four equal sections by lines placed on the video screen at the time of videorecording analysis, and the number of line crossings (with both forepaws) was counted.

### 2.4. Hole-board test

Twenty minutes after the end of post-rotation mice were tested in a hole-board apparatus. Testing was

carried out under red light between 14:00 a.m. and 16:00 p.m. The hole-board (Ugo Basile, Biological Research Apparatus, Varese, I-21025, Italy) consisted of a square unwalled platform (40 × 40 cm), raised 11.5 cm off the floor, containing 4 × 4 equally spaced (7 cm) holes, each 1.5 cm in diameter. The mice were placed individually on the platform and their behaviours videorecorded for 7 min using a Sony VO-5630 apparatus equipped with CH-1400 CE videocameras for red light. Locomotor activity was scored by determining the number of sub-areas crossed, while the number of holes explored was recorded by counting *head-dippings*: scored when the head entered the hole at least up to eye level. The latency to the first *head-dipping*, the number of different holes visited and the number of visits to the four central holes were also recorded. Moreover, an index of exploratory activity was calculated by dividing the total number of visits by the number of visits to the four central holes [18].

### 2.5. Plus-maze test

One day after the rotation mice were tested in a plus-maze apparatus. Tests were conducted under red light between 14:00 a.m. and 16:00 p.m. The elevated plus-maze comprised two open arms (30 × 5 × 0.25 cm) and two closed arms (30 × 5 × 15 cm) that extended from a common central platform (5 × 5 cm). The apparatus was constructed from Plexiglas (black floor, clear walls) and elevated to a height of 60 cm above the floor level. Mice were individually placed on the central platform facing an open arm and allowed to freely explore the maze for 6 min. All sessions were videorecorded by a camera linked to a monitor and VCR (see hole-board test) in the adjacent room and, to avoid unnecessary distractions, the experimenters retreated to this location during testing. Videotapes were scored using the same software adopted for behavioural scoring on the rotational apparatus [16]. Behavioural parameters included both conventional spatiotemporal and ethological measures (according to [19,20]). Conventional measures were the frequencies of total, open and closed entries (arm entry = all four paws into an arm), % open entries [(open/total) × 100], and % time spent in open and closed parts of the maze [e.g. (time open/session duration) × 100]. Ethological measures included frequency and duration scores for *rearing* (vertical movement against the side and/or end of the walls; note that mice very rarely exhibit unsupported rearing), *immobility*, *grooming* (licking, scratching and washing of the head and body), *head-dipping* (exploratory movement of head/shoulders over the side of maze) and *stretched-attend postures* (SAP: exploratory posture in which the body is stretched forward and then retracted to the original position without any forward locomotion) [19,20]. Moreover, in view of the importance of thigmotactic cues to rodent exploration in the plus-maze [21], *head-dipping* and SAP were also differentiated as a function of their occurrence in different parts of the maze. Thus, the closed arms and center platform were designated as "protected" areas (i.e. offering relative security) and the "percent protected" scores for *head-dipping* and SAP calculated as the percentage of these behaviours displayed in the protected areas (e.g. [(protected SAP/total SAP) × 100]). Moreover, as an additional index of animal hypergravity-induced anxiety, has been calculated by the latency to the first entry in the closed arm.

### 2.6. Statistical analyses

Kaolin and food consumption were analysed by a mixed-model ANOVA considering treatment and sex as grouping factors and pre and post-rotational days as repeated measures.

Kruskal–Wallis non-parametric ANOVA was performed on the behavioural parameters separately for pre-rotation, rotation 1st hour, rotation 2nd hour and post-rotation measures. The $\chi^2$ partitioning was then applied to each variable to test the main effects and the interaction of rotation length (when appropriated), hypergravity level and sex (each with one degree of freedom).

Kruskal–Wallis non-parametric ANOVA was also applied to hole-board and plus-maze performances, considering the 10 groups of mice, that is the control (males and females) and the four rotated ones (males and females). The $\chi^2$ partitioning was subsequently used to test the difference between control mice on one hand and the groups of rotated mice on the other hand. The main effects of the length of rotation and the hypergravity level and their interactions within the groups of rotated mice, and the main effect of sex and its interactions with the other factors have been also reported.

## 3. Results

### 3.1. Food and kaolin

Increase in kaolin consumption, but not in food, was evident in post-rotational days in rotated animals (treatment × repeated measures, $F_{(4,90)} = 2.34$; $p = 0.06$; Table 1). In particular, in the 1st day after rotation, all

Table 1
Mean (± SE.) consumption of kaolin in CD-1 mice pre- and post-exposure to 2 G

| | Pre-rotation | | | | Post-rotation | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 1 | Day 2 | Day 3 | Day 4 |
| Stationary controls | | | | | | | | |
| Females | 0.14 ± 0.10 | 0.14 ± 0.10 | 0.03 ± 0.01 | 0.05 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.02 ± 0.00 |
| Males | 0.03 ± 0.01 | 0.12 ± 0.10 | 0.02 ± 0.00 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.00 | 0.01 ± 0.00 |
| 1h rotational controls | | | | | | | | |
| Females | 0.02 ± 0.00 | 0.03 ± 0.00 | 0.05 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 |
| Males | 0.03 ± 0.01 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.03 ± 0.01 | 0.02 ± 0.00 | 0.01 ± 0.00 |
| 1h hypergravity | | | | | | | | |
| Females | 0.24 ± 0.14 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.26 ± 0.15 | 0.14 ± 0.11 | 0.37 ± 0.17 | 0.15 ± 0.11 |
| Males | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.02 ± 0.00 | 0.02 ± 0.01 | 0.12 ± 0.09 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 |
| 2h rotational controls | | | | | | | | |
| Females | 0.12 ± 0.10 | 0.03 ± 0.01 | 0.12 ± 0.11 | 0.03 ± 0.01 | 0.23 ± 0.13 | 0.06 ± 0.01 | 0.05 ± 0.01 | 0.03 ± 0.01 |
| Males | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.00 | 0.03 ± 0.01 | 0.02 ± 0.00 |
| 2h hypergravity | | | | | | | | |
| Females | 0.14 ± 0.10 | 0.23 ± 0.14 | 0.03 ± 0.01 | 0.13 ± 0.10 | 0.23 ± 0.13 | 0.25 ± 0.13 | 0.16 ± 0.10 | 0.04 ± 0.01 |
| Males | 0.03 ± 0.01 | 0.12 ± 0.10 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.00 | 0.02 ± 0.01 |

Kaolin intake is shown as the difference between consecutive days. Rotational Controls, exposure to 1 G; Hypergravity, exposure to 2 G.

female groups, except SC and RC1, showed higher level of kaolin consumption, while only HG1 male showed a slight increase in kaolin consumption.

In the following days, HG females ate more kaolin than the other groups and on the last post-rotational day of observation only HG1 females were still eating more kaolin.

### *3.2. Behavioural observation (frequency of behavioural items are reported in Fig. 1A–B)*

#### *3.2.1. Bar holding*

During the 1st hour of rotation a main effect of sex ($S$) emerged, females performing *bar holding* more often and longer than males ($S$ (freq), $\chi^2_1 = 3.95$; $S$ (dur), $\chi^2_1 = 6.22$; $p < 0.05$; Fig. 1A; data not shown for durations). Moreover, a $S\times$ level of gravity ($G$) interaction also emerged: HG females showing a dramatic reduction of *bar holding* when compared with RC females ($S \times G$ (freq), $\chi^2_1 = 4.87$; $S \times G$ (dur), $\chi^2_1 = 4.42$; $p < 0.05$); a trend toward this difference was also observed in post-rotation ($S$ (freq), $\chi^2_1 = 3.31$; $p = 0.07$; $S$ (dur), $\chi^2_1 = 3.11$; $p = 0.08$). In post-rotation, an interaction between time of exposure ($T$) and $G$ was evident, with HG2 animals, but not HG1, recovering *bar holding* behaviour at the level of RC group ($T \times G$ (freq), $\chi^2_1 = 7.13$; $T \times G$ (dur), $\chi^2_1 = 4.8$; $p < 0.05$).

#### *3.2.2. Exploring*

Frequency of *exploring* behaviour was highly reduced in HG animals during the 1st hour of rotation ($G$ (freq), $\chi^2_1 = 20.87$; $p < 0.05$). Furthermore, HG females spent less time *exploring* the environment than RC ones ($S \times G$ (dur), $\chi^2_1 = 6.07$; $p < 0.05$). In post-rotation, frequency of *exploring* recovered to pre-rotation levels in all groups, except in the HG1 one ($T \times G$ (freq), $\chi^2_1 = 11.38$; $p < 0.05$; $T \times G$ (dur), $\chi^2_1 = 2.93$; $p = 0.09$). Moreover, 2 h rotated females showed higher levels of this behaviour than males ($T \times S$ (dur), $\chi^2_1 = 4.18$; $p < 0.05$; $T \times S$ (freq), $\chi^2_1 = 2.8$; $p = 0.09$).

#### *3.2.3. Rearing and wall rearing*

*Rearing* and *wall rearing* behaviours were overall reduced during rotation. However, a $G$ effect emerged, with HG group reducing these behaviours more consistently than RC during both the 1st hour (*rearing*: $G$ (freq), $\chi^2_1 = 11.28$; $G$ (dur), $\chi^2_1 = 5.93$; $p < 0.05$; *wall rearing*: $G$ (freq), $\chi^2_1 = 29.82$, $G$ (dur), $\chi^2_1 = 30.25$; $p < 0.05$) and the 2nd hour (statistically significant only for *wall rearing*: $G$ (freq), $\chi^2_1 = 6.71$; $G$ (dur), $\chi^2_1 = 6.99$; $p < 0.05$). A $T \times G$ effect was evident in post-rotation, with HG1 performing less *rearing* and *wall rearing* than HG2 group (*rearing*: $T \times G$ (freq), $\chi^2_1 = 13.02$; $T \times G$ (dur), $\chi^2_1 = 12.18$; $p < 0.05$; *wall rearing*: $T \times G$ (freq), $\chi^2_1 = 8.31$; $T \times G$ (dur), $\chi^2_1 = 4.28$; $p < 0.05$). A $T \times S$ trend for *rearing* ($T \times S$ (dur), $\chi^2_1 = 3.21$; $p = 0.07$) and a significant $T \times S$ interaction for *wall rearing* ($T \times S$ (freq), $\chi^2_1 = 3.74$; $p = 0.05$; $T \times S$ (dur), $\chi^2_1 = 4.14$; $p < 0.05$) emerged in post-rotation, females rotated for a longer time showing higher levels of vertical activity than the other rotated groups.

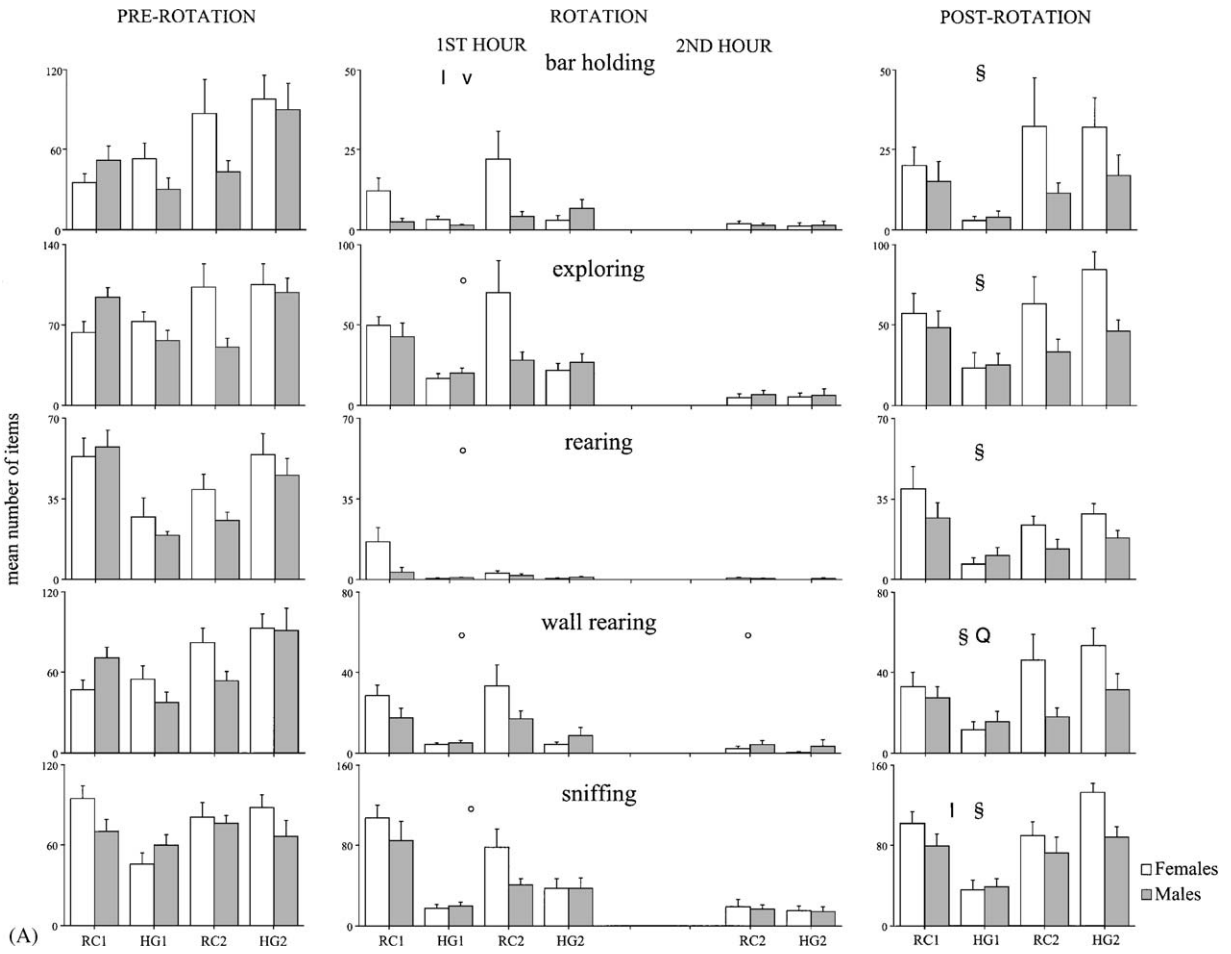


Fig. 1. Behavioural responses of male and female CD-1 mice occurring during 1 h pre-, 1 h or 2 h during and 1 h post-rotation periods. Significant effects ($p < 0.05$): I = main effect of $S$; ◯ = main effect of $G$; Δ = main effect of $T$; V = interaction $S \times G$; Q = interaction $T \times S$; § = interaction $T \times G$; $S$ = sex; $G$ = level of hypergravity (1 G; 2 G); $T$ = exposure duration (1 h; 2 h).

*3.2.4. Sniffing*

Frequency and duration of *sniffing* behaviour were reduced during rotation in HG groups ($G$ (freq), $\chi^2_1 = 28.64$; $G$ (dur), $\chi^2_1 = 21.69$; $p < 0.05$). A $T \times G$ effect was evident in post-rotation, HG1 mice performing less and shorter and HG2 more and longer *sniffing* than the other groups ($T \times G$ (freq), $\chi^2_1 = 17.04$; $T \times G$ (dur), $\chi^2_1 = 6.26$; $p < 0.05$). Moreover, in post-rotation females sniffed the environment more often than males ($S$ (freq), $\chi^2_1 = 4.61$; $p < 0.05$) and this effect was more evident in animals rotated for 1 h.

*3.2.5. Open eyes resting*

This behaviour appeared at the time of rotation and was more pronounced in HG mice than in RC ones ($G$ (freq), $\chi^2_1 = 3.3$; $p = 0.07$; $G$ (dur), $\chi^2_1 = 11.93$; $p < 0.05$; see Fig. 1B; data not shown for duration scores). Since the 1st hour of rotation a difference between males and females emerged; however, this difference became fully evident during the 2nd hour of rotation, when females showed higher values of both frequency and duration of *open eyes resting* than males (1st hour of rotation: $S$ (freq), $\chi^2_1 = 4.88$; $p < 0.05$; $S$ (dur), $\chi^2_1 = 3.89$; $p = 0.05$; 2nd hour of rotation: $S$ (freq), $\chi^2_1 = 11.1$; $S$ (dur), $\chi^2_1 = 11.8$; $p < 0.05$). Moreover, in post-rotation females rotated for 1h continued to rest more with eyes open ($T \times S$ (freq), $\chi^2_1 = 7.88$; $T \times S$ (dur), $\chi^2_1 = 5.24$; $p < 0.05$) than all the other experimental subjects.

*3.2.6. Closed eyes resting*

Overall, this behavioural endpoint considerably increased during rotation. In the course of the 1st hour of exposure RC groups performed *closed eyes resting* more and for a longer time than HG ones ($G$ (freq), $\chi^2_1 = 5.17$;

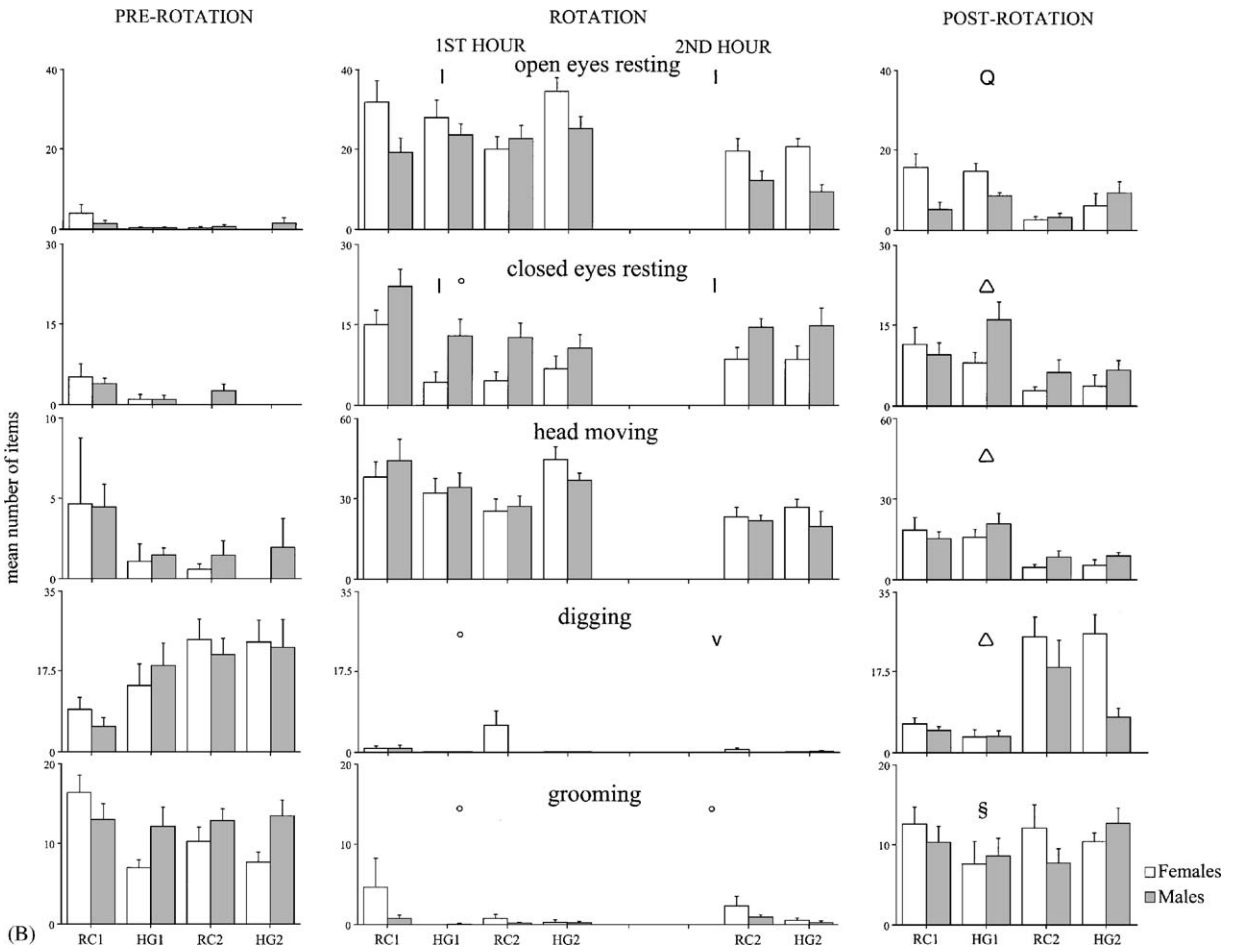


Fig. 1. (*continued*).

$p < 0.05$; $G$ (dur), $\chi^2_1 = 3.22$; $p = 0.07$). Sex differences were fully evident since the 1st hour of rotation throughout the end of the post-rotation phase, male resting with closed eyes more than females (1st hour of rotation ($S$ (freq), $\chi^2_1 = 11.54$; $S$ (dur), $\chi^2_1 = 14.6$; $p < 0.05$; 2nd hour of rotation: $S$ (freq), $\chi^2_1 = 5.19$; $S$ (dur), $\chi^2_1 = 14.14$; $p < 0.05$; post-rotation: $S$ (freq), $\chi^2_1 = 3.68$; $p = 0.05$; $S$ (dur), $\chi^2_1 = 5.67$; $p < 0.05$). Moreover, in post-rotation animals rotated for 1 h rested longer and more often than those rotated for 2 h ($T$ (freq), $\chi^2_1 = 12.77$; $T$ (dur), $\chi^2_1 = 5.46$; $p < 0.05$).

### 3.2.7. Head moving

The frequency of this item steadily increased during rotation with HG showing higher level of this behaviour than RC (statistically significant only for the 1st hour of rotation: $G$ (dur), $\chi^2_1 = 24.75$; $p < 0.05$). In post-rotation phase, a clear $T$ effect emerged, mice rotated for 1 h performing *head moving* behaviour more often and for a longer time than mice rotated for 2 h ($T$ (freq), $\chi^2_1 = 18.15$; $T$ (dur), $\chi^2_1 = 13.16$; $p < 0.05$).

### 3.2.8. Digging

This behaviour dramatically decreased during rotation. In the course of the 1st hour of exposure, *digging* was totally suppressed only in the HG groups. ($G$ (freq), $\chi^2_1 = 4.75$; $p < 0.05$; $G$ (dur), $\chi^2_1 = 3.16$; $p = 0.08$). However, during the 2nd hour of rotation, a consistent reduction of this item was also observed in RC2 mice and particularly in males, which showed a complete suppression of this behaviour ($S \times G$ (freq), $\chi^2_1 = 4.36$; $S \times G$ (dur), $\chi^2_1 = 4.34$; $p < 0.05$). In post-rotation, a recover to pre-rotational levels was evident only in animals rotated for 2 h ($T$ (freq), $\chi^2_1 = 26.3$; $T$ (dur), $\chi^2_1 = 21.4$; $p < 0.05$). In particular, the trend was for females to exhibit this behaviour more often than males ($T \times S$ (freq), $\chi^2_1 = 3.63$; $p = 0.06$; $T \times S$ (dur), $\chi^2_1 = 3.04$; $p = 0.08$).

Table 2
Parameters of exploration of male and female CD-1 mice in a 7 min hole-board test

| | Latency to the first head-dipping[a] | Number of holes visited[a] | Number of visits to the central holes | Total number of visits/Number of visits to the central holes | Number of sub-areas crossed[a] |
|---|---|---|---|---|---|
| Stationary controls | | | | | |
| Females | 14.4 ± 4.3 | 15.4 ± 0.2 | 12.3 ± 1.6 | 3.5 ± 0.4 | 68.1 ± 4.3 |
| Males | 16.0 ± 4.4 | 15.1 ± 0.4 | 15.7 ± 2.5 | 2.7 ± 0.5 | 55.9 ± 6.2 |
| 1 h rotational controls | | | | | |
| Females | 12.7 ± 1.7 | 14.8 ± 0.4 | 11.6 ± 0.1 | 2.8 ± 0.4 | 76.0 ± 4.6 |
| Males | 18.7 ± 5.0 | 12.3 ± 0.9 | 8.2 ± 1.3 | 3.4 ± 0.8 | 74.0 ± 7.8 |
| 1 h hypergravity | | | | | |
| Females | 19.6 ± 5.0 | 14.6 ± 0.5 | 14.0 ± 2.5 | 3.7 ± 1.0 | 57.0 ± 4.6 |
| Males | 18.7 ± 4.9 | 14.0 ± 0.8 | 13.4 ± 2.4 | 2.9 ± 0.6 | 55.8 ± 3.2 |
| 2 h rotational controls | | | | | |
| Females | 32.6 ± 5.9 | 14.0 ± 0.5 | 10.9 ± 2.0 | 3.3 ± 0.5 | 64.2 ± 6.2 |
| Males | 18.3 ± 3.8 | 15.0 ± 0.2 | 11.1 ± 1.5 | 4.0 ± 0.6 | 74.2 ± 6.0 |
| 2 h hypergravity | | | | | |
| Females | 32.0 ± 6.6 | 14.2 ± 0.3 | 13.3 ± 1.8 | 2.6 ± 0.5 | 67.5 ± 6.3 |
| Males | 30.6 ± 8.6 | 14.1 ± 0.6 | 13.6 ± 2.8 | 3.3 ± 0.7 | 72.3 ± 6.1 |

[a]Rotated mice vs. stationary controls, $p < 0.05$. Rotational control, exposure to 1 G; hypergravity, exposure to 2 G.

#### 3.2.9. Grooming

This behaviour was highly suppressed in both the 1st and 2nd hour of rotation in HG mice (1st hour of rotation: $G$ (freq), $\chi^2_1 = 9.96$; $G$ (dur), $\chi^2_1 = 10.52$; $p < 0.05$; 2nd hour of rotation: $G$ (freq), $\chi^2_1 = 6.24$; $G$ (dur), $\chi^2_1 = 6.93$; $p < 0.05$). In post-rotation phase, *grooming* reappeared reaching the pre-rotation levels in all groups, except in the HG1 one ($T \times G$ (freq), $\chi^2_1 = 4.08$; $p < 0.05$).

#### 3.2.10. Locomotor activity

In pre-rotation female were more active than males ($S$, $\chi^2_1 = 7.16$; $p < 0.05$). During the 1st hour of rotation HG mice locomoted less than RC ones ($G$, $\chi^2_1 = 19.9$; $p < 0.05$); however, HG females continued to be more active than HG males ($G \times S$, $\chi^2_1 = 4.14$; $p < 0.05$). Locomotor activity was deeply depressed in the 2nd hour of rotation in RC and HG groups, while in post-rotation, with the exception of HG1 group ($T \times G$, $\chi^2_1 = 11.22$; $p < 0.05$), it quickly recovered with females being more active than males in all groups (data not shown).

### 3.3. Hole-board test

As a whole, rotated animals had longer latencies to the first *head-dipping* than SC ones ($\chi^2_1 = 0.29$; $p < 0.05$) and this was true in particular for RC2 females and both HG2 females and males ($T \times S$, $\chi^2_1 = 6.65$; $p < 0.05$; see Table 2). Rotation per se also affected the number of holes visited, RC mice visiting a minor number of holes than SC ones ($\chi^2_1 = 8.1$; $p < 0.05$). No differences were observed in the number of *head-dippings* in the four central holes and in the ratio total number of visits/number of visits to the four central holes. As for the locomotory activity, measured as number of sub-area crossed, a $T \times S$ effect was evident, females rotated for 2 h being less active than males ($T \times S$, $\chi^2_1 = 4.32$; $p < 0.05$).

### 3.4. Plus-maze test

HG mice entered less often than either RC or SC in both open and closed arms (open arms: $G$ (freq), $\chi^2_1 = 3.74$; $p < 0.05$; closed arms: $G$ (freq); $p < 0.05$). Moreover, with the exception of HG1 group, males were always more active than females, and entered the open arms more often than females ($T \times G \times S$ (freq), $\chi^2_1 = 5.03$; $p < 0.05$; see Fig. 2). A $T \times S$ interaction was evident for both frequency and duration of *rearing*, *head-dipping* and *SAP* behaviours (*Rearing*: $T \times S$ (freq), $\chi^2_1 = 22.05$; $T \times S$ (dur), $\chi^2_1 = 15.97$; $p < 0.05$; *Head-dipping:* $T \times S$ (freq), $\chi^2_1 = 10.44$; $T \times S$ (dur), $\chi^2_1 = 11.22$; $p < 0.05$; *SAP*: $T \times S$ (freq), $\chi^2_1 = 11.63$; $T \times S$ (dur), $\chi^2_1 = 6.75$; $p < 0.05$). In particular, RC2 and HG2 females showed reduced frequency and duration of *rearing* and *head-dipping* while consistently increased *SAP* (Fig. 2). A similar trend was also observed for the percentage of *SAP* performed in the protected areas ($T \times S$, $\chi^2_1 = 3.49$; $p = 0.06$). In addition, for this parameter a $T \times G$ trend also emerged ($T \times G$ (freq), $\chi^2_1 = 3.82$; $p = 0.05$) with HG mice performing *SAP* more than RC and SC.

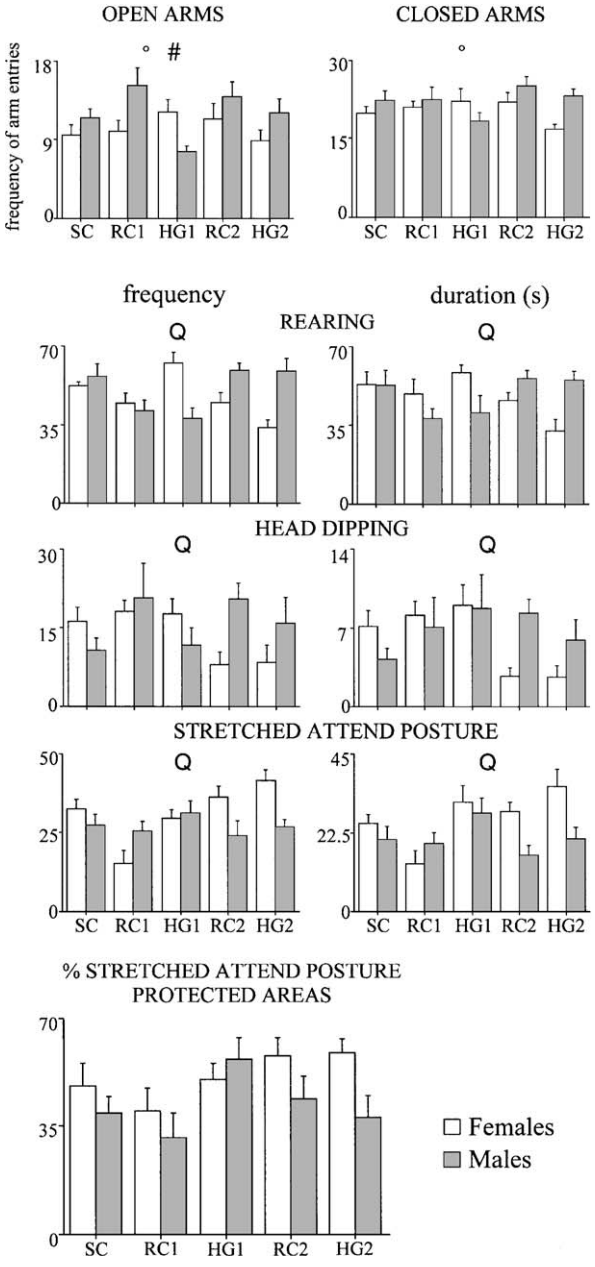


Fig. 2. Plus-maze performance of male and female CD-1 mice. Significant effects ($p < 0.05$): ◯ = main effect of $G$; # = interaction $T \times G \times S$; Q = interaction $T \times S$; $S$ = sex; $G$ = level of hypergravity (1 G; 2 G); $T$ = exposure duration (1 h; 2 h).

## 4. Discussion

The present results clearly indicate that rotation-induced hypergravity influenced the behavioural responses of CD-1 mice, reducing their spontaneous activity and concomitantly increasing their resting behaviour. According to our previous research, this reduction of spontaneous activity may be suggestive of animals experiencing motion sickness syndrome as a consequence of being subjected to rotational stimuli [11,22]. In fact, in the present study pica behaviour, a motion sickness index measured through the consumption of a non-food substance such as kaolin, showed a tendency to increase upon rotation, although the effect was not supported by a statistical significance. Pica behaviour is reportedly difficult to assess due to large interindividual differences in MS susceptibility [23], and this may be the reason for the lack of a clear-cut effect of rotational exposure on kaolin consumption. Animals rotated at 2 G for 1 h seemed the most affected, HG1 females being the only experimental group still eating kaolin in the 4 day after rotation and HG1 males the only male group showing an increase in kaolin intake. These effects confirm that females are more vulnerable than males to the symptoms associated with motion sickness, [11,22]. Moreover in accordance with the effects on behavioural responses (see below) they are suggestive of HG1 mice being less able to recover than HG2 animals.

According to our previous experiments [11] mice spontaneous activity was deeply affected by single hypergravity exposure, and, in most cases, the effects were independent of the exposure duration. Specifically, during rotation, grooming, digging, exploring and vertical activity were almost or totally suppressed in HG mice, while open eyes resting—which in mice has been previously described as a specific index of motion sickness [11]—increased. Interestingly, males spent more time doing resting behaviour than females since the 1st hour of rotation, while in females such behaviour only increased during the second hour of rotation. Moreover, in post-rotation, HG1 females persisted in showing high levels of this behaviour. These data are in accordance with those on pica behaviour pointing out that females are more susceptible than males to the onset of motion sickness.

It should be noted, however, that in HG2 animals the majority of behaviours regained the pre-rotational levels soon after the rotation was terminated, while failed to recover in HG1 mice. In particular grooming behaviour reappeared reaching the pre-rotation levels in all groups except in the HG1 one. Grooming behaviour in rats is a classical measure of displacement activity but also coincides with the period after arousal and rather reflects the process of dearousal due to habituation to a stressful situation [24]. Therefore the reappearance of pre-rotation behavioural profile in the groups of mice rotated for the longest time could be reasonably a consequence of habituation processes taking place during the 2nd hour

of rotation, reducing motion sickness and leading to a recovery in the behavioural profile [12]. This is particularly evident in females which recover better than males, as indicated, in post-rotation, by their higher levels of several behavioural endpoints, such as exploring, vertical activity, digging, and locomotor activity.

Mice exploratory behaviour in the hole-board was impaired, as indicated by a minor number of holes visited and a higher latency to the first head dipping, and the changes appeared to be a consequence of the exposure to the rotational stimuli per se.

By contrast, a clear effect of gravitational environment emerged in the plus maze with HG mice more often avoiding both open and closed arms than the other groups. In addition, they showed a marked tendency to display SAP behaviour especially in protected areas. These results, pointing to alterations in locomotor activity [25], reflects a reduced motivation to explore as a consequence of increased anxiety. SAP behaviour in the elevated plus maze has been related to risk assessment and defensive behaviours [26,27]. Since it has also been shown to be particularly responsive to various anxiolytic drugs, high levels of this response have been considered a reliable index of increased anxiety [25,28,29].

Therefore it appears that rotation *per se* represents a stressful condition affecting the level of exploratory behaviour while hypergravity acts as an additional stimulus selectively affecting their emotional-anxiety profile.

Females appeared generally more susceptible than males to the distressful stimuli associated with rotational/gravitational environment, although they turned out to recover faster than males. They also appeared more anxious than males. In fact, in the plus maze, 2 h-rotated females showed lower levels of rearing and head dipping and higher levels of SAP than males. As documented by an extensive literature on the subject [30–32], sex differences in response to environmental variables are found across species in many behavioural aspects. Such differences can be attributed to proximate (e.g. endocrine, genetic and environmental factors acting on behaviour) as well as ultimate mechanisms such as different adaptive strategies in coping with stressful situations [28,33].

Therefore it appears that exposure to rotation can highlight sex differences in susceptibility and copying to rotational stimuli which, in turn, might be reflected in subsequent behavioural responses.

As a whole the present data confirm the mouse as a good model for future space biology research aimed at understanding the behavioural consequences of the adaptation to different gravity environments. Further studies need to be developed using a protocol of chronic exposure to hypergravity, which may prove useful to assess the consequences of a prolonged permanence in space and to provide important insights about the effects of long-term exposure to altered gravity on adaptive behavioural responses.

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## References

[1] K.E. Money, Motion sickness, Physiological Reviews 50 (1970) 1–39.
[2] B.J. Yates, A.D. Miller, J.B. Lucot, Physiological basis and pharmacology of motion sickness: an update, Brain Research Bulletin 47 (1998) 395–406.
[3] J.L. Homick, M.F. Reschke, E.F. Miller, The effects of prolonged exposure to eightlessness on postural equilibrium, in: R.S. Johnston, L.F. Dietlein (Eds.), Biomedical Results from Skylab, Scientific and Technical Information Office, NASA, Washington, DC, 1997, pp. 104–112.
[4] W.H. Paloski, F.O. Black, M.F. Reschke, D.S. Calkins, C. Shupert, Vestibular ataxia following shuttle flights: effects of microgravity on otolith-mediated sensorimotor control of posture, The American Journal of Otology 14 (1993) 9–17.
[5] M.F. Reschke, D.J. Anderson, J.L. Homick, Vestibulo-spinal response modification as determined with the H-reflex during the Spacelab-1 flight, Experimental Brain Research 64 (1986) 367–379.
[6] D. Mitchell, C. Wells, N. Hoch, K. Lind, S.C. Woods, L.K. Mitchell, Poison induced pica in rats, Physiology and Behavior 17 (1976) 691–697.
[7] D. Mitchell, M.L. Krusemark, D. Hafner, Pica: a species relevant behavioral assay of motion sickness in the rat, Physiology and Behavior 18 (1977) 125–130.
[8] D. Mitchell, J.D. Laycock, W.F. Stephens, Motion sickness-induced pica in the rat, The American Journal of Clinical Nutrition 30 (1977) 147–150.
[9] K.P. Ossenkopp, M.D. Ossenkopp, Animal models of motion sickness: are nonemetic species an appropriate choice?, Physiologist 28 (1985) S61–S62.
[10] N. Takeda, M. Morita, A. Yamatodani, H. Wada, T. Matsunaga, Catecholaminergic responses to rotational stress in rat brain stem: implications for amphetamine therapy of motion sickness, Aviation Space and Environonmental Medicine 61 (1990) 1018–1021.
[11] D. Santucci, G. Corazzi, N. Francia, A. Antonelli, L. Aloe, E. Alleva, Neurobehavioural effects of hypergravity conditions in the adult mouse, Neuroreport 11 (2000) 3353–3356.

[12] N. Francia, D. Santucci, F. Chiarotti, E. Alleva, Cognitive and emotional alterations in periadolescent mice exposed to 2 *g* hypergravity field, Physiology and Behavior 83 (2004) 383–394.
[13] E. Le Bourg, A review of the effects of microgravity and of hypergravity on aging and longevity, Experimental Gerontology 34 (1999) 319–336.
[14] N. Takeda, S. Hasegawa, M. Morita, T. Matsunaga, Pica in rats is analogous to emesis: an animal model in emesis research, Pharmacology Biochemistry and Behavior 45 (1993) 817–821.
[15] N. Takeda, S. Hasegawa, M. Morita, A. Horii, A. Uno, A. Yamatodani, T. Matsunaga, Neuropharmacological mechanisms of emesis. I. Effects of antiemetic drugs on motion- and apomorphine-induced pica in rats, Methods and Findings in Experimental and Clinical Pharmacology 17 (1995) 589–590.
[16] L.P. Noldus, The observer: a software system for collection and analysis of observational data, Behavior Research Methods, Instruments, & Computers 23 (1991) 415–429.
[17] M.L. Terranova, G. Laviola, E. Alleva, Ontogeny of amicable social behavior in the mouse: gender differences and ongoing isolation outcomes, Developmental Psychobiology 26 (1993) 467–481.
[18] G. Calamandrei, S. Pennazza, L. Ricceri, A. Valanzano, Neonatal exposure to anti-nerve growth factor antibodies affects exploratory behavior of developing mice in the hole board, Neurotoxicology and Teratology 18 (1996) 141–146.
[19] C. Fernandes, S.E. File, The influence of open arm ledges and maze experience in the elevated plus-maze, Pharmacology Biochemistry and Behavior 54 (1996) 31–40.
[20] A. Holmes, R.J. Rodgers, Influence of spatial and temporal manipulations on the anxiolytic efficacy of chlordiazepoxide in mice previously exposed to the elevated plus-maze, Neuroscience and Biobehavioral Reviews 23 (1999) 971–980.
[21] D. Treit, J. Menard, C. Royan, Anxiogenic stimuli in the elevated plus-maze, Pharmacology Biochemistry and Behavior 44 (1993) 463–469.
[22] D. Santucci, G. Corazzi, N. Francia, A. Antonelli, L. Aloe, E. Alleva, Neurobehavioural responses to hypergravity environment in the CD-1 mouse, Journal of Gravitational Physiology 9 (2002) 39–40.
[23] S. Hasegawa, N. Takeda, M. Morita, A. Horii, I. Koizuka, T. Kubo, T. Matsunaga, Vestibular, central and gastral triggering of emesis, A study on individual susceptibility in rats, Acta Otolaryngologica 112 (1992) 927–931.
[24] B.M. Spruijt, J.A. van Hooff, W.H. Gispen, Ethology and neurobiology of grooming behavior, Physiological Reviews 72 (1992) 825–852.
[25] P.M. Wall, C. Messier, Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior, Neuroscience and Biobehavioral Reviews 25 (2001) 275–286.
[26] R.J. Rodgers, B.J. Cao, A. Dalvi, A. Holmes, Animal models of anxiety: an ethological perspective, Brazilian Journal of Medical and Biological Research 30 (1997) 289–304.
[27] M. Yang, H. Augustsson, C.M. Markham, D.T. Hubbard, D. Webster, P.M. Wall, R.J. Blanchard, D.C. Blanchard, The rat exposure test: a model of mouse defensive behaviors, Physiology and Behavior 81 (2004) 465–473.
[28] P. Palanza, Animal models of anxiety and depression: how are females different?, Neuroscience and Biobehavioral Reviews 25 (2001) 219–233.
[29] V. Kalueff, P. Tuohimaa, Experimental modelling of anxiety and depression, Acta Neurobiologiae Experimentalis 64 (2004) 439–448.
[30] J.A. Gray, Sex differences in emotional behaviour in mammals including man: endocrine bases, Acta Psychologica (Amst) 35 (1971) 29–46.
[31] J. Archer, Rodent sex differences in emotional and related behavior, Behavioral Biology 14 (1975) 451–479.
[32] R.W. Goy, B.S. McEwen, Sex differences in behavior: rodents, in: R.W. Goy, B.S. McEwen (Eds.), Sexual Differentiation of the Brain, The MIT Press, Cambridge, MA, 1980, pp. 13–73.
[33] A.I. Grigoriev, A.D. Egorov, I.A. Nichiporuk, Neurohumoral mechanism of space motion sickness, Acta Astronautica 17 (1988) 167–172.

# CHAPTER 2

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# Repeated acute exposures to hypergravity during early development subtly affect CD-1 mouse neurobehavioural profile

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**Abstract**

Exposure to altered gravitational environment, especially during critical ontogenetic phases, may induce persistent nervous system modifications and behavioural anomalies. This study evaluated the effects of hypergravity exposure on the development of the nervous system and assessed the relevance of parity in the mother's responses to altered gravitational stimuli. CD-1 mouse pups of both sexes delivered by primiparous and biparous dams were exposed to 1 h of 2 G rotationally induced hypergravity from PND2 to PND9. Sensorimotor responses and somatic growth were daily measured (PND2–PND15), ultrasonic vocalisations recorded on PNDs 2, 5 and 9, and homing behaviour evaluated on PND12. In addition, spatial orientation ability was assessed in a T-maze on PND18, while mice exploratory behaviour and locomotor activity were evaluated in an open-field test (PND21). Long-term effects of hypergravity exposure on both spatial learning (Morris water-maze test) and brain levels of NGF and BDNF were also investigated at adulthood. Rotation per se induced a delay in somatic growth, sensorimotor responses and ultrasonic vocalisation profile, while hypergravity highlighted sex differences in open-field behaviour. Strategies to solve a spatial learning task, rather than learning per se, were affected by early exposure to rotation, while hypergravity selectively altered behavioural profile in the reversal phase of the test. Early exposure to rotation per se also decreased hypothalamic BDNF levels, while hypergravity reduced NGF levels in the frontal cortex. Previous maternal experience did not interact with hypergravity exposure, while differences between offspring of primiparous and biparous dams were observed in sensorimotor development and exploratory behaviour.


*Keywords:* Rodents; Parity; Gravitational environment; Animal behaviour; Neurotrophines

## 1. Introduction

An increasing body of evidence indicates that exposure to altered gravitational environment during ontogeny causes anomalies in vestibular-induced behaviours, supporting the idea that changes in gravitational environment, besides acquisition of knowledge relevant for spaceflight, may represent a tool to gain insight into the mechanisms underlying the development of the nervous system. Although most of the locomotor deficits disappear after few days spent in normal gravity, some anomalies in vestibular-induced behaviours persist into adulthood if the exposure takes place during 'critical' periods of neurobehavioural development. Gimenez y Ribotta et al. [24] found a delay in the development of monoaminergic pathways in the spinal cord in young rats exposed to hypergravity (HG) from gestational day (GD) 11 to postnatal day (PND) 15. Moreover, rats exposed to rotational-generated hypergravity from GD 11 to PND6 or PND21 showed altered cerebellar growth [2,43], while rats flown in 1998 on the space shuttle (Neurolab) from PND8 to PND24 showed an abnormal development of extensor motor neurons [30] and changes in the number and morphology of cortical synapses [17].

Recent data emerging from the literature on early exposure to hypergravity with both acute or chronic paradigms are com-

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monly reporting subtle but consistent alterations in locomotion profile [6], cerebellar circuitry (Purkinje cells [5]), hormones and learning ability in rodents [11] adaptations to changes in gravity supposed to occur in central region of the nervous system.

In particular, Bouet et al. [7] found that hypergravity exposure during the fetal period, and varying period thereafter, induced a delay in both body growth and development of those reflexes, which are mainly dependent upon the vestibular system. After some time spent in normal gravity at PND40, HG rats regained a neurobehavioural profile comparable to that of control animals.

Mammalian development involves bi-directional linkages between mother and offspring [19]. Changes in the mother infant relationship can affect the function, structure and neurochemical architecture of the infant brain [27] and mother's behaviour exerts a regulatory influence on pup activity [28]. It is also known that experienced dams perform higher-quality maternal cares than naïve ones, are more responsive to pup stimuli and afford protection against neonatal losses during exposure to increased gravity [13,14,31,37,39,42].

The aim of the present study was to assess short-, medium- and long-term effects of repeated postnatal exposure to rotationally induced hypergravity on mouse neurobehavioural development. To this purpose, comparing the offspring of primiparous and biparous dams, we evaluated the emergence and maturation of several reflexes during the neonatal and the pre-weaning phases, the appearance of the homing behaviour which require adequate olfactory and motor capabilities [3] and the profile of pup ultrasonic emission on PNDs 2, 5, and 9 (around the time at which this important index of normal postnatal development reaches a peak in mice).

To assess the possible effects of hypergravity exposure on the cholinergic system maturation, pre-weaning mice (PND18) were also tested in a T-maze. In addition, since acute hypergravity exposure can affect the normal behavioural profile of adolescent mice [22], we also assessed mice spontaneous activity during such a critical postnatal phase (PND21).

The long-term effects of postnatal hypergravity exposure were evaluated in a Morris water-maze apparatus using the offspring of primiparous mice. Since changes in neurotrophin levels in the central nervous system upon exposure to rotational stimuli [2,30,43,45] have been reported and given the well-established role of NGF and BDNF in synaptic plasticity [33,50], it was considered of interest to investigate whether altered gravity would also exert long-term effects on such neurobiological determinants.

## 2. Material and methods

### 2.1. Animals

Mice of the outbred Swiss-derived CD-1 strain were obtained as young adults from Charles River Italia (Italy). On arrival they were housed in an air conditioned room maintained at 21 °C and 60% relative humidity on a 12/12-h reversed white-light/red-light cycle (red lights on at 08:30 h). Males and females were housed separately in standard wire-topped Plexiglas cages (42 cm × 27 cm × 14 cm) with up to six individuals per cage. Water and pellet food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, Italy) were available ad libitum.

For breeding, pairs of young adult females were housed together with one male in a home cage (33 cm × 13 cm × 14 cm) for 15 days. Pregnant females were thereafter housed singly and provided with 1.5 g of shredded tissue as nesting material. Dams were checked daily at 12:00 h for delivery (day of birth = postnatal day, PND, 0). Litters were culled to six pups (three females, three males), 24 h after parturition was noted. Biparous dams had completed one previous cycle of pregnancy and lactation at least 20 days before the start of the experiment.

The litters of 24 biparous and 24 primiparous dams were assigned to one of three treatment groups: 2 G hypergravity (HG), 1 G rotational control (RC) and stationary control (SC) ($n = 8$). One female and one male pup randomly selected from each litter was marked daily from PND2 with non-toxic ink for identification and these were the subjects in all subsequent tests.

All experimental procedures have been carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

### 2.2. Rotational device

The apparatus was a custom-made prototype designed and manufactured by Isolceram (Rocca Priora, Italy), consisting of six radial aluminium arms (50 cm long) mounted on a central rotor in the vertical axis of the centrifuge (see Fig. 1). Each arm was fitted with an adjustable bracket designed to hold a single cage of identical dimensions to the home cage (33 cm × 13 cm × 14 cm), such that it hung and swung freely under the arm. To minimize suffering in the delicate phase of lactation, the centrifuge was equipped with six wide-angle videocameras connected to a monitor and six videorecorders for online monitoring of animal behaviour. According to the protocol, the experiment was immediately interrupted when pup huddling (causing thermoregulatory impairment) or nursing by the dams were disturbed. Because of rotation, the centrifuge cage swung-out at a constant angle from the vertical direction, whose magnitude depended on the rotation speed. Since at a given rotation speed the distance along the arm of the bracket from the central rotor (corresponding to the axis of rotation) determined the force generated inside the centrifuge cage, during the experiment, brackets were arranged at two levels: 11 and 45 cm from the central rotor so that rotation at a constant rate of 50 rpm produced a resultant linear acceleration of approximately 1 G (1.09 G) inside the inner cages and approximately 2 G (1.85 G) inside the outer cages. The mice were totally free-moving in the centrifuge cages (Fig. 1).

The centrifuge design necessitated the transferal of animals from their home cage to a centrifuge cage for the duration of each session. To minimize the disturbance to the animals, centrifuge cages were lined with sawdust taken from their home cage. In order to allow adaptation to new environment, mother and pups were transferred to a centrifuge cage almost 1 h before the experimental session.

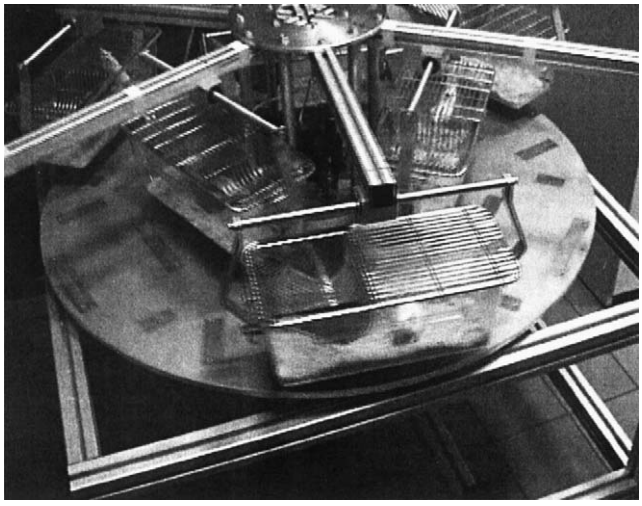

Fig. 1. The centrifuge facility. For a technical description see the text.

### 2.3. Hypergravity exposure

Pups were rotated for 1 h (between 14:00 and 17:00 h) on eight consecutive days (from PND2 to PND9). HG and RC litters were rotated simultaneously while SC litters were positioned on an adjacent surface where they were similarly exposed to the noise and environs of the centrifuge. Litters were transferred with their mothers to a centrifuge cage immediately before the hour of exposure and returned to their home cage immediately afterwards.

### 2.4. Assessment of somatic growth and sensorimotor development

Twelve sensorimotor responses and six measures of somatic growth were recorded for each pup daily from PND2 to PND15 (except PND12). All tests were conducted between 11:00 and 14:00 h by an experimenter blind to the treatment group, following a procedure indicated in Fox [20] and Bignami et al. [4].

Sensorimotor responses, in the order in which they were assessed, were as follows:

- *Righting*: when pup is placed on its back, it instantly turns over to rest with all its feet on the ground;
- *Weak/strong tactile stimuli test*: when a von Frey hair, bending at 0.5 g (weak) or 0.35 g (strong), is stroked across the perioral area on either side of the snout, pup turns its head towards the tactile stimulus;
- *Cliff aversion*: when pup is placed with snout and forepaws over the edge of a flat surface, it immediately withdraws, turning around and crawling away from the edge;
- *Forelimb/hindlimb placing*: contact of the back of the paw against the edge of a piece of card while the pup is suspended by its tail causes the foot to be raised and placed on the surface of the card;
- *Forepaw/hindpaw grasp*: when the shaft of a toothpick is stroked across the inside of the paw, the hand or foot is flexed to grasp the stick;
- *Vibrissae placing*: contact of the vibrissae with a piece of card while the pup is suspended by its tail causes the pup to raise its head and perform a placing response with its forelimb;
- *Ear twitch*: a touch to the external ear with a cotton bud rolled to a point results in a twitch reflex;
- *Pole grasping*: pup firmly embraces a wooden pencil placed under its arms, using both forelimbs and hindlimbs to prevent falling;
- *Level screen test*: pup grips onto a level 2 mm × 2 mm-mesh screen when dragged across it by the tail;
- *Vertical screen test*: as previous but screen is inclined vertically (around 80°) and pup is dragged upwards across mesh;
- *Screen climbing*: when pup is placed head up on the vertically inclined screen it climbs up without hesitation using both fore- and hindlimbs;
- *Auditory startle*: a loud snap of the fingers close to the pup results in an immediate startle response.

Assessment of somatic growth entailed measurements of eyelid and ear opening, incisor eruption and body and tail length. In addition, pups were weighed to the nearest 0.01 g (Mettler PK-300 balance correcting for body movements) every day from PND2 to PND15 then again on PND18 and PND21.

### 2.5. Ultrasonic vocalisations

Ultrasonic vocalisations were monitored before sensorimotor testing on PND2, PND5 and PND9. The pup was taken from the nest and placed in a Petri dish (radius 10 cm) lined with clean paper. After a 15-s pause the number of ultrasonic calls emitted during two consecutive minutes was counted using a hand held Bat Detector (model S25; Ultrasound Advice, London, UK).

### 2.6. Homing test

Homing behaviour was tested on PND12 between 10:00 and 12:00 h.

The testing arena was an open home cage, its floor lined with clean sawdust except for a 4 cm strip at one end which contained sawdust taken from the pup's home cage immediately before testing [3]. The orientation of the arena was alternated randomly to control for unforeseen thermal or olfactory biases in the experimental room.

Before testing the pup was moved from its nest and isolated for 20 min in an empty home cage lined with clean sawdust and warmed by an infrared lamp. It was then placed in the testing arena facing the strip of home sawdust but at the opposite end of the cage. The test stopped when the pup passed the line dividing clean and home sawdust with both front paws, or if 180 s elapsed before this goal was achieved.

### 2.7. T-maze test (spontaneous alternation behaviour)

Spontaneous alternation behaviour (SAB) was tested on PND18 by a T-maze apparatus. Tests were conducted between 14:00 and 16:00 h.

The T-maze apparatus was constructed of grey Plexiglas (BEGI PLASTICA, Roma, Italy) and consisted of a stem, 22 cm long, which diverged into two arms 30 cm. Both stem and arms were 8.5 cm wide and all the walls of the maze were 10 cm high. Guillotine doors closed off the separate arms and the distal 10 cm of the stem, this section serving as a start box. During the experiment the floor of the maze was covered with sawdust taken from the pup's home cage. The maze was placed in a soundproof room dimly lit with red light. All efforts were made to minimize unsymmetrical visual cues around the maze and the experimenter remained at the head of the stem at all times.

On the day before testing (PND17) the two test pups from each litter were placed in the T-maze together and allowed to explore freely for 10 min. At the start of the test the animal remained in the start box for 10 s before the guillotine was raised allowing entrance to the stem. Once the animal had entered an arm, a guillotine was closed behind it and it was confined in the chosen arm for 10 s. It was then replaced in the start box, the guillotine was opened and the procedure repeated. Each animal made a total of five runs in this manner. Correct alternation between consecutive runs scored 1 and failure to alternate scored 0 so that after 5 runs each animal had a score out of 4 for SAB. This score was converted to a percentage (0% no alternation, 50% random choice and 100% alternation at each run).

### 2.8. Open-field test

Mice were tested in the open-field on PND21 between 14:00 and 16:00 h.

The open-field arena was an open-topped cube (60 cm × 60 cm × 60 cm) constructed of black Plexiglas (BEGI PLASTICA, Roma, Italy). The floor was grey and marked with a 12 cm × 12 cm grid. Testing was conducted in a soundproof room dimly lit with red light.

Behavioural performances were video recorded using a digital videocamera (Model TR 7000E, Sony, Tokyo, Japan). The behavioural analysis was carried out from the videotapes, using a commercial software ("The Observer 2.0"; Noldus, Wageningen, The Netherlands; see [38]).

The pup was placed in the centre of the arena and left undisturbed for 20 min. The 20-min session was divided in three blocks of 4 min each (0–4, 8–12 and 16–20). During the 20 min of open-field performance the frequencies and durations of the following behavioural parameters were scored: *wall-rearing*, standing on the hindlimbs and touching the walls of the apparatus with the forelimbs; *rearing*, standing with the body inclined vertically, forequarters raised; *grooming*, licking and mouthing its own fur, sometimes helping itself with its forepaws; *immobility*, self-explaining (s.e.); *sniffing*, s.e. To evaluate locomotor activity, the number of *crossings* of the square limits with both forepaws were also recorded during three blocks of 2 min each (0–2, 9–11 and 18–20); moreover, frequency of *crossings* in the peripheral area (6 cm to the walls) of the apparatus and time spent in such area were also evaluated as a measure of thigmotaxis.

### 2.9. Morris water-maze test

At the age of 12 months, 5 primiparous dams litters for each treatment group were culled to 2 males and the subjects were tested in a Morris water-maze task (BEGI PLASTICA, Roma, Italy).

The Morris water-maze apparatus consisted of a black Plexiglas circular pool 150 cm in diameter and 50 cm in height designed according to Morris [35], and placed in the middle of an experimental room (dimension 5 m × 4 m × 3 m).

The pool was filled with water kept at a temperature of 24–26 °C. A plastic black platform (15 cm in diameter) was placed 0.5 cm below the water surface and 15 cm from the edge of the pool. Distant visual cues for navigation were provided by four geometric-shaped black and white posters put on the four room walls.

The Morris water-maze test was conducted under dim light between 13:30 and 17:30 h. The entire procedure took 5 days: the position of the hidden platform remained fixed for the first 3 days (acquisition phase), while on the days 4 and 5 the platform was placed at the opposite location with the respect to the position of the acquisition phase (reversal phase). On the first day of the reversal phase (first trial of fourth day test) the platform was removed and each mouse was tested for a 60 s period (probe trial).

Each subject was allowed six individual trials a day. To avoid visual orientation prior to release, mice were transferred from their cage into the pool in a nontransparent plastic cup, from which they glided into the water facing the pool wall. Release points were balanced across eight symmetrical positions on the pool perimeter. Animals were left swimming until either they found the platform or 60 s had elapsed. Platform findings were defined as staying for at least 3 s on it. If mice crossed the platform without stopping (jumping immediately into the water), they were left swimming until they met the above criterion. After staying for about 10 s on the platform, the mice were given the opportunity to climb on a wire-mesh grid. Between trials the animals were placed under infrared lamps and allowed to warm up and dry for about 3 min. Inter-trial times varied between 40 and 50 min.

The swim path of the mice was recorded by means of a computer-based video-tracking system Ethovision (Noldus, Wageningen, The Netherlands). The variables recorded were length of swim path (total distance moved), latency to reach the platform (escape latency), mean swimming speed, and relative turn angle (clockwise or counterclockwise turn movement).

### 2.10. Brain levels of neurotrophines

One day after the end of the Morris water-maze test, brain levels of NGF and BDNF were assessed in five males for each treatment group. Mice were sacrificed by decapitation and hippocampus, hypothalamus and frontal cortex were quickly dissected and stored at −70 °C.

Hippocampal, hypothalamic and frontal cortex levels of NGF were measured by a highly sensitive two-site immunoenzymatic assay, which recognizes both murine and human NGF and does not cross-react with BDNF. The exogenous NGF yield was calculated by subtracting the amount of this NGF from the endogenous variety. Under these conditions the recovery of NGF on our assay ranged from 80 to 90% [8,51].

Hippocampal, hypothalamic and frontal cortex levels of BDNF were measured following the procedure suggested by the manufacturer (Emaxtm ImmunoAssay System number G6891 by Promega, Madison, WI, USA), using a monoclonal anti-mouse-BDNF antibody and a polyclonal anti-human-BDNF antibody. BDNF concentration was determined from the regression line for the BDNF standard curve (ranging from 7.8 to 500 pg/ml-purified mouse BDNF) incubated under similar conditions in each assay. The sensitivity of the assay is about 15 pg/ml of BDNF, and the cross-reactivity with other related neurotrophic factors (NGF, NT-3, and NT-4) is considered nil [1].

### 2.11. Statistical analyses

Fox's battery, homing and T-maze performances were evaluated using Kruskal–Wallis non-parametric ANOVA to analyze the main effect of sex (S), parity (P), and treatment (T), and the interactions of P × S, T × S, P × T and P × T × S. Data on body weight, body and tail length, as well as ultrasonic vocalisation and open-field performances, were analyzed using a mixed-model ANOVA, considering T and P as grouping factors, litter as a random blocking factor, S as within-litter factor, and postnatal days or the blocks of the behavioural observations as repeated measures (R). Morris water-maze performances were analyzed using a mixed-model ANOVA considering T as grouping factors, litter as a random blocking factor and the daily behavioural session as repeated measures. Post hoc comparisons were performed using the Tukey HSD test. When separate ANOVAs were performed day by day, Bonferroni's correction was applied to correct for the decrease in type I error probability due to repeated testing. Differences in brain neurotrophin levels were analyzed by non-parametric Kruskal–Wallis analysis of variance. Mann–Whitney *U*-test with Bonferroni's correction was used for multiple comparisons.

## 3. Results

### 3.1. Somatic growth and sensorimotor development

Parity (P) affected both somatic and neurobehavioural development. Specifically, biparous offspring showed higher body weight gain [P × R (repeated measures: PNDs), $F(15,630) = 21.23$; $P < 0.05$. Post hoc comparisons statistically significant starting from PND9, $P < 0.05$; see Table 1] and opened eyes earlier than primiparous one (day of adult-like response: $\chi^2_1 = 4.01$; $P = 0.05$; see Table 2). In addition they exhibited both accelerated adult-like responses and a higher overall score of *swift righting* performance (respectively, $\chi^2_1 = 9.60$ and 4.14; $P < 0.05$), *weak tactile stimulation* (respectively, $\chi^2_1 = 10.23$ and 16.47; $P < 0.05$), *forelimb* (respectively, $\chi^2_1 = 13.23$ and 9.69; $P < 0.05$) and *hindlimb placing* (respectively, $\chi^2_1 = 3.82$; $P = 0.05$ and $\chi^2_1 = 20.39$; $P < 0.05$), and *climbing* (respectively, $\chi^2_1 = 5.77$ and 5.94; $P < 0.05$). Conversely, primiparous offspring performed accelerated adult-like responses in the *vibrissae placing* test ($\chi^2_1 = 5.90$; $P < 0.05$), and they totalled a higher overall score in *pole embrace* assessment ($\chi^2_1 = 5.17$; $P < 0.05$; Table 2).

Rotation per se altered some somatic growth measures, rotated pups showing a delay in hair growth [day of adult-like response: treatment effect, T, $\chi^2_1 = 5.88$; $P = 0.05$; overall score: SC versus (RC + HG), $\chi^2_1 = 3.96$; $P < 0.05$] and ears opening [day of adult-like response: SC versus (RC + HG), $\chi^2_1 = 6.13$; overall score: SC versus (RC + HG), $\chi^2_1 = 6.13$; $P < 0.05$] when compared to SC ones (Table 2). Rotation also influenced sensorimotor development. Indeed, when compared to SC mice, rotated animals showed both delayed adult-like responses appearance and a lower overall score in *auditory startle* (respectively, $\chi^2_1 = 5.99$ and 5.03; $P < 0.05$), *ear twitch* (respectively, $\chi^2_1 = 3.88$ and 3.88; $P < 0.05$) and *pole embrace* (respectively, $\chi^2_1 = 4.11$ and 5.51; $P < 0.05$) tests, while they displaying a lower overall score in the *level screen* test performance ($\chi^2_1 = 4.27$; $P < 0.05$; see Table 2).

Hypergravity exposure differentially affected the tail growth of primiparous and biparous pups. Specifically, among primiparous offspring HG mice showed a shorter tail than SC ones (SC versus HG comparison statistically significant on PND15), while HG biparous offspring had a longer tail compared to SC biparous ones [T × P × R, $F(24,50) = 1.64$; $P < 0.05$; see Table 1].

Moreover, among rotated pups, RC males and HG females showed a delay in the *climbing* test [day of adult-like response: RC versus HG × sex (S), $\chi^2_1 = 5.53$; $P < 0.05$; see Table 2].

### 3.2. Ultrasonic vocalisations

Differences in ultrasonic vocalisation profile were observed especially on PND9, when rotated pups maintained still higher call levels than SC ones [T × R (PNDs), $F(4,84) = 2.80$; $P < 0.05$; see Fig. 2].

Table 1
Body weight gain, body and tail length in offspring of primiparous and biparous dams exposed to 1 h of 2 G hypergravity from PND2 to PND9

| | PND2 | PND3 | PND4 | PND5 | PND6 | PND7 | PND8 | PND9 | PND10 | PND11 | PND12 | PND13 | PND14 | PND15 | PND18 | PND21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Body weight gain (g)[a]** | | | | | | | | | | | | | | | | |
| **Stationary Controls** | | | | | | | | | | | | | | | | |
| Primiparous offsp. | 2.7±0.1 | 3.3±0.1 | 3.9±0.1 | 4.7±0.1 | 5.4±0.1 | 6.1±0.1 | 6.8±0.2 | 7.4±0.1 | 8.1±0.2 | 8.6±0.2 | 9.2±0.2 | 9.6±0.2 | 9.9±0.2 | 10.2±0.2 | 12.5±0.3 | 15.3±0.4 |
| Biparous offsp. | 2.6±0.0 | 3.2±0.0 | 3.9±0.0 | 4.7±0.0 | 5.4±0.1 | 6.2±0.1 | 6.9±0.1 | 7.6±0.1 | 8.3±0.1 | 9.0±0.1 | 9.6±0.1 | 10.3±0.1 | 10.7±0.1 | 11.3±0.1 | 13.6±0.1 | 16.4±0.2 |
| **Rotational Controls** | | | | | | | | | | | | | | | | |
| Primiparous offsp. | 2.6±0.1 | 3.2±0.1 | 3.8±0.1 | 4.4±0.1 | 5.0±0.2 | 5.6±0.2 | 6.3±0.2 | 6.9±0.2 | 7.4±0.2 | 7.9±0.2 | 8.4±0.2 | 8.9±0.2 | 9.4±0.2 | 9.8±0.2 | 11.7±0.3 | 14.8±0.3 |
| Biparous offsp. | 2.6±0.1 | 3.1±0.1 | 3.8±0.1 | 4.6±0.1 | 5.2±0.1 | 5.9±0.1 | 6.7±0.1 | 7.3±0.1 | 8.0±0.1 | 8.6±0.1 | 9.2±0.1 | 9.8±0.1 | 10.3±0.2 | 10.8±0.1 | 13.1±0.2 | 16.2±0.3 |
| **Hypergravity** | | | | | | | | | | | | | | | | |
| Primiparous offsp. | 2.4±0.1 | 3.0±0.1 | 3.6±0.1 | 4.2±0.1 | 4.9±0.1 | 5.6±0.2 | 6.2±0.2 | 6.8±0.2 | 7.3±0.2 | 7.9±0.2 | 8.5±0.2 | 9.0±0.2 | 9.5±0.2 | 9.9±0.2 | 12.0±0.3 | 14.5±0.4 |
| Biparous offsp. | 2.6±0.1 | 3.2±0.1 | 3.9±0.1 | 4.7±0.1 | 5.4±0.1 | 6.1±0.1 | 6.8±0.2 | 7.5±0.2 | 8.2±0.2 | 8.8±0.2 | 9.4±0.2 | 10.0±0.2 | 10.5±0.2 | 11.1±0.2 | 13.6±0.3 | 16.3±0.3 |
| **Body length (mm)** | | | | | | | | | | | | | | | | |
| **Stationary Controls** | | | | | | | | | | | | | | | | |
| Primiparous offsp. | 30.6±0.6 | 33.4±0.4 | 35.1±0.3 | 36.8±0.2 | 38.9±0.3 | 41.1±0.4 | 42.3±0.5 | 44.1±0.5 | 45.8±0.5 | 47.3±0.7 | - | 51.4±0.6 | 53.6±0.6 | 55.8±1.0 | - | - |
| Biparous offsp. | 30.8±0.4 | 32.8±0.3 | 34.8±0.3 | 36.4±0.3 | 38.2±0.3 | 40.0±0.3 | 40.9±0.8 | 43.9±0.3 | 45.2±0.4 | 46.8±0.4 | - | 50.3±0.5 | 52.8±0.7 | 56.1±0.8 | - | - |
| **Rotational Controls** | | | | | | | | | | | | | | | | |
| Primiparous offsp. | 30.4±0.4 | 32.8±0.3 | 34.4±0.4 | 37.4±0.5 | 38.3±0.5 | 39.6±0.5 | 40.9±0.5 | 43.3±0.6 | 44.7±0.5 | 45.7±0.5 | - | 49.8±0.6 | 52.3±0.7 | 53.9±0.6 | - | - |
| Biparous offsp. | 30.6±0.3 | 32.9±0.4 | 34.7±0.3 | 36.3±0.4 | 38.4±0.4 | 40.8±0.2 | 41.7±0.3 | 43.1±0.5 | 44.9±0.5 | 46.3±0.4 | - | 50.5±0.7 | 53.5±0.6 | 56.1±0.5 | - | - |
| **Hypergravity** | | | | | | | | | | | | | | | | |
| Primiparous offsp. | 30.6±0.4 | 32.3±0.3 | 34.4±0.4 | 36.8±0.4 | 37.8±0.4 | 39.3±0.5 | 41.0±0.5 | 43.2±0.6 | 43.9±0.6 | 45.6±0.7 | - | 48.9±0.4 | 51.1±0.7 | 53.6±0.8 | - | - |
| Biparous offsp. | 30.9±0.4 | 33.0±0.5 | 34.9±0.4 | 37.3±0.5 | 38.6±0.5 | 40.3±0.4 | 42.1±0.5 | 43.1±0.3 | 44.8±0.4 | 46.5±0.6 | - | 50.5±0.9 | 52.4±0.8 | 55.6±0.8 | - | - |
| **Tail length (mm)[b]** | | | | | | | | | | | | | | | | |
| **Stationary Controls** | | | | | | | | | | | | | | | | |
| Primiparous offsp. | 1.8±0.0 | 2.0±0.0 | 2.2±0.0 | 2.5±0.1 | 2.8±0.0 | 3.1±0.1 | 3.4±0.1 | 3.7±0.1 | 4.0±0.0 | 4.3±0.1 | - | 4.8±0.1 | 5.0±0.1 | 5.3±0.1 | - | - |
| Biparous offsp. | 1.7±0.0 | 2.0±0.0 | 2.2±0.0 | 2.5±0.0 | 2.8±0.0 | 3.0±0.0 | 3.3±0.0 | 3.6±0.0 | 3.9±0.0 | 4.3±0.0 | - | 4.8±0.1 | 4.9±0.1 | 5.2±0.1 | - | - |
| **Rotational Controls** | | | | | | | | | | | | | | | | |
| Primiparous offsp. | 1.8±0.0 | 2.0±0.0 | 2.2±0.1 | 2.4±0.1 | 2.7±0.1 | 3.0±0.1 | 3.3±0.1 | 3.6±0.1 | 3.9±0.1 | 4.2±0.1 | - | 4.7±0.1 | 4.9±0.1 | 5.2±0.1 | - | - |
| Biparous offsp. | 1.6±0.0 | 1.9±0.0 | 2.1±0.0 | 2.4±0.0 | 2.7±0.0 | 3.0±0.0 | 3.3±0.1 | 3.5±0.1 | 3.8±0.1 | 4.1±0.1 | - | 4.6±0.1 | 4.9±0.1 | 5.2±0.1 | - | - |
| **Hypergravity** | | | | | | | | | | | | | | | | |
| Primiparous offsp. | 1.7±0.0 | 1.9±0.0 | 2.1±0.0 | 2.4±0.0 | 2.6±0.0 | 2.9±0.0 | 3.2±0.0 | 3.5±0.1 | 3.8±0.1 | 4.1±0.1 | - | 4.6±0.0 | 4.8±0.1 | 5.0±0.1 | - | - |
| Biparous offsp. | 1.7±0.0 | 1.9±0.0 | 2.2±0.0 | 2.5±0.1 | 2.8±0.1 | 3.1±0.0 | 3.4±0.0 | 3.7±0.0 | 4.0±0.1 | 4.2±0.1 | - | 4.8±0.1 | 5.0±0.1 | 5.4±0.1 | - | - |

Stationary Controls = unrotated, Rotational Controls = exposure to 1 G, Hypergravity = exposure to 2 G. Primiparous offsp. = offspring of primiparous dams, Biparous offsp. = offspring of biparous dams. Significant effects ( $P < 0.05$). P = parity (primiparous or biparous), T = treatment (unrotated or rotated to 1 G or 2 G), R = repeated measures (postnatal day, PND). Data are means ± S.E. $n = 8$ in each group. [a]interaction P x R, [b]interaction T x P x R.

Table 2
Assessment of somatic growth and neurobehavioural development in offspring of primiparous and biparous dams exposed to 1 h of 2 G hypergravity from PND2 to PND9

**Day of adult-like response**

| | Slow righting | Swift righting | Weak tactile stimulus | Strong tactile stimulus | Cliff aversion | Vibrissae placing | Forelimb placing | Hindlimb placing | Forelimb grasp | Hindlimb grasp |
|---|---|---|---|---|---|---|---|---|---|---|
| | | * | * | | | * | * | * | | |
| **Stationary Controls** | | | | | | | | | | |
| Primiparous offsp. | 5.9±0.3 | 10.9±0.4 | 5.5±0.5 | 5.8±0.4 | 3.8±0.6 | 4.3±0.3 | 5.1±0.3 | 11.2±0.4 | 6.7±0.4 | 12.5±0.4 |
| Biparous offsp. | 5.6±0.4 | 8.8±0.4 | 4.1±0.5 | 4.8±0.6 | 3.4±0.3 | 5.6±0.5 | 3.6±0.2 | 11.3±0.6 | 6.5±0.4 | 12.8±0.3 |
| **Rotational Controls** | | | | | | | | | | |
| Primiparous offsp. | 6.3±0.5 | 10.8±0.5 | 5.3±0.3 | 5.9±0.4 | 4.8±0.6 | 4.4±0.4 | 5.1±0.2 | 12.6±0.4 | 6.8±0.6 | 13.3±0.4 |
| Biparous offsp. | 6.1±0.4 | 10.3±0.4 | 4.0±0.3 | 5.6±0.4 | 3.9±0.5 | 4.6±0.4 | 4.3±0.3 | 11.4±0.5 | 7.3±0.4 | 12.8±0.3 |
| **Hypergravity** | | | | | | | | | | |
| Primiparous offsp. | 5.4±0.3 | 11.0±0.4 | 5.9±0.4 | 6.4±0.4 | 3.9±0.4 | 4.4±0.4 | 5.2±0.4 | 12.1±0.5 | 7.4±0.4 | 13.7±0.3 |
| Biparous offsp. | 5.7±0.4 | 9.3±0.5 | 4.3±0.5 | 5.4±0.5 | 3.7±0.4 | 5.2±0.3 | 3.9±0.3 | 10.6±0.6 | 6.6±0.4 | 13.0±0.3 |

| | Pole embrace | Level screen | Vertical screen | Climbing | Ear twitch | Auditory startle | Ears open | Eyes open | Incisor eruption | Hair growth |
|---|---|---|---|---|---|---|---|---|---|---|
| | $ | | | *# | $ | $ | $ | * | | ¶ |
| **Stationary Controls** | | | | | | | | | | |
| Primiparous offsp. | 9.9±0.4 | 11.6±0.3 | 13.4±0.3 | 12.1±0.7 | 3.2±0.2 | 11.3±0.3 | 11.4±0.2 | 14.1±0.2 | 10.5±0.2 | 13.3±0.1 |
| Biparous offsp. | 10.5±0.6 | 12.2±0.4 | 13.4±0.2 | 10.4±0.5 | 3.4±0.3 | 12.1±0.3 | 11.3±0.2 | 13.8±0.1 | 9.9±0.1 | 13.3±0.2 |
| **Rotational Controls** | | | | | | | | | | |
| Primiparous offsp. | 11.1±0.4 | 12.1±0.3 | 13.0±0.5 | 12.0±0.5 | 3.6±0.3 | 12.1±0.3 | 11.8±0.3 | 14.3±0.2 | 10.8±0.3 | 13.5±0.2 |
| Biparous offsp. | 10.9±0.4 | 12.6±0.4 | 13.4±0.3 | 10.9±0.4 | 4.4±0.4 | 12.4±0.3 | 11.9±0.3 | 14.2±0.1 | 10.4±0.2 | 13.4±0.1 |
| **Hypergravity** | | | | | | | | | | |
| Primiparous offsp. | 11.6±0.4 | 12.2±0.3 | 13.6±0.3 | 12.3±0.6 | 3.5±0.3 | 12.8±0.2 | 12.4±0.2 | 14.4±0.2 | 10.8±0.3 | 13.6±0.1 |
| Biparous offsp. | 11.4±0.4 | 12.1±0.5 | 13.6±0.4 | 11.0±0.5 | 4.6±0.3 | 12.5±0.3 | 12.1±0.3 | 13.9±0.2 | 10.3±0.2 | 13.8±0.2 |

**Overall score**

| | Slow righting | Swift righting | Weak tactile stimulus | Strong tactile stimulus | Cliff aversion | Vibrissae placing | Forelimb placing | Hindlimb placing | Forelimb grasp | Hindlimb grasp |
|---|---|---|---|---|---|---|---|---|---|---|
| | | * | * | | | | * | * | | |
| **Stationary Controls** | | | | | | | | | | |
| Primiparous offsp. | 32.7±0.6 | 22.5±0.6 | 2.1±0.5 | 1.6±0.3 | 36.6±0.7 | 34.3±0.7 | 33.1±0.7 | 15.9±0.8 | 33.4±0.5 | 17.6±0.9 |
| Biparous offsp. | 33.3±0.8 | 25.1±0.8 | 4.1±0.7 | 2.3±0.6 | 37.0±0.6 | 32.6±0.7 | 36.0±0.6 | 21.6±1.2 | 33.2±0.6 | 18.4±0.7 |
| **Rotational Controls** | | | | | | | | | | |
| Primiparous offsp. | 31.3±0.9 | 21.8±1.1 | 2.4±0.5 | 1.8±0.4 | 36.1±0.5 | 34.4±0.8 | 33.4±0.5 | 14.5±0.7 | 32.7±0.8 | 15.0±0.8 |
| Biparous offsp. | 32.2±0.8 | 22.7±1.1 | 5.0±0.5 | 2.9±0.7 | 37.1±0.6 | 33.9±0.9 | 35.1±0.6 | 19.9±1.1 | 32.1±0.6 | 16.6±0.9 |
| **Hypergravity** | | | | | | | | | | |
| Primiparous offsp. | 32.3±0.7 | 23.0±0.9 | 1.9±0.4 | 1.4±0.4 | 37.1±0.4 | 34.3±0.8 | 33.3±0.8 | 16.0±0.8 | 31.8±0.6 | 14.9±0.9 |
| Biparous offsp. | 33.4±0.8 | 24.4±1.1 | 4.9±0.7 | 3.3±0.7 | 37.2±0.5 | 33.3±0.7 | 35.4±0.7 | 21.7±1.4 | 32.4±0.6 | 16.7±0.9 |

| | Pole embrace | Level screen | Vertical screen | Climbing | Ear twitch | Auditory startle | Ears open | Eyes open | Incisor eruption | Hair growth |
|---|---|---|---|---|---|---|---|---|---|---|
| | *$ | $ | | * | $ | $ | $ | | | $ |
| **Stationary Controls** | | | | | | | | | | |
| Primiparous offsp. | 26.3±0.5 | 20.8±0.5 | 16.7±0.7 | 19.6±1.0 | 35.4±0.6 | 12.0±0.6 | 11.4±0.4 | 7.4±0.3 | 13.7±0.5 | 19.4±0.4 |
| Biparous offsp. | 23.3±1.1 | 20.4±0.4 | 15.6±0.6 | 22.6±0.8 | 34.9±1.0 | 10.5±0.7 | 11.6±0.4 | 7.9±0.2 | 15.4±0.4 | 19.6±0.4 |
| **Rotational Controls** | | | | | | | | | | |
| Primiparous offsp. | 23.9±0.6 | 18.9±0.4 | 15.7±0.5 | 18.6±1.0 | 34.1±0.9 | 10.3±0.4 | 10.9±0.4 | 6.6±0.4 | 13.3±0.7 | 18.6±0.5 |
| Biparous offsp. | 22.3±0.8 | 18.8±0.7 | 15.0±0.6 | 21.3±0.7 | 31.9±1.3 | 9.9±0.7 | 10.5±0.5 | 6.7±0.4 | 13.7±0.5 | 18.9±0.3 |
| **Hypergravity** | | | | | | | | | | |
| Primiparous offsp. | 23.4±0.8 | 19.1±0.5 | 14.8±0.6 | 17.6±1.2 | 34.5±0.8 | 9.4±0.3 | 9.9±0.4 | 6.2±0.5 | 13.1±0.7 | 18.4±0.4 |
| Biparous offsp. | 22.6±0.7 | 20.1±0.6 | 14.9±0.9 | 19.9±0.9 | 31.3±1.0 | 9.6±0.6 | 10.3±0.4 | 7.6±0.5 | 14.3±0.5 | 18.4±0.5 |

Stationary Controls = unrotated, Rotational Controls = exposure to 1 G, Hypergravity = exposure to 2 G. Primiparous offsp. = offspring of primiparous dams, Biparous offsp. = offspring of biparous dams. Significant effects ($P < 0.05$). P = parity (primiparous or biparous), T = treatment (unrotated or rotated to 1 G or 2 G), S = sex. Data are means ± S.E. $n = 8$ in each group. * main effect of P, ¶ main effect of T, $ stationary controls *vs* rotated mice, # rotational controls *vs* hypergravity x S.

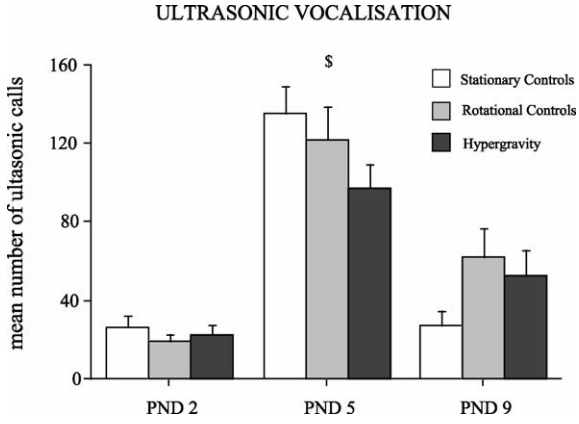


Fig. 2. Ultrasonic calling rate of CD-1 pup mice exposed to 1 h of 2 G hypergravity from PND2 to PND9. Stationary controls = unrotated, rotational controls = exposure to 1 G, hypergravity = exposure to 2 G. Significant effects ($P < 0.05$): $^{\$}$interaction T × R. T = treatment (unrotated or rotated to 1 or 2 G), R = repeated measures (postnatal day, PND). Data are means ± S.E. $n = 8$ in each group.

### 3.3. Homing test

No differences were observed in homing test performance (data not shown).

### 3.4. T-maze test

Primiparous female offspring performed higher percentage of spontaneous alternation than males, while no sex (S) differences emerged for biparous offspring (P × S, $\chi^2_1 = 4.62$; $P < 0.05$; Table 3). Moreover, among rotated pups, RC females showed a better performance than males, and no differences were observed between females and males of the HG group (RC versus HG × S, $\chi^2_1 = 3.91$; $P < 0.05$; see Table 3).

### 3.5. Open-field test

Primiparous offspring showed higher frequency of *sniffing* behaviour than biparous one [P, $F(1,40) = 11.62$; $P < 0.05$] and a similar trend for *sniffing* duration was also observed [P, $F(1,40) = 2.98$; $P = 0.09$; see Fig. 3].

Moreover, when compared to the other groups, primiparous female offspring showed a trend to sniff the open-field arena for longer during the first 4-min block [P × S × R (4-min blocks), $F(2,80) = 3.11$; $P = 0.05$; data not shown for repeated measures].

Parity condition also affected *grooming* and *wall-rearing* durations with primiparous offspring performing longer *grooming* and shorter *wall-rearing* than biparous one [*grooming*, $F(1,40) = 5.51$, $P < 0.05$; *wall-rearing*, $F(1,40) = 22.31$, $P < 0.05$; Fig. 3]. Specifically, over the course of the test session primiparous offspring increased *grooming* while they decreased *wall-rearing* duration [P × R interaction, respectively, $F(2,80) = 2.56$, $P = 0.08$ and $F(2,80) = 3.29$, $P < 0.05$; primiparous versus biparous offspring, for both the second and the third 4-min blocks, $P < 0.05$]. Similarly, over the course of the test session primiparous offspring performed more frequent and longer *immobility* than biparous one [frequency: P × R, $F(2,80) = 5.15$; $P < 0.05$; duration: P × R, $F(2,80) = 4.69$, $P < 0.05$; primiparous versus biparous offspring for both the second and the third 4-min blocks, $P < 0.05$].

Primiparous offspring spent longer time in the central area of the open-field [P, $F(1,40) = 6.59$; $P < 0.05$] and performed less *crossings* over the course of the session [P × R (2-min blocks), $F(2,80) = 6.67$; $P < 0.05$] when compared to biparous one (data not shown). Conversely, throughout the course of the test session, biparous offspring spent longer time in the peripheral area of the arena [P, $F(1,40) = 6.59$; $P < 0.05$] and performed more *crossings* in this area [P × R, $F(2,80) = 7.63$; $P < 0.05$] than primiparous one (data not shown).

Hypergravity affected both *sniffing* and *grooming* behaviours. In particular, HG females sniffed the open-field arena longer than HG males [T × S, $F(2,40) = 3.44$; $P < 0.05$], while HG males performed *grooming* more frequently than HG females [T × S, $F(2,40) = 3.12$; $P = 0.05$; see Fig. 3]. Moreover, during the first 4 min of the test session, HG animals showed the highest frequency of *wall-rearing* [T × R (4-min blocks), $F(4,80) = 2.71$; $P < 0.05$]. The interaction between T and R was also significant for *immobility* behaviour, HG and RC groups being more inactive during the first and the second 4-min blocks, respectively, than the other groups, while SC animals being more inactive than the other groups during the last 4 min of test [$F(4,80) = 3.88$; $P < 0.05$].

Hypergravity affected locomotor activity as well (data not shown). In particular, HG males showed higher frequency of *crossings* in both the peripheral and central areas than HG females [respectively, T × S, $F(2,40) = 3.55$ and $F(2,40) = 3.57$, $P < 0.05$; HG males versus HG females, $P < 0.05$]. During the first 2-min block of the test session, HG mice crossed both the peripheral and central areas more times than RC and SC ones, while during the second 2-min block the highest frequency of *crossings* was observed in the RC group [T × R, respectively, $F(4,80) = 4.19$ and $F(4,80) = 3.57$; $P < 0.05$].

Table 3
T-maze performance in 18-day-old-male and female CD-1 mice exposed to 1 h of 2 G hypergravity from PND2 to PND9

| | Interaction P × S, rotational controls vs. hypergravity × S | |
|---|---|---|
| | Primiparous offsp. | Biparous offsp. |
| SC females | 75.0 ± 6.7 | 78.1 ± 7.4 |
| SC males | 53.1 ± 10.0 | 68.8 ± 7.8 |
| RC females | 90.6 ± 6.6 | 75.0 ± 6.7 |
| RC males | 65.6 ± 6.6 | 59.4 ± 6.6 |
| HG females | 78.1 ± 7.4 | 59.4 ± 11.5 |
| HG males | 62.5 ± 10.6 | 78.1 ± 5.7 |

SC = stationary controls, unrotated; RC = rotational controls, exposure to 1 G; HG = hypergravity, exposure to 2 G. Significant effects ($P < 0.05$). P = parity (primiparous or biparous), S = sex. Data are means ± S.E. $n = 8$ in each group.

### 3.6. Morris water-maze test

During the acquisition phase, differences in latencies to find the platform location were observed only on day 2, RC mice showing longer latency to locate the platform than HG or SC ones [T, $F(2,12) = 4.768$; $P < 0.05$; see Fig. 4]. Moreover, on day 2, RC animals tended to spend more time in the central area of the

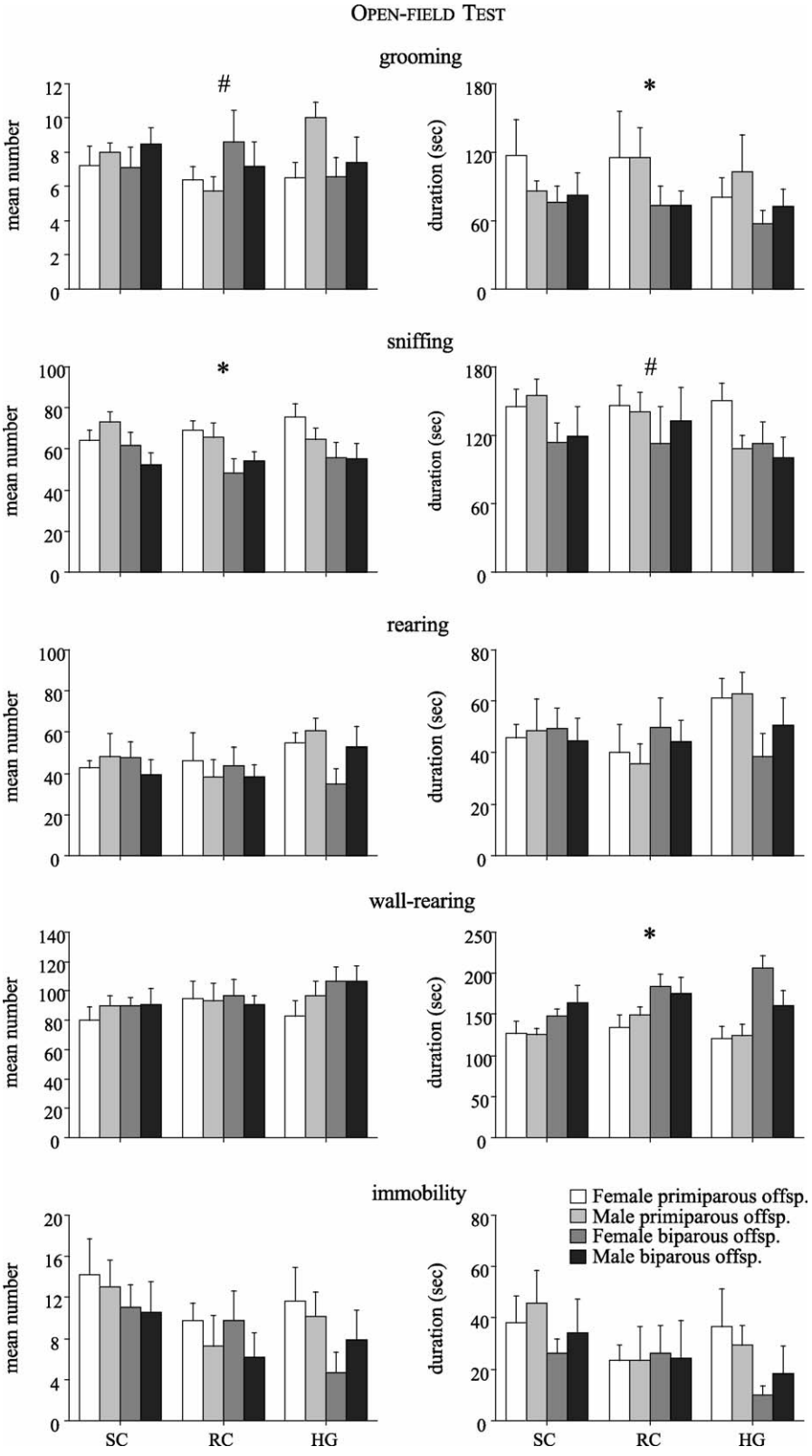


Fig. 3. Open-field performance in 21-day-old male and female CD-1 mice exposed to 1 h of 2 G hypergravity from PND2 to PND9. SC = stationary controls, unrotated; RC = rotational controls, exposure to 1 G; HG = hypergravity, exposure to 2 G. Female primiparous offsp. = offspring female of primiparous dams, male primiparous offsp. = offspring male of primiparous dams, female biparous offsp. = offspring female of biparous dams, male biparous offsp. = offspring male of biparous dams. Significant effects ($P \leq 0.05$): $^{*}$main effect of P, $^{\#}$interaction T × S. P = parity (primiparous or biparous), T = treatment (unrotated or rotated to 1 or 2 G), S = sex. Data are means ± S.E. $n = 8$ in each group.

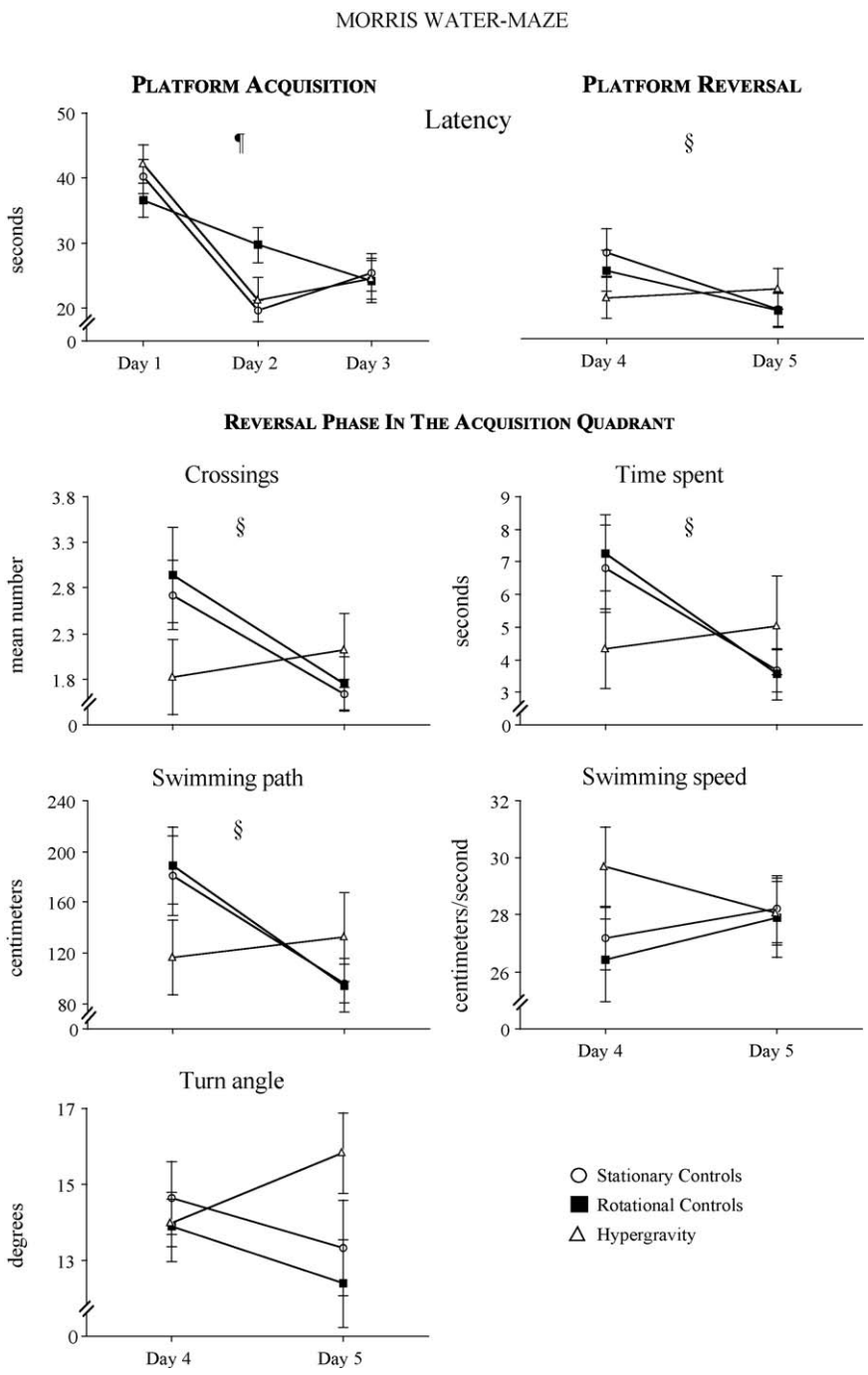


Fig. 4. Morris water-maze performance in 12-month-old-male CD-1 mice exposed to 1 h of 2 G hypergravity from PND2 to PND9. Stationary controls = unrotated, rotational controls = exposure to 1 G, hypergravity = exposure to 2 G. Significant effects ($P \leq 0.05$): ¶main effect of T, §interaction T × R. T = treatment (unrotated or rotated to 1 or 2 G), R = repeated measures (day of test). Data are means ± S.E. $n = 5$ in each group.

arena than HG or SC groups [T × R (test days), $F(8,48) = 1.95$; $P = 0.07$]. No differences were found in swimming path, swimming speed or turn angle, nor were observed variations in probe test performances.

During the 2 days of reversal phase, HG animals showed a different trend over time in latency to find the new platform location when compared to RC or SC mice [T × R, $F(2,12) = 3.79$; $P = 0.05$; Fig. 4]. Analogously, when considering mice performance in the acquisition quadrant, HG animals showed different profile over time in time spent [T × R, $F(2,12) = 6.27$; $P < 0.05$], crossings [T × R, $F(2,12) = 5.04$; $P < 0.05$], and swimming path [T × R, $F(2,12) = 5.58$; $P < 0.05$] when compared to SC or RC ones (Fig. 4).

### 3.7. Brain NGF and BDNF levels

NGF concentration was lower in the frontal cortex of HG mice than in the frontal cortex of both SC and RC mice [$H_{(2)} = 6.18$; $P < 0.05$; see Fig. 5]. Moreover, when compared to SC animals, both RC and HG groups had lower levels

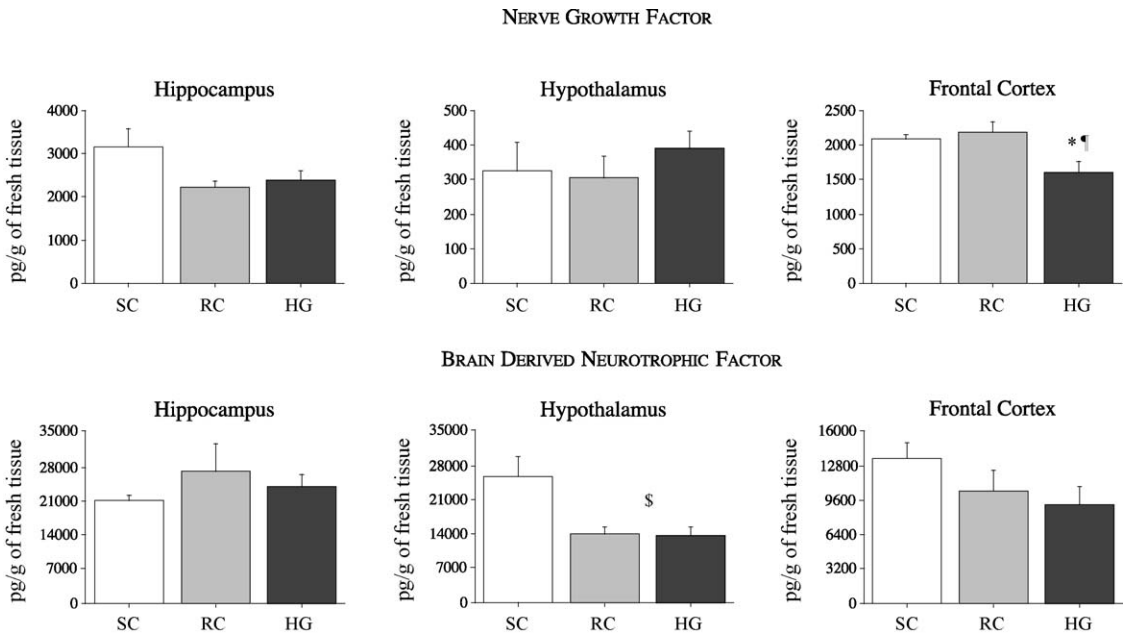


Fig. 5. Brain levels of NGF and BDNF in 12-month-old-male CD-1 mice exposed to 1 h of 2 G hypergravity from PND2 to PND9. SC = stationary controls, unrotated; RC = rotational controls, exposure to 1 G; HG = hypergravity, exposure to 2 G. Significant effects ($P < 0.05$): hypergravity vs. stationary controls (*) or rotational controls (¶), $ stationary controls vs. rotated mice. Data are means ± S.E. $n = 5$ in each group.

of BDNF in the hypothalamus [$H_{(2)} = 6.62$; $P < 0.05$; see Fig. 5].

## 4. Discussion

The present study indicates that repeated acute exposures to a rotational environment during the postnatal phase affect mouse neurobehavioural development. In particular, independently from hypergravity exposure, rotation per se delayed somatic growth (hair growth and ears opening), sensorimotor responses (*ear twich*, *auditory startle*, *pole embrace* and *level screen test*), and the ultrasonic vocalisation profile.

Hypergravity did not exert specific effects on pup development. Interestingly, when comparing our results with the findings recently reported by Bouet et al. [7], clear differences arise likely due to differences in time and modality of hypergravity exposure. In Bouet et al.'s experiment, the treatment protocol was designed to expose animals to hypergravity from conception until varying periods after birth. They reported a postnatal delay in body growth and in the development of the righting response and negative geotaxis, motor aspects mainly dependent upon the vestibular system. In our experiment a very different exposure schedule was applied—i.e. 1 h daily exposure from PND2 to PND9. Thus, it is possible that the different exposure durations could account for the lack of hypergravity-induced effects on the development of the specific behavioural endpoints. In fact, all the major effects on the vestibular system development reported so far resulted from exposure to hypergravity during the prenatal or both the prenatal and the postnatal phase [10,12,23,32,54]. Moreover, in Bouet et al.'s experiment, rotational controls were not included, making it difficult to draw conclusions about the specificity of hypergravity effects.

In order to control for rotational stimulus, in our experiments, a control group was rotated at 1 G (Earth gravitational field). Indeed, acute exposure to rotation represents a stressful condition per se, as we have already shown in adult and adolescent mice, where it can induce motion sickness syndrome and increase the levels of emotional/anxiety behaviour [22,45]. Stressors repeatedly applied to neonatal rodents induce alterations of central nervous system functions, persisting up to the adult age, due to the sensitive periods in the formation of brain circuitry associated with early development [34,49]. Therefore it can be hypothesized that the changes here reported might reflect the influence of enhanced stress levels on development. The high rate of ultrasonic calling found in rotated pups, in the absence of a reduction in body weight gain, seems consistent with this hypothesis because in altricial rodents ultrasonic emission has been correlated with stressful experiences (see [9], for reference).

In the open-field test, carried out on PND21, HG males were more active in both the peripheral and central area and performed more *wall-rearing* during the initial phase of the test than HG females. Moreover HG males sniffed the novel environment less than females. Exploratory behaviour in adult rodents is found to be sexually dimorphic with males being less active and showing more rapid habituation than their female counterparts [16,18,26,36]. Moreover, during adolescence gender differences in the response to environmental challenges have been reported across various mammalian species [48]. Hypergravity during critical postnatal phase seems to affect the shape of males and females behavioural profile at periadolescence, apparently enhancing such differences.

Rotation-induced effects turned out to be independent from those exerted by parity condition. In fact, no interaction between

rotational environment and dam parity has been found, except for the tail length which showed a higher growth rate in HG biparous litters when compared to SC biparous ones. However, since that change was not accompanied by any other somatic growth change, it seems difficult to ascribe it to any general impairment of somatic growth.

Maternal reproductive experience per se exerted a strong influence on pup development and several somatic, including body weight, and sensorimotor parameters were accelerated in the offspring of biparous dams. Moreover, primiparous female offspring showed a better performance in the T-maze test and apparently both males and females were less anxious than biparous one in the open-field.

"Maternal effects" are often associated with variations in the quality of the maternal environment which alters the nature of the dam–offspring interaction and thus the phenotype of the offspring [55]. It is also known that pup behaviour can exert a strong influence on dam responsiveness which in turn is affected by previous nursing experience. Stressed pups elicit different levels of maternal behaviour in primiparous and multiparous rat dams [53]. If pups are stressed during early infancy, maternal behaviour of multiparous dams increases during the first 2 days of treatment and then becomes stable. By contrast, primiparous mothers are consistently responsive to stressed pup cues, showing a sort of "mother on demand" profile but, after termination of stress treatment, and in the presence of minimal pup stress cues, frequency of nursing declines markedly.

Multiparous and primiparous mothers respond differently to the cues of stressed pups and these differences have been correlated to differences in the open-field behaviour of the offspring [52].

Repeated acute exposure to the altered gravity environment during the postnatal phase also exerted slight but significant long-term effects on learning performance in the Morris water-maze. In fact, on the second day of the acquisition trials, RC mice spent more time searching the platform location, and, in the reversal phase, HG mice performed differently when compared to RC or SC mice in all the parameters examined, although some missed statistical significance. The impairment showed by RC mice seems in line with other findings suggesting that stress early in life can induce slight, but significant impairments on acquisition in the Morris water-maze task at adulthood [29]. The reason why the Morris water-maze performance from mice exposed to 1 g but not to 2 g rotation was affected is not clear. However, the same effect has been reported in adolescent mice which underwent to the same treatment [22] suggesting the possibility that HG represents an additional specific stress which leads to several compensatory mechanisms eventually resulting in a different response strategy.

HG reversal has been extensively used to evaluate the ability to shift strategies to task demands [46], since animals have to shift from an acquired response to a different one. Altered performances in Morris water-maze during this phase have been related to changes in attentional process and/or in behavioural flexibility following environmental changes [46]. Thus, the different trend over time in latency to find the new platform location as well as in time spent, number of crossings and swimming path in the acquisition quadrant during the reversal phase, may reflect changes in behavioural responding to changes in task requirements in mice exposed to hypergravity during development. Moreover, it has been recently reported that early exposure to hypergravity induces a facilitation in locomotor behaviour, in particular in the appearance of antigravity activities [6] and Cao et al. [11] have reported that both expression of somatostatin levels in hippocampus and learning performance of rats were differently affected by exposure to different G-levels during the early phases of development.

Following the Morris water-maze test, decreased NGF levels in the frontal cortex of HG mice and decreased BDNF levels in the hypothalamus of both RC and HG mice were found. It is likely that plastic changes in the central nervous system (e.g. neuromodulation, changes in synaptic efficacy, "rewiring", sprouting, reorganisation of neural network) underlie sensorimotor adaptation to changes in gravitational environment [21]. Moreover, during early development and in the adult life, they are implicated in mechanisms regulating synaptogenesis, neuronal organisation, both under normal conditions and following neuronal injury, and environmental challenges [15]. BDNF participates in synaptic plasticity and the adaptive changes in the strength of communication between neurons thought to underlie aspects of behavioural adaptation [25]. Thus, the altered levels of brain NGF and BDNF could represent persistent changes of an early compensatory/recovery mechanism following hypergravity exposure. The possibility that they might be correlated with the slight effects on learning performance in the Morris water-maze cannot be also ruled out. In fact, NGF and BDNF have been reportedly involved in neuronal plasticity underlying cognitive function [50] and long-term consequences on NGF expression has been shown to be induced by stressors administered to rats during postnatal development [40]. Moreover, the reduction in BDNF in the hypothalamus has been reported as a consequence of being exposed to stress suggesting that BDNF may play a role in plasticity processes related to the stress response [41,47] and further experiments should be devoted in the future to assess other stress related hormonal parameters in an hypergravity paradigm.

In analogy with other studies, effects here reported are minor to moderate. Several studies have shown the detrimental effects of microgravity exposure on neurodevelopment in young rodents. The opposite situation, i.e. hypergravity, which strongly stimulates several sensory systems and in particular the vestibular system, may allow, as other environmental mild stressors, to the reported effects on the CNS and behavioural outcomes [44].

Overall, this study confirms and extends previous findings on the neurobehavioural development of rodents subjected to hypergravity, suggesting a role for factors other than changes in the gravito-inertial vector, with stress and relative effects on central nervous system development seeming suitable candidates. Maternal experience confirms its fundamental role in affecting offspring development, apparently without interacting with the specific environmental challenges. Mice thus represent a particularly good model for future space biology research aimed at clarifying the role of altered gravitational environment on development. Further ground-based studies with earlier and

prolonged exposure to hypergravity during critical phases of development should be carried out in mice to better understand neurological and behavioural consequences of adaptation to different gravity environments, as well as developing models to test side-effects of permanence in altered gravity in humans.

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## References

[1] L. Aloe, F. Properzi, L. Probert, K. Akassoglou, G. Kassiotis, A. Micera, M. Fiore, Learning abilities, NGF and BDNF brain levels in two lines of TNF-alpha transgenic mice, one characterized by neurological disorders, the other phenotypically normal, Brain Res 840 (1999) 125–137.

[2] L.A. Baer, G.H. Li, A.E. Ronca, C.E. Wade, Early and late effects of perinatal hyper-gravity exposure on developing CNS, Grav Space Biol Bull 14 (2000) 23.

[3] G. Bignami, Economical test methods for developmental neurobehavioral toxicity, Environ Health Perspect 104 (Suppl. 2) (1996) 285–298.

[4] G. Bignami, E. Alleva, F. Chiarotti, G. Laviola, Selective changes in mouse behavioral development after prenatal benzodiazepine exposure: a progress report, Prog Neuropsychopharmacol Biol Psychiatry 16 (1992) 587–604.

[5] V. Bouet, F. Dijk, J. Ijkema-Paassen, R.J. Wubbels, J.J. van der Want, A. Gramsbergen, Early hypergravity exposure effects calbindin-D28k and inositol-3-phosphate expression in Purkinje cells, Neurosci Lett 382 (2005) 10–15.

[6] V. Bouet, J. Ijkema-Paassen, R. Wubbels, A. Gramsbergen, Development of the locomotor system in 2 G exposed rats, J Gravit Physiol 11 (2004) P165–P166.

[7] V. Bouet, R.J. Wubbels, H.A. de Jong, A. Gramsbergen, Behavioural consequences of hypergravity in developing rats, Brain Res Dev Brain Res 153 (2004) 69–78.

[8] L. Bracci-Laudiero, L. Aloe, R. Levi-Montalcini, M. Galeazzi, D. Schilter, J.L. Scully, U. Otten, Increased levels of NGF in sera of systemic lupus erythematosus patients, Neuroreport 4 (1993) 563–565.

[9] I. Branchi, D. Santucci, A. Vitale, E. Alleva, Ultrasonic vocalizations by infant laboratory mice: a preliminary spectrographic characterization under different conditions, Dev Psychobiol 33 (1998) 249–256.

[10] L.L. Bruce, Adaptations of the vestibular system to short and long-term exposures to altered gravity, Adv Space Res 32 (2003) 1533–1539.

[11] X.S. Cao, X.Q. Sun, Y.H. Wu, X.Y. Wu, T.S. Liu, S. Zhang, Changes of hippocampus somatostatin and learning ability in rats after + Gz exposure, Space Med Med Eng (Beijing) 18 (2005) 79–83.

[12] C. Chabbert, A. Brugeaud, G. Lennan, J. Lehouelleur, A. Sans, Electrophysiological properties of the utricular primary transducer are modified during development under hypergravity, Eur J Neurosci 17 (2003) 2497–2500.

[13] J. Cohen, R.S. Bridges, Retention of maternal behaviour in nulliparous and primiparous rats: effects of duration of previous maternal experience, J Comp Physiol Psychol 95 (1981) 450–459.

[14] C. Cohen-Salmon, M. Carlier, P. Roubertoux, Differences in patterns of pup care in Mus musculus domesticus lI—effects of parity on eight inbred strains, Biol Behav 7 (1982) 337–346.

[15] B. Connor, M. Dragunow, The role of neuronal growth factors in neurodegenerative disorders of the human brain, Brain Res Brain Res Rev 27 (1998) 1–39.

[16] C. de Cabo de la Vega, A. Pujol, M. Paz Viveros, Neonatally administered naltrexone affects several behavioral responses in adult rats of both genders, Pharmacol Biochem Behav 50 (1995) 277–286.

[17] J. DeFelipe, J.I. Arellano, A. Merchan-Perez, M.C. Gonzalez-Albo, K. Walton, R. Llinas, Spaceflight induces changes in the synaptic circuitry of the postnatal developing neocortex, Cereb Cortex 12 (2002) 883–891.

[18] M. Dubovicky, E. Ujhazy, P. Kovacovsky, I. Rychlik, T. Kalnovicova, J. Navarova, P. Turcani, M. Durisova, A. Gajdosik, Effect of long-term administration of stobadine on exploratory behaviour and on striatal levels of dopamine and serotonin in rats and their offspring, J Appl Toxicol 17 (1997) 63–70.

[19] A.S. Fleming, D.H. O'Day, G.W. Kraemer, Neurobiology of mother–infant interactions: experience and central nervous system plasticity across development and generations, Neurosci Biobehav Rev 23 (1999) 673–685.

[20] W.M. Fox, Reflex-ontogeny and behavioural development of the mouse, Anim Behav 13 (1965) 234–241.

[21] R.A. Fox, N.G. Daunton, M.L. Corcoran, Study of adaptation to altered gravity through systems analysis of motor control, Adv Space Res 22 (1998) 245–253.

[22] N. Francia, D. Santucci, F. Chiarotti, E. Alleva, Cognitive and emotional alterations in periadolescent mice exposed to 2 g hypergravity field, Physiol Behav 83 (2004) 383–394.

[23] S. Gaboyard, A. Sans, J. Lehouelleur, Differential impact of hypergravity on maturating innervation in vestibular epithelia during rat development, Brain Res Dev Brain Res 143 (2003) 15–23.

[24] M. Gimenez y Ribotta, F. Sandillon, A. Privat, Influence of hypergravity on the development of monoaminergic systems in the rat spinal cord, Brain Res Dev Brain Res 111 (1998) 147–157.

[25] J.A. Gorski, S.A. Balogh, J.M. Wehner, K.R. Jones, Learning deficits in forebrain-restricted brain-derived neurotrophic factor mutant mice, Neuroscience 121 (2003) 341–354.

[26] R.W. Goy, B.S. McEwen, Sex differences in behavior: rodents, birds, and primates, in: R.W. Goy, B.S. McEwen (Eds.), Sexual Differentiation of the Brain, The MIT Press, Cambridge, 1980, pp. 13–73.

[27] N. Gunzenhauser, Infant Stimulation: for Whom, What Kind, When and How Much? Johnson and Johnson Baby, Biscayne Bay, FL, 1987, 206 pp.

[28] M.A. Hofer, Relationships as regulators: a psychobiologic perspective on bereavement Psychosom Med 46 (1984) 183–197.

[29] R.L. Huot, P.M. Plotsky, R.H. Lenox, R.K. McNamara, Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats, Brain Res 950 (2002) 52–63.

[30] E.M. Inglis, K.E. Zuckerman, M. Roberts, R.G. Kalb, Altered dendritic morphology in a population of motoneurons in rats reared in microgravity, Abstr Soc Neurosci 91 (1999) 9.

[31] G. Laviola, S. Petruzzi, J. Rankin, E. Alleva, Induction of maternal behavior by mouse neonates: influence of dam parity and prenatal oxazepam exposure, Pharmacol Biochem Behav 49 (1994) 871–876.

[32] D.J. Lim, J.A. Stith, C.W. Stockwell, J. Oyama, Observations on saccules of rats exposed to long-term hypergravity, Aerosp Med 45 (1974) 705–710.

[33] R.M. Lindsay, Role of neurotrophins and trk receptors in the development and maintenance of sensory neurons: an overview, Philos Trans R Soc Lond B Sci 351 (1996) 365–373.

[34] A. Loizzo, A. Capasso, G. Galietta, C. Severini, G. Campana, S. Spampinato, Vas deferens response to selective opioid receptor agonists in adult mice is impaired following postnatal repeated mild stress, Eur J Pharmacol 458 (2003) 201–205.

[35] R. Morris, Developments of a water-maze procedure for studying spatial learning in the rat, J Neurosci Methods 11 (1984) 47–60.

[36] A.G. Nasello, C. Machado, J.F. Bastos, L.F. Felicio, Sudden darkness induces a high activity-low anxiety state in male and female rats, Physiol Behav 63 (1998) 451–454.

[37] E. Noirot, Changes in responsiveness to young in the adult mouse. 3. The effect of immediately preceding performances, Behaviour 24 (1965) 318–325.

[38] L.P.J.J. Noldus, The observer: a software system for collection and analysis of observational data, Behav Res Methods Instum Comput 23 (1991) 415–429.
[39] B.G. Orpen, A.S. Fleming, Experience with pups sustains maternal responding in postpartum rats, Physiol Behav 40 (1987) 47–54.
[40] U. Otten, J.B. Baumann, J. Girard, Stimulation of the pituitary–adrenocortical axis by nerve growth factor, Nature 282 (1979) 413–414.
[41] F. Rage, L. Givalois, F. Marmigere, L. Tapia-Arancibia, S. Arancibia, Immobilization stress rapidly modulates BDNF mRNA expression in the hypothalamus of adult male rats, Neuroscience 112 (2002) 309–318.
[42] A.E. Ronca, L.A. Baer, N.G. Daunton, C.E. Wade, Maternal reproductive experience enhances early postnatal outcome following gestation and birth of rats in hypergravity, Biol Reprod 65 (2001) 805–813.
[43] E.M. Sajdel-Sulkowska, G.H. Li, A.E. Ronca, L.A. Baer, G.M. Sulkowski, N. Koibuchi, C.E. Wade, Effects of hypergravity exposure on the developing central nervous system: possible involvement of thyroid hormone, Exp Biol Med (Maywood) 226 (2001) 790–798.
[44] E.M. Sajdel-Sulkowska, K. Nguon, Z.L. Sulkowski, G.D. Rosen, M.G. Baxter, Purkinje cell loss accompanies motor impairment in rats developing at altered gravity, Neuroreport 16 (2005) 2037–2040.
[45] D. Santucci, G. Corazzi, N. Francia, A. Antonelli, L. Aloe, E. Alleva, Neurobehavioural effects of hypergravity conditions in the adult mouse, Neuroreport 11 (2000) 3353–3356.
[46] G. Smith, Animal models of Alzheimer's disease: experimental cholinergic denervation, Brain Res 472 (1988) 103–118.
[47] M.A. Smith, S. Makino, S.Y. Kim, R. Kvetnansky, Stress increases brain-derived neurotropic factor messenger ribonucleic acid in the hypothalamus and pituitary, Endocrinology 136 (1995) 3743–3750.
[48] L.P. Spear, The adolescent brain and age-related behavioral manifestations, Neurosci Biobehav Rev 24 (2000) 417–463.
[49] W.F. Sternberg, C.G. Ridgway, Effects of gestational stress and neonatal handling on pain, analgesia, and stress behavior of adult mice, Physiol Behav 78 (2003) 375–383.
[50] H. Thoenen, Neurotrophins and neuronal plasticity, Science 270 (1995) 593–598.
[51] G. Weskamp, U. Otten, An enzyme-linked immunoassay for nerve growth factor (NGF): a tool for studying regulatory mechanisms involved in NGF production in brain and in peripheral tissues, J Neurochem 48 (1987) 1779–1786.
[52] L. Wright, R.W. Bell, Interactive effects of parity and early pup stress on the open field behavior of laboratory rats, Dev Psychobiol 11 (1978) 413–418.
[53] L.L. Wright, R.W. Bell, H.L. Schreiber, R. Villescas, L. Conely, Interactive effects of parity and pup stress on the maternal behavior of *Rattus norvegicus*, Dev Psychobiol 10 (1977) 331–337.
[54] R.J. Wubbels, J. van Marle, H.N. Sondag, H.A. de Jong, Effects of hypergravity on the morphological properties of the vestibular sensory epithelium. II. Life-long exposure of rats including embryogenesis, Brain Res Bull 58 (2002) 575–580.
[55] T.Y. Zhang, C. Parent, I. Weaver, M.J. Meaney, Maternal programming of individual differences in defensive responses in the rat, Ann NYAS 1032 (2004) 85–103.

# CHAPTER 3

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Research report

# A mouse model of neurobehavioural response to altered gravity conditions: An ontogenetical study

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ABSTRACT

To determine the influence of gravity during critical periods of development is important in the perspective of long-term spaceflight and exploration, data coming from this kind of studies providing insight into basical biological phenomena underlying the development of the nervous system and its plasticity.

Aim of the present study was to evaluate neurobehavioural responses to hypergravity exposure in CD-1 mice at different stage of development. Early adolescent (postnatal day 28, PND 28), adolescent (PND 42) and young-adult (PND 60) male and female mice were exposed to acute 2*g* rotational-generated hypergravity. Motion sickness index and behavioural performances pre, during and after rotation were recorded, and long-lasting effects on exploratory behaviour (hole-board test) and emotional/anxiety-like responses (plus-maze test) were investigated. Furthermore, in order to correlate behavioural changes with alterations in central levels of neurotrophins, brain amounts of Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF) were also assessed on PND 90, following a re-exposure to hypergravity. Age and sex differences were observed, females being more vulnerable than males to motion sickness, and susceptibility to hypergravity increasing with age of exposure. Moreover, mice showed a general reduction in spontaneous activity during the rotation, while recovery time after rotation became progressively longer with increasing age of the experimental subjects. Long-term effects on exploratory behaviour and emotional/anxiety-like response were also observed, behavioural profiles mainly changing in those animals experiencing hypergravity as young-adults. Finally, major changes in brain levels of NGF and BDNF were detected in mice firstly exposed as young-adults.



## 1. Introduction

With the advent of long-term interplanetary missions, space biology has become an emerging field. Exposure to altered gravity has been extensively reported to cause motion sickness (MS) syndrome in mammals, somatosensory inputs in space contradicting or differing from those predicted from experience [42,73], and a variety of mammalian studies have been focused on this syndrome as well as on the neuro-physiological response to changes in gravitational conditions [30].

In rats, pica behaviour (eating non-nutritive substances) upon hypergravity exposure has been considered an index of MS [40,45,62]. MS has been recently characterized also in the mouse (a more suitable species for space missions): exposure to 2*g* induced picaism and pronounced changes in specific behavioural endpoints [53,54].

Susceptibility to MS, and – more in general – the vulnerability of the vestibular system upon exposure to rotational stimuli, changes with age [7,36,42,58,68,72]. In rats, effects of exposure to hypergravity resulted particularly marked just after birth and again at weaning. Moreover, age and gender-specific changes in cerebellar protein as well as subtle alterations in vestibular functions, early motor-reflexes development, exploratory behaviour,

*Abbreviations:* A, age; ADO, adolescent; BDNF, Brain Derived Neurotrophic Factor; CNS, central nervous system; MS, motion sickness; NGF, Nerve Growth Factor; NT-4, neurotrophic factor 4; NT-3, neurotrophic factor 3; PND, postnatal day; R, repeated measures; S, sex; SAP, stretched-attend postures; T, treatment; WEAN, early adolescent; Y-A, young-adult.

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and in spatial learning performance, have been reported both in adolescent and adult rats and mice early exposed to hypergravity [4,8,20,21,23,47,51,52,65].

The aim of the present study was to establish age-related differences in the neurobehavioural response to hypergravity exposure in the CD-1 mouse strain during late postnatal development. Overall, the study was aimed at using exposure to gravitational field as a psycho-physiological challenge to provide insights in the dynamical interaction between the nervous system and environmental cues. In particular, although sensory system development at this age is almost concluded, modification in stressor-sensitive region to environmental experience during adolescence might lead to substantial and long-lasting neurobehavioural alterations.

The occurrence of rotation-induced MS was evaluated in both male and female mice on PND 28, 42 or 60. Ethological scoring before, during, and after exposure to 2*g* was performed to finely evaluate the effect of changes in the gravitational environment on the behavioural repertoire. Moreover, long-lasting effects on exploratory behaviour and emotional/anxiety-like response were also assessed.

Acute exposure experiments are generally aimed to gaining insight on the effects of short episodes of hypergravity, which mimic what normally occurs at launch.

Moreover, changes in neurotrophin levels in the central nervous system (CNS) and altered cerebellar growth upon exposure to rotational stimuli have been reported [4,20,21,28,52,54]. A variety of studies have shown that Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF), in addition to their trophic function on neuronal survival and differentiation, act as modulators of synaptic plasticity [55,64], influencing remodelling of nerve terminals, neurotrasmitter and neuropeptide synthesis and release [12,34], playing a role in some critical aspects of cochlear and vestibular neurons during development [22]. It was therefore considered of interest to investigate whether altered gravity would affect such neurobiological determinants, and NGF and BDNF levels in frontal and parietal cortex, hippocampus, hypothalamus, and olfactory bulbs were evaluated in rotated mice.

## 2. Methods

### 2.1. Animal breeding and fostering

A total of 36 mice (12 males and 24 females) of an outbred Swiss-derived strain (CD-1) weighing 30–33 g (virgin males) or 28–30 g (virgin nulliparous females) were purchased from a commercial breeder (Charles River Italy, I-22050 Calco, Italy). Upon arrival at the laboratory, animals were kept in an air-conditioned room (temperature 21 ± 1 °C, relative humidity 60 ± 10%; lights on from 08:30 p.m. to 08:30 a.m.). Males and females were housed separately in groups of 5–7 in 42 cm × 27 cm × 14 cm Plexiglas boxes with sawdust as bedding. Pellet food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, I-20019, Italy) and tap water were continuously available. Two weeks later, they were coupled in triads (1 male and 2 females) and 15 days later, males were removed and pregnant females singly housed in 33 cm × 13 cm × 14 cm box. On PND 1 (day of delivery = PND 0) all litters were reduced to six pups (three males and three females). Dams remained with pups until weaning, which took place on PND 21. On this day males and females of each litter were housed separately in groups of three. Females were monitored for oestrus cycle throughout the experiment, resulting highly synchronized. Eight litters (three males and three females) for each developmental stage were used.

### 2.2. Motion sickness evaluation

Appearance of rotation-induced motion sickness syndrome was evaluated in early adolescent (WEAN, PND 28), adolescent (ADO, PND 42) and young-adult (Y-A, PND 60) mice. Eight litters (three males and three females per litter) for each ontogenetic age were tested. For 7 days preceding rotation day (adaptation period) animals were adapted by daily exposure to the presence of kaolin (Pharmaceutical grade kaolin hydrate aluminium silicate, Sigma, Milan, I-20151, Italy).

From adaptation days 1–3 animals were maintained in groups of three with free access to pellet food, kaolin and water, while from adaptation day 4 to post-rotational day 5 kaolin access was limited to 2 h/day (from 12:00 a.m. to 02:00 p.m.), during which animals were singly housed in 33 cm × 13 cm × 14 cm Plexiglas boxes. Food and kaolin were weighed daily before and after the 2 h of exposure to the nearest 0.1 g (to evaluate consumption rate) and refilled. Moreover, spilled food and kaolin pieces were collected and weighed to obtain correct consumption values [39–41,54,62,63]. Kaolin paste was prepared according to refs. [39,40,54].

Non-rotational littermate controls for food and kaolin consumption (eight males and eight females for each age) were placed close to the rotation apparatus during the 3 h of the rotation test (see below) to be subjected to the noise and vibration of the turntable apparatus but not be rotated.

### 2.3. Rotational device, procedures and spontaneous behaviour observations

The apparatus is a custom-made prototype designed and manufactured by Isolceram, Rocca Priora, I-00040, Italy, consisting of a turntable (radius = 50 cm) set in motion by a central rotor, number of turns per minutes being adjustable by means of a digital switch. The turntable can hold up to six home cages and during the experiment it was rotated at a constant rate: an angular velocity of 336° $s^{-1}$ (56 rpm) produced a resultant linear acceleration of 2*g*. During rotation, in addition to linear acceleration, mice were exposed to variable Coriolis forces depending on speed and direction of animal motion within the centrifuge cages. Moreover, home cages, were not placed in black larger boxes and animals were submitted to a rotating visual scene.

After adaptation, two females and two males per litter per age were rotated in their individual home cages for 1 h at 2*g*, and their behaviours scored for 1 h before, 1 h during, and 1 h after rotation over 5 min × 5 min-blocks (0–5, 10–15, 25–30, 40–45, 55–60). The presence of the following behavioural items was recorded by an instantaneous (15 s interval) sampling procedure (for details see ref. [54]): *exploring*: moving around the cage, exploring the environment; *wall rearing*: standing on hind legs and placing forelimbs on the wall of the cage; *rearing*: standing on hind legs; *bar holding*: grasping the metal top of cage holding itself above the level of the ground; *running*: moving quickly around the cage without stopping to explore the environment; *sniffing*: sniffing the environment; *digging*: digging in the sawdust, pushing and kicking it around using the snout and/or the fore and hindpaws, mostly moving around the cage and sometimes changing the whole arrangement of the substrate material; *head moving*: making small, rapid, up and down movements with the head; *resting* (either with *closed* or *open eyes*): no visible movements, eyes closed or open. In particular, the open eyes resting is a passive behaviour completely different form the freezing one, which is an active posture; *scratching*: scratching ear with hind leg; *face washing*: self-explanatory; *self-grooming*: wiping, licking, combing any part of the body; *food* or *kaolin eating, drinking* and *sawdust chewing*: self-explanatory.

### 2.4. Hole-board test

On PND 70, mice rotated on PND 28, 42 or 60 and corresponding controls were tested in a hole-board apparatus. The test was carried out under red light between 10:00 a.m. and 01:00 p.m. The hole-board (Ugo Basile, Biological Research Apparatus, Varese, I-21025, Italy) consisted of a square unwalled platform (40 cm × 40 cm), raised 11.5 cm, containing 4 × 4 equally spaced (7 cm) holes, each 1.5 cm in diameter. The mice were placed individually on the platform and their behaviours video-recorded for 7 min using a Sony VO-5630 apparatus equipped with CH-1400 CE videocameras for red light. Locomotor activity was scored by determining the number of sub-areas crossed, while *dipping* behaviour was scored by the number of holes explored (*head-dipping*). A *head-dipping* was scored if the head entered the hole at least up to eyes level. The latency to the first *head-dipping*, the number of different holes visited and the number of visits to the four central holes were also recorded. Moreover, an index of exploratory activity was calculated by dividing the total number of visits by the number of visits to the four central holes [10].

### 2.5. Plus-maze test

On PND 80 mice rotated on PND 28, 42 or 60 and corresponding controls were tested in a plus-maze apparatus. Tests were conducted under red light between 10:00 a.m. and 01:00 p.m. The elevated plus-maze comprised two open arms (30 cm × 5 cm × 0.25 cm) and two closed arms (30 cm × 5 cm × 15 cm) that extended from a common central platform (5 cm × 5 cm). The apparatus was constructed from Plexiglas (black floor, clear walls) and elevated to a height of 60 cm above the floor level. Mice were individually placed on the central platform facing an open arm and allowed to freely explore the maze for 6 min. All sessions were videorecorded by a camera linked to a monitor and VCR (see hole-board test) in the adjacent room and, to avoid unnecessary distractions, the experimenters retreated to this location during testing. Videotapes were scored by a highly trained observer using dedicated ethological software ("The Observer"; [43]). Behavioural parameters included both conventional spatiotemporal and ethological measures according to [18,27]. Conventional measures were the frequencies of total, open and closed entries (arm entry = all four paws into an arm), % open entries [(open/total) × 100], and % time spent in open, closed and central parts of the maze [e.g. (time open/session duration) × 100]. Ethological measures included frequency and duration scores for *rearing* (vertical movement against the side and/or end of the walls; note that mice very rarely exhibit unsupported rearing), *immobility, grooming* (licking, scratching and washing of the head and body), *head-dipping* (exploratory

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movement of head/shoulders over the side of maze) and *stretched-attend postures* (SAP: exploratory posture in which the body is stretched forward and then retracted to the original position without any forward locomotion; [18,27]). Moreover, in view of the importance of thigmotactic cues to rodent exploration in the plus-maze [67], *head-dipping* and SAP were also differentiated as a function of their occurrence in different parts of the maze. Thus, the closed arms and central platform were designated "protected" areas (i.e. offering relative security) and the "percent protected" scores for *head-dipping* and SAP calculated as the percentage of these behaviours displayed in the protected areas (e.g. [(protected SAP/total SAP) × 100]).

### 2.6. NGF and BDNF determination

On PND 90 male mice rotated on PND 28, 42 or 60 underwent a 1 h-long second rotation at 2*g* in order to assess changes in central NGF and BDNF levels induced by the two rotational sessions. Twenty minutes after rotation, animals were sacrificed with an overdose of Nembutal (Abbott Laboratories, North Chicago, IL 60064, USA) and brain region (frontal and parietal cortex, hippocampus, hypothalamus, and olfactory bulbs) quickly removed and stored at −70 °C. Brain sections were removed following the method previously described [3] and samples were used for NGF and BDNF determination. The levels of NGF were measured by a highly sensitive two-site immunoenzymatic assay, which recognizes both murine and human NGF and does not cross-react with BDNF. The exogenous NGF yield was calculated by subtracting the amount of this NGF from the endogenous variety. Under these conditions the recovery of NGF on our assay ranged from 80% to 90% (see refs. [9,70]). BDNF levels were measured following the procedure suggested by the manufacturer (Emaxtm ImmunoAssay System number G6891 by Promega, Madison, WI, USA), using a monoclonal anti-mouse-BDNF antibody and a polyclonal anti-human-BDNF antibody. BDNF concentration was determined from the regression line for the BDNF standard curve (ranging from 7.8 to 500 pg/ml-purified mouse BDNF) incubated under similar conditions in each assay. The sensitivity of the assay is about 15 pg/ml of BDNF, and the cross-reactivity with other related neurotrophic factors (NGF, NT-3, and NT-4) is considered nil (see also ref. [2]).

### 2.7. Statistical analyses

Kaolin and food consumption were evaluated using a mixed-model parametric ANOVA considering age as between-litter factor, litter as random blocking factor nested under age, sex as within-litter factor, and pre- and post-rotation as repeated measures. For behavioural parameters, we used Kruskal–Wallis non-parametric ANOVA for independent groups to analyze the main effect of age and the interactions of age (A) × sex (S) and A × treatment (T), and Friedman non-parametric ANOVA for repeated measures to analyze the main effect and interactions of within-litter factors. Hole-board and plus-maze performances were evaluated by a mixed-model parametric ANOVA, considering age of exposure as between-litter factor, litter as random blocking factor nested under age and sex as within-litter factor. Differences in neurotrophin levels in the central nervous system were assessed by ANOVA considering age of first exposure and treatment as grouping factor and different brain areas as repeated measures.

## 3. Results

### 3.1. Food and kaolin consumption

As a whole, food consumption on post-rotational days was not affected by prior rotational-generated hypergravity stimuli.

Picaism was evidenced in mice, a significant increase in kaolin consumption being observed in rotated mice on post-rotational days ($F(1,21) = 4.5$; $p < 0.05$), the difference becoming more pronounced after the third day (data not shown for repeated measures; see Fig. 1).

Moreover, significant age differences emerged in kaolin consumption with Y-A consuming more kaolin than ADO, and ADO more than WEAN ($F(2,21) = 6.16$; $p < 0.05$). A sex effect was also observed, females consuming more kaolin than males ($F(1,21) = 4.13$; $p < 0.05$; data not shown).

### 3.2. Spontaneous behavioural observations

#### 3.2.1. Exploring

At all ages considered, rotation elicited a short-lasting (first two or three 5 min-blocks) increase (T × repeated (R); $p < 0.05$) in exploration. Females showed especially high levels of this behaviour, particularly during and following rotation (T × S, $\chi^2 = 10.67$, d.f. = 2; $p < 0.05$). Quite interestingly, a similar pattern emerged when rotation was stopped, and mice re-entered the normal gravitational field (see Fig. 2; data for repeated measures not shown). This was the only item in which sex differences were evident.

#### 3.2.2. Wall rearing

A T × A effect emerged for this behaviour, higher levels being observed in ADO during the first 5 min-block when compared with WEAN and Y-A (T × A, $\chi^2 = 7.66$, d.f. = 2; $p < 0.05$). During rotation and in post-rotation, performance dramatically decreased, apparently more so in ADO and Y-A than in WEAN (T × A × R; $p < 0.05$).

#### 3.2.3. Rearing

Rotation completely abolished it, which rapidly reappeared in the post-rotational phase nearly reaching pre-rotational levels. No age differences were evident.

#### 3.2.4. Bar holding

This item, having full habituation profile even in the youngest animals, showed a decrease during pre-rotation, was nearly abolished by rotation, and re-emerged in post-rotation (T × R, $\chi^2 = 40.52$, d.f. = 8; $p < 0.05$). The increase in post-rotation was much more rapid in ADO than in either WEAN or Y-A animals (A × T, $\chi^2 = 11.30$, d.f. = 2; $p < 0.05$).

#### 3.2.5. Running

Among locomotor-type behaviours, this was the most affected in the rotation phase, reaching very high levels in the first half of the rotation session, both in WEAN and ADO animals. Animals of all ages showed no significant changes during the second half of the session, with a complete abatement at the end of the session (A × T, $\chi^2 = 15.15$, d.f. = 2; $p < 0.05$).

#### 3.2.6. Sniffing

This was continuously present in pre-rotation, decreased substantially during rotation and then rebounded in the first half of the post-rotation session, decreasing again in the second half (T, $\chi^2 = 87.52$, d.f. = 2; $p < 0.05$; T × R, $\chi^2 = 57.57$, d.f. = 8; $p < 0.05$). The rebound trend in post-rotation was more marked in WEAN and ADO than in Y-A animals (A × T, $\chi^2 = 4.85$, d.f. = 2; $p = 0.08$).

#### 3.2.7. Digging

At all ages this behaviour was present in pre-rotation, then totally suppressed during rotation. In post-rotation it became progressively more pronounced, so that pre-rotational levels have been achieved by the end (T, $\chi^2 = 129.67$, d.f. = 2; $p < 0.05$).

#### 3.2.8. Head moving

This was rather rare in pre-rotation, but consistently increased during rotation, particularly in older animals (A × T, $\chi^2 = 15.45$, d.f. = 2; $p < 0.05$).

#### 3.2.9. Open eyes resting

This never occurred in pre-rotation; however, in rotation it was expressed consistently at all ages (T, $\chi^2 = 31.70$, d.f. = 2; $p < 0.05$). An age-dependent pattern of sensitivity emerged during post-rotation, with WEAN mice recovering faster and better than both ADO and Y-A subjects (A × T, $\chi^2 = 15.48$, d.f. = 2; $p < 0.05$).

#### 3.2.10. Closed eyes resting

This behaviour, present in pre-rotation, increased progressively through both rotational and post-rotational sessions. WEAN subjects showed higher frequency of this behaviour than ADO and Y-A (A × T, $\chi^2 = 7.99$, d.f. = 2; $p < 0.05$).

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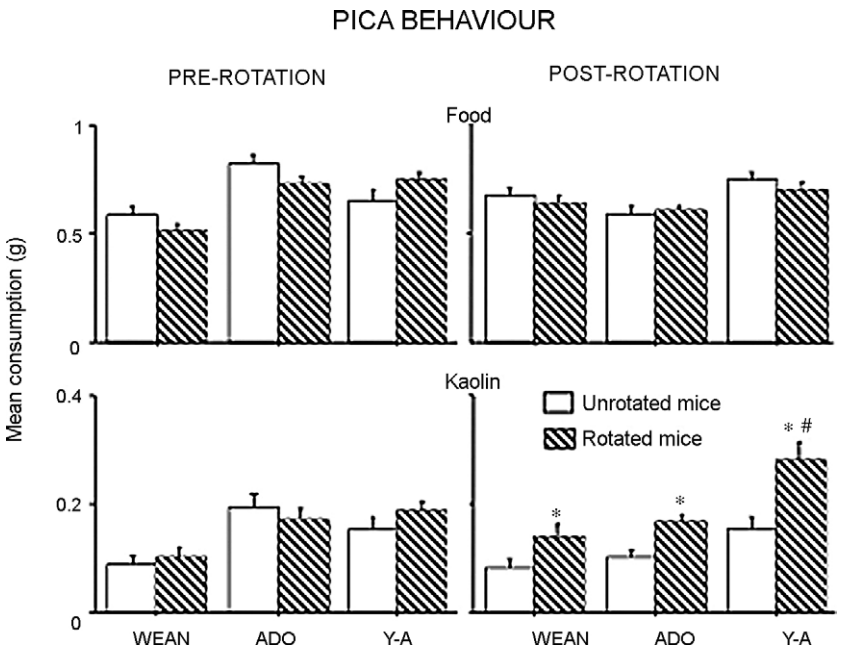


**Fig. 1.** Daily total consumption of food and kaolin by male and female mice exposed to 1 h-2*g* hypergravitational stimuli when early adolescent (WEAN, PND 28), adolescent (ADO, PND 42) or young-adult (Y-A, PND 60). Data are means (±S.E.) of eight litters. *Rotated vs. unrotated mice; #Y-A vs. WEAN or ADO; $p < 0.05$.

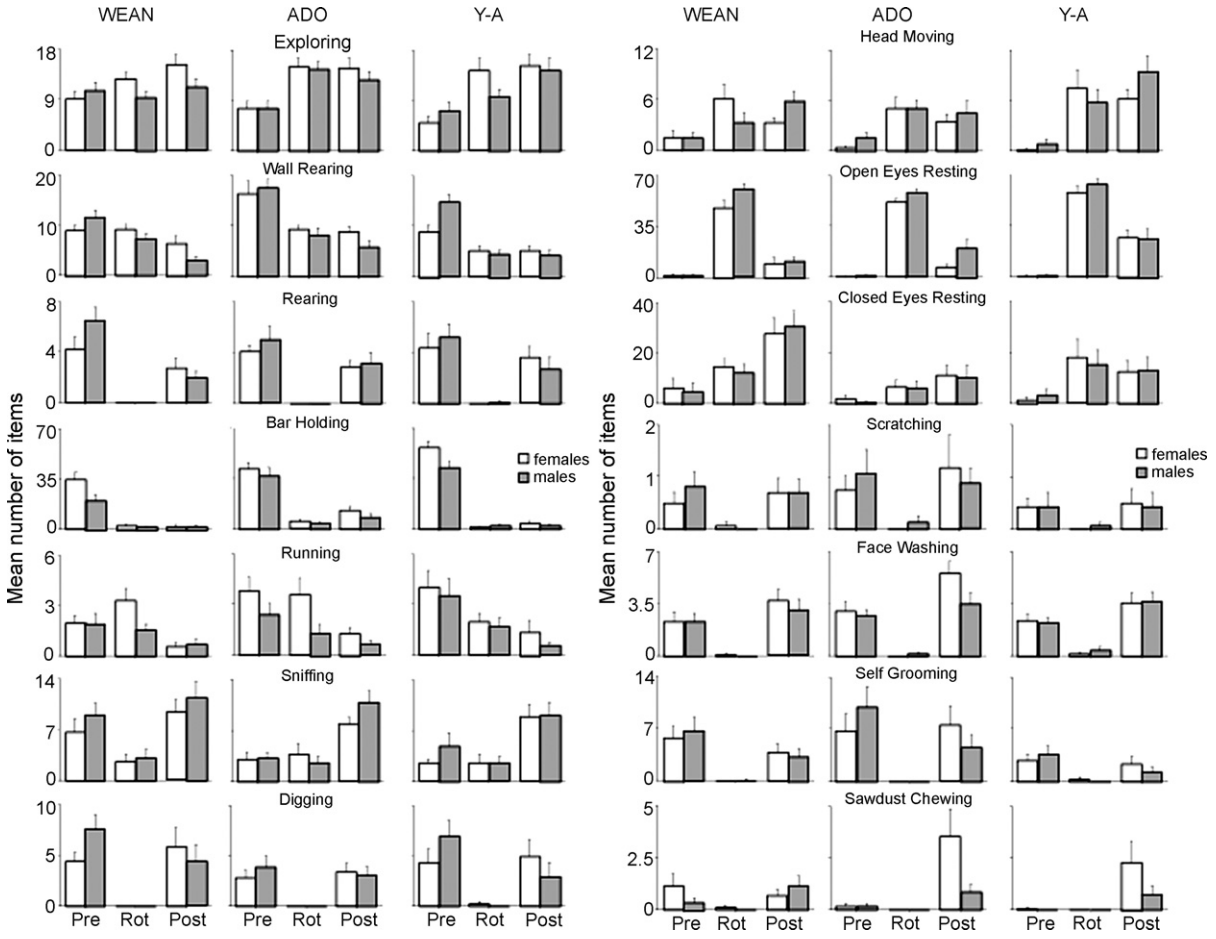


**Fig. 2.** Behavioural responses of early adolescent (WEAN, PND 28), adolescent (ADO, PND 42) and young-adult (Y-A, PND 60) male and female CD-1-mice occurring during 60 min pre-, during and post-rotation periods. Data are means (±S.E.) of eight litters. Significant values are reported in the results section.

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**Table 1**
Parameters of exploration in a hole-board test by 70-day-old male and female mice exposed to 1 h-2*g* hypergravitational stimuli when early adolescents (WEAN, PND 28), adolescents (ADO, PND 42), or young-adults (Y-A, PND 60)

| | Latency to the first visit (s) | Different holes visited | Visits to the central holes | Total visits/visits to the cental holes | Number of crossings |
|---|---|---|---|---|---|
| WEAN | [a] | | | | [a,b] |
| Unrotated females | 7.4 ± 1.3 | 14.0 ± 1.0 | 49.2 ± 4.6 | 11.7 ± 1.9 | 68.4 ± 15.9 |
| Rotated females | 8.6 ± 2.5 | 15.0 ± 0.3 | 51.2 ± 4.4 | 14.2 ± 2.3 | 84.0 ± 15.8 |
| Unrotated males | 10.9 ± 3.0 | 14.0 ± 0.1 | 57.5 ± 5.6 | 9.2 ± 2.5 | 95.6 ± 11.4 |
| Rotated males | 12.5 ± 3.0 | 15.0 ± 1.0 | 55.5 ± 5.8 | 14.7 ± 1.9 | 100.6 ± 11.8 |
| ADO | [a] | | | [a] | [a,b] |
| Unrotated females | 9.4 ± 2.1 | 15.0 ± 0.4 | 50.2 ± 4.5 | 21.0 ± 1.6 | 61.4 ± 5.5 |
| Rotated females | 16.6 ± 2.6 | 15.0 ± 0.5 | 50.4 ± 4.7 | 12.7 ± 2.0 | 72.1 ± 10.8 |
| Unrotated males | 13.2 ± 3.4 | 15.0 ± 0.2 | 50.2 ± 4.0 | 17.0 ± 2.8 | 77.7 ± 6.6 |
| Rotated males | 22.6 ± 8.3 | 15.0 ± 0.4 | 56.4 ± 4.3 | 13.7 ± 1.7 | 85.1 ± 8.8 |
| Y-A | [c] | | | | [a,b] |
| Unrotated females | 17.4 ± 3.3 | 14.5 ± 0.3 | 50.1 ± 7.7 | 17.1 ± 2.1 | 46.9 ± 7.1 |
| Rotated females | 26.9 ± 7.6 | 13.5 ± 0.8 | 58.1 ± 2.3 | 15.6 ± 2.6 | 47.0 ± 8.0 |
| Unrotated males | 14.0 ± 3.3 | 15.0 ± 0.3 | 53.7 ± 4.3 | 10.9 ± 1.8 | 74.2 ± 3.7 |
| Rotated males | 15.8 ± 4.3 | 15.0 ± 0.2 | 54.5 ± 6.0 | 15.0 ± 2.0 | 65.0 ± 10.8 |

Data are mean ± S.E. with of eight litters.
[a] Rotated vs. unrotated mice.
[b] Female vs. male mice.
[c] Rotated vs. unrotated females; $p < 0.05$.

#### 3.2.11. Scratching

Almost totally suppressed during rotation at all ages, *scratching* was more evident in WEAN animals both in pre- and post-rotation (A × T, $\chi^2 = 7.46$, d.f. = 2; $p < 0.05$).

#### 3.2.12. Face washing

This was expressed in animals of all ages in pre- and post-rotation, while during rotation it was shown only by Y-A animals (A × T, $\chi^2 = 17.82$, d.f. = 2; $p < 0.05$).

#### 3.2.13. Self-grooming

This behaviour disappeared almost totally during rotation in mice of all ages. During both pre- and post-rotation phases it was performed at higher levels by WEAN and ADO than by Y-A (A × T, $\chi^2 = 14.78$, d.f. = 2; $p = 0.05$).

#### 3.2.14. Drinking and food eating

Both these were almost totally suppressed during the rotation, the few expressions being limited to ADO and Y-A animals in the first 5 min-block (data not shown).

#### 3.2.15. Sawdust chewing

This behaviour was very rarely observed; it was performed at low levels by WEAN in pre- and post-rotation, while ADO and Y-A chewed sawdust only in post-rotation (A × T, $\chi^2 = 17.19$, d.f. = 2; $p < 0.05$).

### 3.3. Hole-board test

Rotated animals showed longer latencies to the first *head-dipping* ($F(1,21) = 4.73$; $p < 0.05$). This trend was observed in either males and females exposed to rotation as WEAN or ADO, while of animals exposed as Y-A only females showed this tendency ($F(2,21) = 4.42$; $p < 0.05$). Sex, hypergravity stimuli and age of exposure did not affect the total number of *head-dippings* or number of visits to the four central holes (see Table 1).

However, with respect to the ratio, total number of visits/number of visits to the four central holes, a T × A of exposure effect emerged, animals rotated when ADO visiting more consistently the central holes ($F(2,21) = 8.32$; $p < 0.05$).

Females were less active than males ($F(1,21) = 9.30$; $p < 0.05$). Moreover, animals undergoing hypergravity challenge at different ontogenetical stages showed change in levels of spontaneous activity behaviour, number of sub-areas crossed decreasing with increasing age of exposure ($F(2,21) = 6.33$; $p < 0.05$).

### 3.4. Plus-maze test

Mice rotated when ADO, and related controls, showed high levels of open and closed arms entries ($F(2,21) = 4.56$ and $F(2,21) = 4.44$, respectively; $p < 0.05$; see Fig. 3). Age of exposure appeared to have no effect on time spent in the different parts of the maze; however, an A × T effect emerged in the percentage of time spent in open and closed arms ($Fs(2,21) = 4.19$; 5.6; $p < 0.05$). Specifically, mice rotated as WEAN or ADO stayed longer than controls in the open arms, whereas the opposite trend was observed for mice rotated at adulthood (data not shown for duration). Overall, males entered the open arms more frequently than females ($F(1,21) = 19.78$; $p < 0.05$), and a T × S effect ($F(1,21) = 5.66$; $p < 0.05$) on percentage of time spent in the closed arms was observed, rotated females spending less time and rotated males spending more than their un-rotated counterparts (data not shown).

As for behavioural items, animals rotated as WEAN showed a lower frequency (but not a shorter duration) of *immobility* ($F(2,21) = 4.35$; $p < 0.05$), males showed a higher frequency and longer duration of *rearing* behaviour than females ($Fs(1,21) = 7.47$; 6.12; $p < 0.05$; see Fig. 3), while no age, treatment, or sex effect was observed for *grooming* behaviour.

Differences among groups were observed in the frequency and duration of *head-dipping*. Specifically, mice exposed to hypergravity challenge as Y-A performed *head-dipping* more frequently and for longer than those exposed as WEAN ($Fs(2,21) = 3.85$; 3.27; $p < 0.05$). Overall, males performed more *head-dippings* than females ($F(1,21) = 6.17$; $p < 0.05$). However, percentage of *head-dippings* in the protected areas was influenced by an A × T effect, with animals rotated as Y-A increasing the number of protected *head-dipping*, while the opposite trend was observed for animals rotated as WEAN or ADO.

Moreover, it appears that in the protected areas, overall females expressed *head-dipping* more often than males ($F(1,21) = 5.53$; $p < 0.05$), while rotated females did less *head-dipping* than unrotated ones ($F(1,21) = 5.92$; $p < 0.05$).

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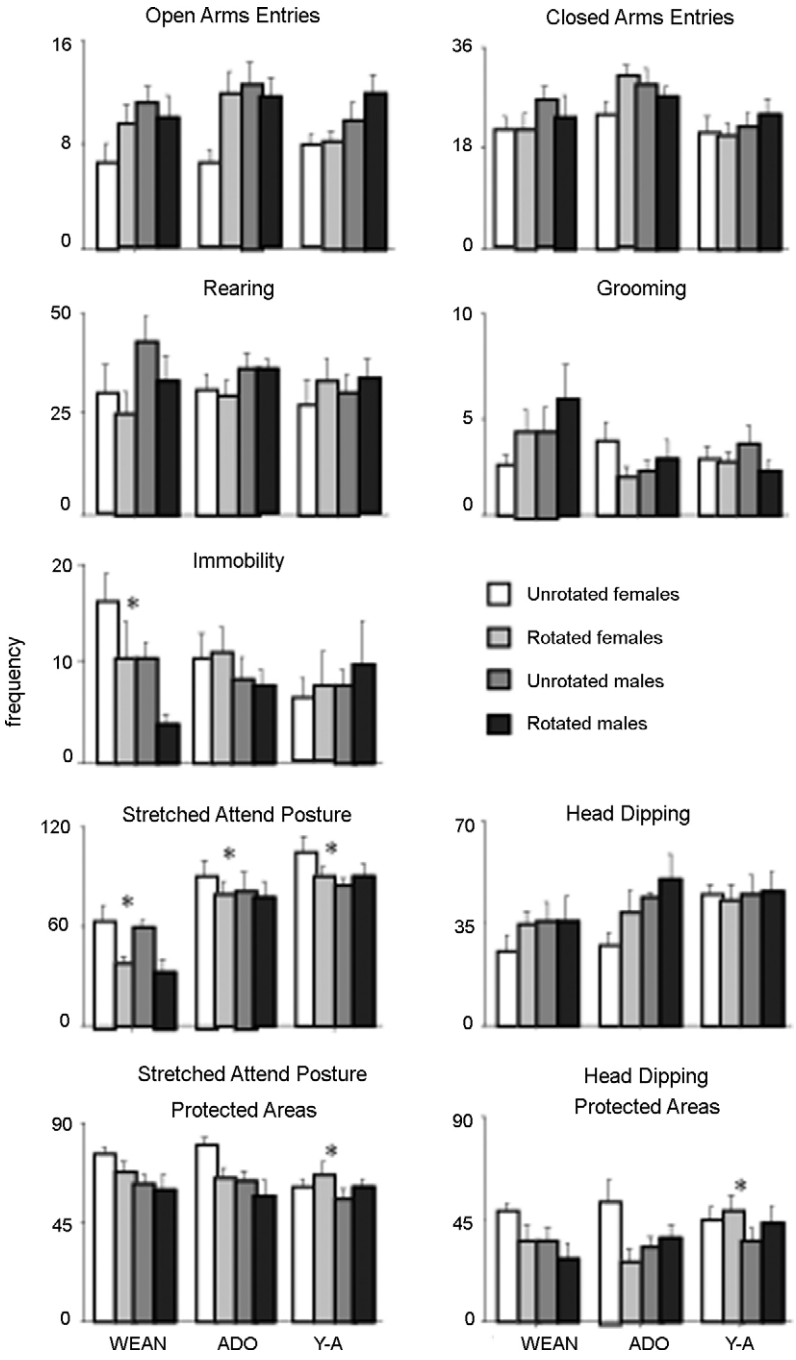


**Fig. 3.** Plus-maze behavior in 80-day-old male and female mice exposed to 1 h-2*g* hypergravitational stimuli when early adolescents (WEAN, PND 28), adolescents (ADO, PND 42) or young-adults (Y-A, PND 60). Data are means (±S.E.) of eight litters. *Rotated vs. unrotated mice; $p < 0.05$.

Animals rotated at all ages showed lower levels of SAP behaviour than unrotated controls. Moreover, an A × T effect was observed for frequency and duration of SAP. Animals rotated as WEAN or ADO performed fewer and longer SAPs than their age-matched controls: $F(2,21) = 3.63$, statistically significant only for duration; $p < 0.05$. By contrast, animals rotated when Y-A showed higher levels of protected SAP ($F(2,21) = 3.61$; $p < 0.05$). A sex effect was also observed, females assuming this posture for longer than males ($F(1,21) = 6.15$; $p < 0.05$). Analogously, a sex effect was observed for the percentage of SAPs occurring in the protected area, with females still consistently displaying these postures more than males ($F(1,21) = 15.35$; $p < 0.05$).

### 3.5. NGF and BDNF

A clear increase in NGF levels was evident in the hippocampus and in the olfactory bulbs of all rotated animals (*F*s(1,36) = 87.5; 14.2, respectively; $p < 0.05$), while a trend for an increase in BDNF levels in the hypothalamus was observed (see Fig. 4) only in animals exposed to the first rotation when Y-A ($F(1,36) = 4.5$; $p < 0.05$). Among rotated animals, age of first exposure was relevant to the effects of rotation. Specifically, animals exposed when WEAN had higher NGF levels than those exposed when ADO or Y-A in the frontal and parietal cortex and in the olfactory bulbs, while animals exposed when Y-A showed the highest levels of this neurotrophin

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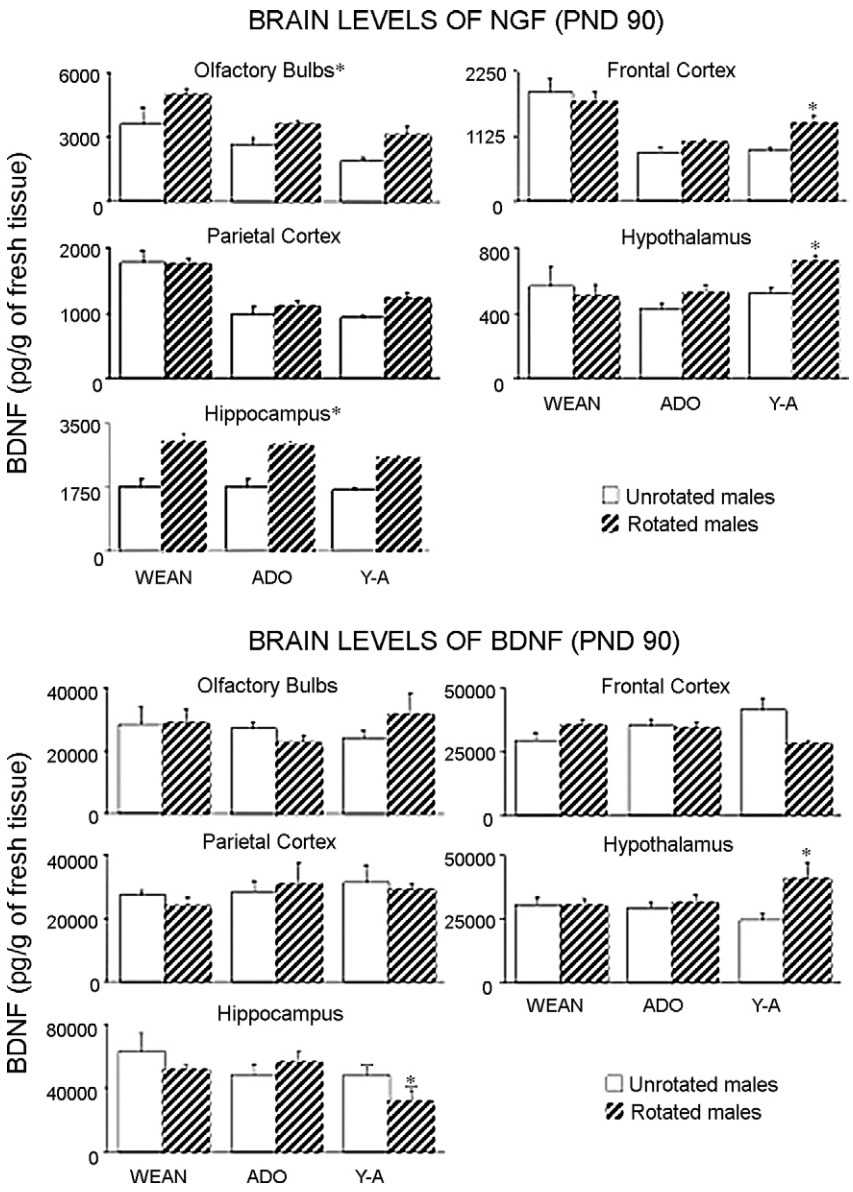


**Fig. 4.** Brain NGF and BDNF levels (expressed as pg/g of fresh tissue) in unrotated and rotated 90-day-old CD-1 male mice. Rotated subjects underwent 1 h-2*g* hypergravitational stimuli when early adolescents (WEAN, PND 28), adolescents (ADO, PND 42) or young-adults (Y-A, PND 60). *Rotated vs. unrotated mice; $p < 0.05$.

in the hypothalamus. Moreover, mice first exposed to rotation when Y-A and rotated again on PND 90 showed lower levels of BDNF in the hippocampus ($F(2,18) = 5.2$; $p < 0.05$) than animals first exposed as WEAN or ADO.

## 4. Discussion

Exposure to rotation-induced hypergravitational stimuli highly affected the behavioural repertoire of developing CD-1-mice as well as brain levels of neurotrophins.

### 4.1. Kaolin consumption

At all ages, pica behaviour was observed in mice exposed to acute rotational stimuli and was more pronounced in females than males, this latter effect being in agreement with data previously reported for rodents [54], insectivora [29] and humans [42]. Concerning age differences in kaolin consumption, a study performed on rats [36] reports 20-month-old rats engaging in significantly less kaolin consumption than either 2- or 11-month-old animals. In the present mouse study, we found that Y-A (2 months old) eat more kaolin than ADO and WEAN combined, all data suggesting a non-linear profile in MS response over life span. Age related changes in the pattern of vulnerability to MS have been reported in humans [13,42,68], with neonates appearing almost unaffected and children being most affected. A reduction in such vulnerability has then been reported up to the age of 40, after when MS syndrome increases again.

### 4.2. Behavioural observations

Overall, and in agreement with data previously reported for rodents [17,46,54,59], squirrel monkeys [71] and humans [24,42], a decrease in spontaneous behaviour was evident during and immediately after the rotation, with several activities almost or totally suppressed during the rotation. For the present study, it should be also taken into account that considered the relatively small diameter of the centrifuge, some changes in behavioural per-

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formance upon centrifugation should be related to the Coriolis effect.

Differences in behavioural repertoire among the three age groups were evident during pre-rotation, while, with the exception of exploring behaviour, no major sex differences were observed. Upon or after rotation, ADO and Y-A mice showed the highest number of changes in behavioural responses, suggesting a lower susceptibility in youngest (WEAN) animals to changes in gravitational environment. *Bar-holding* behaviour performed at high level by ADO, may in fact indicate discomfort in distressed animals, while eating sawdust by ADO and Y-A, may be explained as age-typical performance of pica behaviour. Moreover, WEAN recovered faster from *open eyes resting* in post-rotation—this item being an established index of MS in mice [54]. A role for changes in vestibular nuclei, whose full maturation continues until the end of the first month of life, as well as in projections from these nuclei versus relevant areas of the central nervous system should be taken into account for the observed effects.

When considering long-lasting effects of rotational exposure, a delay in the onset of exploratory behaviour in a novel environment was observed in rotated animals of all ages. Moreover, mice rotated when WEAN and ADO showed a reduction in emotional/anxiety-like response. By contrast, animals rotated when Y-A showed increased number of head dipping and SAP when in the protected area (closed arms) of the maze, suggesting an elevated level of anxiety-like behaviours in these animals. SAP are considered a primary index of risk assessment, a group of behaviours that are thought to facilitate information gathering in potentially dangerous environments, and seem particularly sensitive to anti- or proanxiety drugs (see refs. [48,50]). Behavioural observations on stress-like (or emotional-like) response related to changes in gravitational conditions report an increase in defecation [44], a reduction in exploratory behaviour as well as vibrissae activation and breathing [16] in rats subjected to changes in gravito-inertial force for 60–90 min. With repeated or chronic exposure, animals seem to habituate to the rotation [44], and no changes in defecation rate have been reported after long-term exposure to 2.5*g* condition in hamsters [59]. Moreover, hypothalamo–pituitary–adrenal axis activation (see below) and alterations in brain serotonin metabolism [6] have been reported in microgravity animals.

Long-lasting effects of repeated exposure to hypergravity also include altered locomotor activity and impaired swimming navigation in rats [19] and hamsters [59], in this latter species the effect being more severe in subject exposed at adulthood than at younger ages. Moreover, brains from rat pups born and raised under prolonged hypergravity conditions (23 h/day) show relevant changes in forebrain and cerebellum sizes, general developmental effects being greatest just after birth and again at weaning [4]. Interestingly, rats exposed to microgravity during spaceflight (14 days in microgravity since postnatal day 8) did not develop the normal mature righting behaviour after landing, with an infantile pattern still present when tested 110 days later [26].

### 4.3. Neurobiological markers

Neural processes involved in both short- and long-term neurobehavioural adaptation to hyper- or microgravity are though to be similar to those involved in learning and/or in recovery from neural damage, i.e., experience-dependent neural plasticity [35,56,69]. It has been suggested [19] that it is likely that plastic changes in the CNS (e.g., neuromodulation, changes in synaptic efficacy, "rewiring", sprouting, reorganization of neural network) underlie sensori-motor adaptation to changes in gravitational environment. NGF and BDNF are two neurotrophins that play a critical role on neuronal survival and phenotypic differentiation [32,64]. During early development and in adult life, they are implicated in mechanisms regulating synaptogenesis, neuronal organization both under normal conditions and following neuronal injury and environmental challenges [15].

Frontal cortex is a region involved in MS syndrome [38,73], and plastic neural changes in corticovestibular pathways may explain the observed NGF increase, and further studies should be devoted to evaluate possible alteration in BDNF and NGF in these areas. For example, a dramatic increase in Fos-positive cells in this area has been reported in rats following exposure to 90 min hypergravity [16], and it has been suggested that, in addition to other otolith input, signals from the vestibular system to the cortex may contribute to cognitive functions like body scheme, spatial cognition, and navigation, as well as visual spatial constancy [5,25,66].

NGF and to a lesser extent BDNF, were affected in mice re-exposed to rotational stimuli when 90-day-old. NGF increased in the hippocampus, olfactory bulbs and in both parietal and frontal cortex of rotated animals, confirming a role of this peptide in brain "precognitive" areas, characterized by higher plasticity and fast turnover, in the context of adaptive responses to hypergravitational stimuli. Previous data in which adult mice were once exposed to 1 h at 2*g* [54] did not show any NGF increase in olfactory bulbs and in the parietal cortex, suggesting that previous experience with hypergravity might be required for later changes in neuronal plasticity to occur. In addition, at weaning, animals appear to be more prone to show changes in neurotrophin levels in response to external manipulation, both rotated and control subjects showing a dramatic NGF elevation in the parietal and frontal cortex. Ontogenetical data on brain function during adolescence suggest that prominent neural change occurs in this period in regions such as the frontal cortex not only in humans but also across mammalian species ranging from rodents to non-human primates [31,49,60,61]. Therefore, manipulation *per se* (in the present study consisting, in the non-rotated controls, in the daily experimental procedure for kaolin adaptation and consumption evaluation) at adolescence seems relevant to the long-lasting changes in NGF levels observed at adulthood, such an effect potentially overwhelming any possible changes due to the exposure to rotational stimuli. Indeed, there is evidence that specific neuronal response and survival might be associated to individual neurotrophic factor released either by target-innervated tissues or through paracrine or autocrine mechanism [11]. This neurotrophic dependency of CNS neurons can be altered not only by surgical or chemical manipulation, but also by behavioural responding. It is therefore possible that the different response in neurotrophin distribution observed in our studies is part of this homeodynamic mechanism. However, these and other questions arising by the present findings need to be addressed with future studies. Likewise the influence of NGF on peripheral NGF responsive cells, the effect on immunocompetent cells, the effect on undifferentiated stem cells localized in the brain ventricles, olfactory bulbs and in the hippocampal formation [37] remains to be explored. In addition, the possible involvement of NGF in the adaptation of the vestibular system to changes of gravitational field needs to be investigated in the future.

Finally, animals rotated as Y-A showed higher levels of NGF and BDNF in the hypothalamus. Elevations in NGF levels in this area has already been related to stressful stimuli [1,14], this peptide being a key element in controlling neuroendocrine responses [32], and increased levels of BDNF in the hypothalamus have been reported in animals subjected to acute or repeated stress (such as immobilization; [57]), this latter neurotrophic factor overlapping distributions and functions in several brain areas with NGF [33]. Thus, such NGF and BDNF increase in animals exposed as Y-A appear to parallel behavioural observations indicating that older animals experience a higher level of stress upon centrifugation. Further experiments are

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in progress to verify if these neurobehavioural responses correlate to changes in circulating levels of corticosterone.

## 5. Conclusions

Behavioural and neurobiological changes upon altered gravitational stimuli have been reported for several different species in different experimental paradigms. All data suggest the existence of critical developmental phases during which an organism is particularly adaptable/susceptible to alterations in this relevant aspect of their environment. Exposure to this "new" condition during an early stage of development induces functional rearrangements and long-lasting neurobehavioural adaptations, representing a very powerful tool for developmental neurobiology investigation.

The study confirms and extends previous findings on the neurobehavioural response of rodents to hypergravitational stimuli. Stress, coping and relative plastic changes in selected CNS areas, besides alterations of the gravito-inertial vector, might significantly contribute for the observed behavioural changes.

Finally, mice appear to represent a particularly good model for future space biology research aimed at understanding neurological and behavioural consequences of the adaptation and re-adaptation to different gravity environments. Further ground-based studies with prolonged and/or earlier exposure of this small-sized mammalian species should be developed in the view of prospective microgravity space missions.

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## References

[1] Alleva E, Petruzzi S, Cirulli F, Aloe L. NGF regulatory role in stress and coping of rodents and humans. Pharmacol Biochem Behav 1996;54:65–72.
[2] Aloe L, Properzi F, Probert L, Akassoglou K, Kassiotis G, Micera A, et al. Learning abilities, NGF and BDNF brain levels in two lines of TNF-alpha transgenic mice, one characterized by neurological disorders, the other phenotypically normal. Brain Res 1999;840:125–37.
[3] Aloe L, Fiore M, Santucci D, Amendola T, Antonelli A, Francia N, et al. Effect of hypergravity on the mouse basal expression of NGF and BDNF in the retina, visual cortex and geniculate nucleus: correlative aspects with NPY immunoreactivity. Neurosci Lett 2001;302:29–32.
[4] Baer LA, Li GH, Ronca AE, Wade CE. Early and late effects of perinatal hypergravity exposure on developing CNS. Grav Space Biol Bull 2000;14:23.
[5] Berthoz A. How does the cerebral cortex process and utilize vestibular signals? In: Baloh RW, Halmagyu GM, editors. Disorder of the vestibular system. New York: Oxford UP; 1996. p. 113–25.
[6] Blanc S, Somody L, Gharib A, Gauquelin G, Gharib C, Sarda N. Counteraction of spaceflight-induced changes in the rat central serotonergic system by adrenalectomy and corticosteroid replacement. Neurochem Int 1998;33:375–82.
[7] Bouët V, Gahery Y, Lacour M. Behavioural changes induced by early and long-term gravito-inertial force modification in the rat. Behav Brain Res 2003;139:97–104.
[8] Bouët V, Wubbels RJ, de Jong HA, Gramsbergen A. Behavioural consequences of hypergravity in developing rats. Brain Res Dev Brain Res 2004;153:69–78.
[9] Bracci-Laudiero L, Aloe L, Levi-Montalcini R, Galeazzi M, Schilter D, Scully JL, et al. Increased levels of NGF in sera of systemic lupus erythematosus patients. Neuroreport 1993;4:563–5.
[10] Calamandrei G, Pennazza S, Ricceri L, Valanzano A. Neonatal exposure to anti-nerve growth factor antibodies affects exploratory behavior of developing mice in the hole board. Neurotoxicol Teratol 1996;18:141–6.
[11] Canals JM, Checa N, Marco S, Akerud P, Michels A, Perez-Navarro E, et al. Expression of brain-derived neurotrophic factor in cortical neurons is regulated by striatal target area. J Neurosci 2001;21:117–24.
[12] Cellerino A, Pinzon-Duarte G, Carroll P, Kohler K. Brain-derived neurotrophic factor modulates the development of the dopaminergic network in the rodent retina. J Neurosci 1998;18:3351–62.
[13] Chinn HI, Smith PK. Motion sickness. Pharmacol Rev 1955;7:33–82.
[14] Cirulli F, Alleva E, Antonelli A, Aloe L. NGF expression in the developing rat brain: effects of maternal separation. Brain Res Dev Brain Res 2000;123:129–34.
[15] Connor B, Dragunow M. The role of neuronal growth factors in neurodegenerative disorders of the human brain. Brain Res Brain Res Rev 1998;27:1–39.
[16] Dit Duflo GS, Gestreau C, Lacour M. Fos expression in the rat brain after exposure to gravito-inertial force changes. Brain Res 2000;861:333–44.
[17] Eskin A, Riccio D. The effects of vestibular stimulation on spontaneous activity in the rat. Psychol Rec 1966;16:523–7.
[18] Fernandes C, File SE. The influence of open arm ledges and maze experience in the elevated plus-maze. Pharmacol Biochem Behav 1996;54:31–40.
[19] Fox RA, Daunton NG, Corcoran ML. Study of adaptation to altered gravity through systems analysis of motor control. Adv Space Res 1998;22:245–53.
[20] Francia N, Santucci D, Chiarotti F, Alleva E. Cognitive and emotional alterations in periadolescent mice exposed to 2*g* hypergravity field. Physiol Behav 2004;83:383–94.
[21] Francia N, Simeoni M, Petruzzi S, Santucci D, Aloe L, Alleva E. Repeated acute exposures to hypergravity during early development subtly affect CD-1 mouse neurobehavioural profile. Brain Res Bull 2006;69:560–72.
[22] Fritzsch B, Silos-Santiago I, Bianchi LM, Farinas I. The role of neurotrophic factors in regulating the development of inner ear innervation. Trends Neurosci 1997;20:159–64.
[23] Gimenez y Ribotta M, Sandillon F, Privat A. Influence of hypergravity on the development of monoaminergic systems in the rat spinal cord. Brain Res Dev Brain Res 1998;111:147–57.
[24] Graybiel A, Kennedy RS, Knoblock EC, Guedry Jr FE, Mertz W, McLeod ME, et al. Effects of exposure to a rotating environment (10 rpm) on four aviators for a period of twelve days. Aerosp Med 1965;36:733–54.
[25] Grusser OJ. Information theory and signal processing in sense organs and the central nervous system. Naturwissenschaften 1972;59:436–47.
[26] Highstein SM, Cohen B. Neurolab mission. Curr Opin Neurobiol 1999;9:495–9.
[27] Holmes A, Rodgers RJ. Influence of spatial and temporal manipulations on the anxiolytic efficacy of chlordiazepoxide in mice previously exposed to the elevated plus-maze. Neurosci Biobehav Rev 1999;23:971–80.
[28] Inglis EM, Zuckerman KE, Roberts M, Kalb RG. Altered dendritic morphology in a population of motoneurons in rats reared in microgravity. Abstr Soc Neurosci 1999;91:9.
[29] Javid FA, Naylor RJ. Variables of movement amplitude and frequency in the development of motion sickness in Suncus murinus. Pharmacol Biochem Behav 1999;64:115–22.
[30] Kalb R, Solomon D. Space exploration, mars, and the nervous system. Arch Neurol 2007;64:485–90.
[31] Laviola G, Adriani W, Terranova ML, Gerra G. Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models. Neurosci Biobehav Rev 1999;23:993–1010.
[32] Levi-Montalcini R. The nerve growth factor 35 years later. Science 1987;237:1154–62.
[33] Lindsay RM. Brain-derived neurotrophic factor: an NGF-related neurotrophin. In: Loughlin SE, Fallon JH, editors. Neurotrophic factors. San Diego (CA): Academic Press; 1993. p. 257–84.
[34] Lindsay RM, Harmar AJ. Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons. Nature 1989;337:362–4.
[35] Luneburg U, Flohr H. Effects of melanocortins on vestibular compensation. Prog Brain Res 1988;76:421–9.
[36] McCaffrey RJ, Graham G. Age-related differences for motion sickness in the rat. Exp Aging Res 1980;6:555–61.
[37] McEwen BS, Sapolsky RM. Stress and cognitive function. Curr Opin Neurobiol 1995;5:205–16.
[38] Miller AD, Rowley HA, Roberts TP, Kucharczyk J. Human cortical activity during vestibular- and drug-induced nausea detected using MSI. Ann N Y Acad Sci 1996;781:670–2.
[39] Mitchell D, Laycock JD, Stephens WF. Motion sickness-induced pica in the rat. Am J Clin Nutr 1977;30:147–50.
[40] Mitchell D, Krusemark ML, Hafner D. Pica: a species relevant behavioral assay of motion sickness in the rat. Physiol Behav 1977;18:125–30.
[41] Mitchell D, Wells C, Hoch N, Lind K, Woods SC, Mitchell LK. Poison induced pica in rats. Physiol Behav 1976;17:691–7.
[42] Money KE. Motion sickness. Physiol Rev 1970;50:1–39.
[43] Noldus LPJJ. The observer: a software system for collection and analysis of observational data. Behav Res Methods Instrum Comput 1991;23:415–29.
[44] Ossenkopp KP, Frisken NL. Defecation as an index of motion-sickness in the rat. Physiol Psychol 1982;10:355–60.
[45] Ossenkopp KP, Ossenkopp MD. Animal models of motion sickness: are nonemetic species an appropriate choice? Physiologist 1985;28:S61–62.
[46] Ossenkopp KP, Rabi YJ, Eckel LA, Hargreaves EL. Reductions in body temperature and spontaneous activity in rats exposed to horizontal rotation: abolition following chemical labyrinthectomy. Physiol Behav 1994;56:319–24.
[47] Oyama J, Platt WT. Reproduction and growth of mice and rats under conditions of simulated increased gravity. Am J Physiol 1967;212:164–6.

Please cite this article in press as: Santucci D, et al. A mouse model of neurobehavioural response to altered gravity conditions: An ontogenetical study. Behav Brain Res (2008), doi:10.1016/j.bbr.2008.08.008

[48] Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav 1986;24:525–9.
[49] Primus RJ, Kellogg CK. Developmental influence of gonadal function on the anxiolytic effect of diazepam on environment-related social interaction in the male rat. Behav Pharmacol 1990;1:437–46.
[50] Rodgers RJ, Johnson NJ. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol Biochem Behav 1995;52:297–303.
[51] Sajdel-Sulkowska EM. Brain development, environment and sex: what can we learn from studying graviperception, gravitransduction and the gravireaction of the developing CNS to altered gravity? Cerebellum 2007:1–17.
[52] Sajdel-Sulkowska EM, Nguon K, Sulkowski ZL, Rosen GD, Baxter MG. Purkinje cell loss accompanies motor impairment in rats developing at altered gravity. Neuroreport 2005;16:2037–40.
[53] Santucci D, Francia N, Aloe L, Alleva E. Neurobehavioural responses to hypergravity environment in the CD-1 mouse. J Gravit Physiol 2002;9:P39–40.
[54] Santucci D, Corazzi G, Francia N, Antonelli A, Aloe L, Alleva E. Neurobehavioural effects of hypergravity conditions in the adult mouse. Neuroreport 2000;11:3353–6.
[55] Schinder AF, Poo M. The neurotrophin hypothesis for synaptic plasticity. Trends Neurosci 2000;23:639–45.
[56] Singer W. Neurobiology. Striving for coherence. Nature 1999;397:391–3.
[57] Smith MA, Makino S, Kim SY, Kvetnansky R. Stress increases brain-derived neurotropic factor messenger ribonucleic acid in the hypothalamus and pituitary. Endocrinology 1995;136:3743–50.
[58] Sondag HN, de Jong HA, Oosterveld WJ. Altered behaviour in hamsters conceived and born in hypergravity. Brain Res Bull 1997;43:289–94.
[59] Sondag HN, de Jong HA, Oosterveld WJ. Behaviour of adult hamsters subjected to hypergravity. J Vestib Res 1999;9:13–8.
[60] Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 2000;24:417–63.
[61] Spear LP, Brake SC. Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. Dev Psychobiol 1983;16:83–109.
[62] Takeda N, Hasegawa S, Morita M, Matsunaga T. Pica in rats is analogous to emesis: an animal model in emesis research. Pharmacol Biochem Behav 1993;45:817–21.
[63] Takeda N, Hasegawa S, Morita M, Horii A, Uno A, Yamatodani A, et al. Neuropharmacological mechanisms of emesis I. Effects of antiemetic drugs on motion- and apomorphine-induced pica in rats. Methods Find Exp Clin Pharmacol 1995;17:589–90.
[64] Thoenen H. Neurotrophins and neuronal plasticity. Science 1995;270:593–8.
[65] Thullier F, Hayzoun K, Dubois M, Lestienne F, Lalonde R. Exploration and motor activity in juvenile and adult rats exposed to hypergravity at 1.8*G* during development: a preliminary report. Physiol Behav 2002;76:617–22.
[66] Tomko DL, Barbaro NM, Ali FN. Effect of body tilt on receptive field orientation of simple visual cortical neurons in unanesthetized cats. Exp Brain Res 1981;43:309–14.
[67] Treit D, Menard J, Royan C. Anxiogenic stimuli in the elevated plus-maze. Pharmacol Biochem Behav 1993;44:463–9.
[68] Tyler DB, Bard P. Motion sickness. Physiol Rev 1949;29:311–69.
[69] Tyler WJ, Petzold GC, Pal SK, Murthy VN. Experience-dependent modification of primary sensory synapses in the mammalian olfactory bulb. J Neurosci 2007;27:9427–38.
[70] Weskamp G, Otten U. An enzyme-linked immunoassay for nerve growth factor (NGF): a tool for studying regulatory mechanisms involved in NGF production in brain and in peripheral tissues. J Neurochem 1987;48:1779–86.
[71] Wilpizeski CR, Lowry LD, Contrucci RB, Green SJ, Goldman WS. Effects of head and body restraint on experimental motion-induced sickness in squirrel monkeys. Aviat Space Environ Med 1985;56:1070–3.
[72] Wubbels RJ, de Jong HA. Vestibular-induced behaviour of rats born and raised in hypergravity. Brain Res Bull 2000;52:349–56.
[73] Yates BJ, Miller AD, Lucot JB. Physiological basis and pharmacology of motion sickness: an update. Brain Res Bull 1998;47:395–406.

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# GENERAL DISCUSSION

## General discussion

As reported in the Introduction, the main objective of the present project was to characterize the short-, medium- and long-lasting effects of hypergravity exposure on central nervous system and behaviour in the CD-1 mouse model. Data coming from ground-based researches using the hypergravity paradigm can help to predict changes in behavioural and neurobiological responses by mammalian species to microgravity or weightlessness exposure experienced by these animals while orbiting in the space. Indeed, we provided a refined mouse model to study the physiological effects of altered gravity exposure, with in mind the perspective of a smooth transfer of this animal model from ground-based settings to weightlessness aboard of the ISS.

### 4.1 Chapter 1: brief results and discussion

In Chapter 1, a detailed characterization of the behavioural responses of adult CD-1 mice exposed to acute 2*g* hypergravity was performed. Sex differences in MS vulnerability were also investigated.

Overall, results from this study clearly indicated that rotationally-induced hypergravity influenced the short-term behavioural responses of CD-1 mice, reducing their spontaneous activity and concomitantly increasing their resting behaviour. According to our previous research, this reduction of spontaneous activity may be suggestive of animals experiencing MS syndrome as a consequence of being subjected to rotational stimuli (Santucci et al., 2000). In fact, in the present study pica behaviour, a MS index measured through the consumption of a non-food substance such as kaolin, showed a tendency to increase upon rotation. Animals rotated at 2*g* for 1h (HG1) seemed the most affected, HG1 females being the only experimental group still eating kaolin in the fourth day after rotation and HG1 males the only male group showing an increase in kaolin intake. These effects confirm that females are more vulnerable than males to

the symptoms associated with MS (Santucci et al., 2000). Moreover in accordance with the effects on behavioural responses (see below) they are suggestive of HG1 mice being less able to recover than HG2 (rotated at 2*g* for 2h) animals.

According to our previous experiments (Santucci et al., 2000; Francia et al., 2004) mice spontaneous activity was deeply affected by single hypergravity exposure, and, in most cases, the effects were independent of the exposure duration. Specifically, during rotation, grooming, digging, exploring and vertical activity were almost or totally suppressed in HG mice while open eyes resting – which in mice has been previously described as a specific behavioural index of MS (Santucci et al., 2000) – increased. Interestingly, females spent more time in the characteristic open eyes resting behaviour than males since the first hour of rotation. Moreover, in post-rotation, females rotated for 1h persisted in showing high levels of this behaviour. These data are in accordance with those on pica behaviour pointing out that females are more susceptible than males to the onset of motion sickness.

It should be noted, however, that in HG2 animals the majority of behaviours regained the pre-rotational levels soon after the rotation was terminated, while HG1 mice failed to recover. In particular, grooming behaviour reappeared reaching the pre-rotation levels in all groups except in the HG1 one. Grooming behaviour in rats is a classical measure of displacement activity but also coincides with the period after arousal and rather reflects the process of dearousal due to habituation to a stressful situation (Spruijt et al., 1992). Therefore the reappearance of pre-rotation behavioural profile in the groups of mice rotated for the longest time could be reasonably a consequence of habituation processes taking place during the 2nd hour of rotation, reducing motion sickness and leading to a recovery in the behavioural profile (Francia et al., 2004). This is particularly evident in females which recover better than males, as indicated by their higher levels of several behavioural endpoints, such as exploring, vertical activity, digging, and locomotor activity in post-rotation.

Mice exploratory behaviour in the hole board apparatus resulted impaired, as indicated by a minor number of holes visited and a long latency to the first head dipping. These changes appeared to be a consequence of the exposure to the rotational stimuli *per se*.

By contrast, a clear effect of gravitational environment emerged in the plus maze test, with HG mice more often avoiding both open and closed arms than the other groups. In addition, they showed a marked tendency to display SAP behaviour (stretched-attend postures) especially in the protected areas. These results, pointing to alterations in locomotor activity (Wall and Messier, 2001), reflect a reduced motivation to explore as a consequence of increased anxiety. SAP behaviour in the elevated plus maze has been related to risk assessment and defensive behaviours (Pellow and File, 1986; Rodgers and Johnson, 1995). Since it has also been shown to be particularly responsive to various anxiolytic drugs, high levels of this response have been considered a reliable index of increased anxiety (Palanza, 2001; Wall and Messier, 2001).

Therefore it appears that rotation *per se* represents a stressful condition affecting the level of exploratory behaviour, while hypergravity acts as an additional stimulus selectively affecting their emotional-anxiety profile.

Females appeared generally more susceptible than males to the distressful stimuli associated with rotational/gravitational environment, although they turned out to recover faster than males. They also appeared more anxious than males. In fact, in the plus maze, 2h-rotated females showed lower levels of rearing and head dipping and higher levels of SAP than males. As documented by an extensive literature on the subject (see for example: Gray, 1971; Archer, 1975; Goy and McEwen, 1980), sex differences in response to environmental variables are found across species in many behavioural aspects. Such differences can be attributed to proximate (e.g. endocrine, genetic and environmental factors acting on behaviour) as well as ultimate mechanisms such as different adaptive strategies in coping with stressful situations (Grigoriev et al., 1988; Palanza, 2001).

Therefore, it appears that exposure to rotation can highlight sex differences in susceptibility and copying to rotational stimuli, which, in turn, might be reflected in subsequent behavioural responses.

## 4.2 Chapter 2: brief results and discussion

In the Chapter 2, we investigated the short-, medium- and long-term consequences of repeated acute exposure to hypergravity during early postnatal development. In this study, the relevance of maternal experience on pups' neurobehavioural responses to altered gravitational stimuli was also assessed. To this purpose, CD-1 mouse pups of both sexes delivered by primiparous and biparous dams were exposed to 1h of 2*g* rotationally-induced hypergravity (HG group) from PND2 to PND9. Sensorimotor responses, somatic growth, ultrasonic vocalisations and homing behaviour were evaluated during the first two weeks of life. In addition, locomotor activity, exploratory behaviour and spatial orientation ability were also evaluated. Long-term effects of hypergravity exposure on both spatial learning (Morris water-maze test) and brain levels of NGF and BDNF were investigated at adulthood.

Taken as a whole, findings from this experiment indicate that repeated acute exposures to a rotational environment during the early postnatal phase affect mouse neurobehavioural development. In particular, independently from hypergravity exposure, rotation *per se* delayed somatic growth (hair growth and ears opening), sensorimotor responses (ear twich, auditory startle, pole embrace and level screen test), and the ultrasonic vocalization profile.

Hypergravity did not exert specific effects on pup development. Interestingly, when comparing our results with the findings recently reported for example by Bouët and coworkers (2004), clear differences arise likely due to differences in time and modality of hypergravity exposure. In Bouët and coworkers' experiments, the treatment protocol was designed to expose animals to hypergravity from conception until varying periods after

birth. They reported a postnatal delay in body growth and in the development of the righting response and negative geotaxis, motor aspects mainly dependent upon the vestibular system. In our experiment a very different exposure schedule was applied - i.e. one hour daily exposure from PND 2 to PND 9. Thus, it is possible that the different exposure durations could account for the lack of hypergravity-induced effects on the development of the specific behavioural endpoints. In fact, all the major effects on the vestibular system development reported so far resulted from exposure to hypergravity during the prenatal or both the prenatal and the postnatal phases (Lim et al., 1996; Wubbels et al., 2002; Bruce, 2003; Chabbert et al., 2003; Gaboyard et al., 2003). Moreover, in Boüet and coworkers' experiment, rotational controls were not included, making it difficult to draw conclusions about the specificity of hypergravity effects.

To assess for effects related to rotational stimulus *per se*, in our experiments a control group was rotated at 1*g* (Earth gravitational field). Indeed, acute exposure to rotation represents a stressful condition *per se*, as we have already shown in adult and adolescent mice, where it can induce MS syndrome and increase the levels of emotional/anxiety behaviour (Santucci et al., 2000; Francia et al., 2004). Stressors repeatedly applied to neonatal rodents induce alterations of central nervous system functions, persisting up to the adult age, due to the sensitive periods in the formation of brain circuitry associated with early development (Loizzo et al., 2003; Sternberg and Ridgway, 2003). Therefore it can be hypothesized that the changes here reported might reflect the influence of enhanced stress levels on development. The high rate of ultrasonic calling found in rotated pups, in the absence of a reduction in body weight gain, seems consistent with this hypothesis because in altricial rodents ultrasonic emission has been correlated with stressful experiences (Branchi et al., 1998).

In the open-field test, carried out on PND 21, HG males were more active in both the peripheral and central area and performed more wall-rearing during the initial phase of the test than HG females. Moreover HG males sniffed the novel environment less than females. Exploratory behaviour in adult rodents is found to be sexually dimorphic with males

being less active and showing more rapid habituation than their female counterparts (Goy and McEwen, 1980; de Cabo de la Vega et al., 1995; Nasello et al., 1998). Moreover, during adolescence gender differences in the response to environmental challenges have been reported across various mammalian species (Spear, 2000). Hypergravity during critical postnatal phase seems to affect the shape of males and females behavioural profile at periadolescence, apparently enhancing such differences.

Rotation-induced effects turned out to be independent from those exerted by parity condition. Indeed, no interaction between rotational environment and dam parity has been found. Maternal reproductive experience *per se* exerted a strong influence on pups' development and several somatic, including body weight, and sensorimotor parameters were accelerated in the offspring of biparous dams. Moreover, primiparous female offspring showed a better performance in the T-maze test and apparently both males and females were less anxious than biparous offspring in the open-field.

"Maternal effects" are often associated with variations in the quality of the maternal environment, which alters the nature of the dam-offspring interaction and thus the phenotype of the offspring (Zhang et al., 2004). It is also known that pups' behaviour can exert a strong influence on dam responsiveness, which in turn is affected by previous nursing experience. Stressed pups elicit different levels of maternal behaviour in primiparous and multiparous rat dams (Wright et al., 1978). If pups are stressed during early infancy, maternal behaviour of multiparous dams increases during the first two days of treatment and then becomes stable. By contrast, primiparous mothers are consistently responsive to stressed pup cues, showing a sort of "mother on demand" profile but, after termination of stress treatment, and in the presence of minimal pup stress cues, frequency of nursing declines markedly.

Multiparous and primiparous mothers respond differently to the cues of stressed pups and these differences have been correlated to differences in the open-field behaviour of the offspring (Wright et al., 1978).

Repeated acute exposure to the altered gravity environment during the postnatal phase also exerted slight but significant long-term effects on learning performance in the Morris water-maze. In fact, on the 2nd day of the acquisition trials, RC mice spent more time searching the platform location, and, in the reversal phase, HG mice performed differently when compared to RC or unrotated mice in all the parameters examined. The impairment showed by RC mice seems in line with other findings suggesting that stress early in life can induce slight, but significant impairments on acquisition in the Morris water-maze task at adulthood (Huot et al., 2002).

Morris water-maze reversal phase has been extensively used to evaluate the ability to shift strategies to task demands (Smith, 1988), since animals have to shift from an acquired response to a different one. Altered performances in Morris water-maze during this phase have been related to changes in attentional process and/or in behavioural flexibility following environmental changes (Smith, 1988). Thus, the different trend over time in latency to find the new platform location as well as in time spent, number of crossings and swimming path in the acquisition quadrant during the reversal phase, may reflect changes in behavioural responding to changes in task requirements in mice exposed to hypergravity during development.

Following the Morris water-maze test, decreased NGF levels in the frontal cortex of HG mice and decreased BDNF levels in the hypothalamus of both RC and HG mice were found. It is likely that plastic changes in the central nervous system (e. g., neuromodulation, changes in synaptic efficacy, “rewiring”, sprouting, reorganization of neural network) underlie sensorimotor adaptation to changes in gravitational environment (Fox, 1965). Moreover, during early development and in the adult life, neurotrophins are implicated in mechanisms regulating synaptogenesis and neuronal organisation, both under normal conditions and following neuronal injury, as well as under environmental challenges (see for example Connor and Dragunow, 1998). BDNF participates in synaptic plasticity and the adaptive changes in the strength of communication between neurons thought to underlie aspects of behavioural adaptation (Gorski et al., 2003).

Thus, the altered levels of brain NGF and BDNF could represent persistent changes of an early compensatory/recovery mechanism following hypergravity exposure. The possibility that they might be correlated with the slight effects on learning performance in the Morris water-maze cannot be also ruled out. In fact, NGF and BDNF have been reportedly involved in neuronal plasticity underlying cognitive function (Thoenen, 1995) and long-term consequences on NGF and BDNF expression have been shown be induced by stressors administered to rats during postnatal development (Otten et al., 1979; Smith et al., 1995; Rage et al., 2002). Further experiments should be devoted in the future to assess other stress-related hormonal parameters in an hypergravity paradigm.

In analogy with other studies, effects here reported are minor to moderate. Several studies have shown the detrimental effects of microgravity exposure on neurodevelopment in young rodents. The opposite situation, i.e. hypergravity, which strongly stimulates several sensory systems and in particular the vestibular system, may lead, as other environmental mild stressors, to the reported effects on the central nervous system and behavioural outcomes (Sajdel-Sulkowska et al., 2005).

Overall, this study confirms and extends previous findings on the neurobehavioural development of rodents subjected to hypergravity, suggesting a role for factors other than changes in the gravito-inertial vector, with stress and relative effects on central nervous system development seeming suitable candidates. Maternal experience confirms its fundamental role in affecting offspring development, apparently without interacting with the specific environmental challenges.

### 4.3 Chapter 3: brief results and discussion

In Chapter 3, studies to evaluate the neurobehavioural response to hypergravity exposure during late postnatal development were performed. Early adolescent (WEAN, PND 28), adolescent (ADO, PND 42) and young-adult (Y-A, PND 60) male and female CD-1 mice were exposed to acute 2*g* rotationally-generated hypergravity. MS index and behavioural performances before, during and after rotation were recorded, and long-lasting effects on exploratory behaviour (hole-board test) and emotional/anxiety-like responses (plus-maze test) were investigated. Furthermore, in order to correlate behavioural changes with alterations in central levels of neurotrophins, brain amounts of NGF and BDNF were also assessed on PND 90, following a re-exposure to hypergravity.

Exposure to rotation-induced hypergravitational stimuli highly affected the behavioural repertoire of developing CD-1 mice as well as brain levels of neurotrophins.

In particular, at all ages, pica behaviour was observed in mice exposed to acute rotational stimuli and was more pronounced in females than males, this latter effect being in agreement with data previously reported for rodents (Santucci et al., 2000), insectivora (Javid and Naylor, 1999) and humans (Money, 1970). Concerning age differences in kaolin consumption, a study performed on rats (McCaffrey and Grham, 1980) reports 20 months old rats engaging in significantly less kaolin consumption than either 2 or 11 months old animals. In the present mouse study we found that Y-A (2 months old) subjects eat more kaolin than ADO and WEAN combined, all data suggesting a non-linear profile in MS response over life span. Age-related changes in the pattern of vulnerability to MS have been reported in humans (Tyler and Bard, 1949; Chinn and Smith, 1955; Money, 1970), with neonates appearing almost unaffected and children being most affected. A reduction in such vulnerability has then been reported up to the age of 40 years, after when MS syndrome increases again.

In agreement with data previously reported for rodents (Eskin and Riccio, 1966; Ossenkopp et al., 1994; Sondag et al., 1999; Santucci et al.,

2000; Francia et al., 2004), squirrel monkeys (Wilpizeski et al., 1985) and humans (Graybiel et al., 1965; Money, 1970), a decrease in spontaneous behaviour was evident during and immediately after the rotation, with several activities almost or totally suppressed during the rotation.

Differences in behavioural repertoire among the three age groups were evident during pre-rotation, while, with the exception of exploring behaviour, no major sex differences were observed. Upon or after rotation, ADO and Y-A mice showed the highest number of changes in behavioural responses, suggesting a lower susceptibility in youngest (WEAN) animals to changes in gravitational environment. Bar-holding behaviour performed at high levels by ADO group, may in fact indicate discomfort in distressed animals, while eating sawdust by ADO and Y-A subjects, may be explained as age-typical performance of pica behaviour. Moreover, WEAN group recovered faster from open eyes resting in post rotation - this item being an established behavioural index of MS in mice (Santucci et al., 2000). A role for changes in vestibular nuclei, whose full maturation continues until the end of the first month of life, as well as in projections from these nuclei versus relevant areas of the central nervous system should be taken into account for the observed effects.

When considering long-lasting effects of rotational exposure, a delay in the onset of exploratory behaviour in a novel environment was observed in rotated animals of all ages. Moreover, mice rotated when WEAN and ADO showed a reduction in emotional/anxiety-like response. By contrast, animals rotated when Y-A showed increased number of head dipping and SAP (stretched-attend postures) when in the protected area (closed arms) of the plus maze apparatus, suggesting an elevated level of anxiety-like behaviours in these animals. SAP is considered a primary index of risk assessment, a group of behaviours that are thought to facilitate information gathering in potentially dangerous environments, and seems particularly sensitive to anti- or proanxiety drugs (see Pellow and File, 1986; Rodgers and Johnson, 1995). Behavioural observations on stress-like (or emotional-like) response related to changes in gravitational conditions report an increase in defecation (Ossenkopp and Frisken, 1982), a reduction in

exploratory behaviour as well as vibrissae activation and breathing (Dit Duflo et al., 2000) in rats subjected to changes in gravito-inertial force for 60-90 minutes. With repeated or chronic exposure, animals seem to habituate to the rotation (Ossenkopp and Frisken, 1982), and no changes in defecation rate have been reported after long-term exposure to 2.5*g* condition in hamsters (Sondag et al., 1999). Moreover, hypothalamo-pituitary-adrenal axis activation (see below) and alterations in brain serotonin metabolism (Blanc et al., 1998) have been reported in microgravity animals.

Long-lasting effects of repeated exposure to hypergravity also include altered locomotor activity and impaired swimming navigation in rats (Fox et al., 1998) and hamsters (Sondag et al., 1999), in this latter species the effect being more severe in subjects exposed at adulthood than at younger ages. Moreover, brains from rat pups born and raised under prolonged hypergravity conditions (23h/day) show relevant changes in forebrain and cerebellum sizes, general developmental effects being greatest just after birth and again at weaning (Bear et al., 2000). Interestingly, rats exposed to microgravity during spaceflight (14 days in microgravity since PND 8) did not develop the normal mature righting behaviour after landing, with an infantile pattern still present when tested 110 days later (Highstein et al., 1999).

Neural processes involved in both short- and long-term neurobehavioral adaptation to hyper- or microgravity are though to be similar to those involved in learning and/or in recovery from neural damage, i.e., experience-dependent neural plasticity (Luneburg and Flohr, 1988; Singer, 1999; Tyler et al., 2007). It has been suggested (Fox et al., 1998) that it is likely that plastic changes in the central nervous system (e.g., neuromodulation, changes in synaptic efficacy, “rewiring”, sprouting, reorganization of neural network) underlie sensori-motor adaptation to changes in gravitational environment. NGF and BDNF are two neurotrophins that play a critical role on neuronal survival and phenotypic differentiation (Levi-Montalcini, 1987; Thoenen, 1995). During early development and in adult life, they are implicated in mechanisms regulating

synaptogenesis, neuronal organisation both under normal conditions and following neuronal injury and environmental challenges (Connor and Dragunow, 1998).

Frontal cortex is a region involved in MS syndrome (Miller et al., 1996; Yates et al., 1998), and plastic neural changes in corticovestibular pathways may explain the observed NGF increase, and further studies should be devoted to evaluate possible alteration in BDNF and NGF in these areas. For example, a dramatic increase in Fos-positive cells in this area has been reported in rats following exposure to hypergravity for 90min (Dit Duflo et al., 2000), and it has been suggested that, in addition to other otolith input, signals from the vestibular system to the cortex may contribute to cognitive functions like body scheme, spatial cognition, and navigation, as well as visual spatial constancy (Grusser, 1972; Tomko et al., 1981; Berthoz, 1996).

NGF, and to a lesser extent BDNF, was affected in mice re-exposed to rotational stimuli when 90 days old. NGF increased in the hippocampus, olfactory bulbs and in both parietal and frontal cortex of rotated animals, confirming a role of this peptide in brain "precognitive" areas, characterized by higher plasticity and fast turnover, in the context of adaptive responses to hypergravitational stimuli. Previous data in which adult mice were once exposed to 1h at 2*g* (Santucci et al., 2000) did not show any NGF increase in olfactory bulbs and in the parietal cortex, suggesting that previous experience with hypergravity might be required for later changes in neuronal plasticity to occur. In addition, at weaning, animals appear to be more prone to show changes in neurotrophin levels in response to external manipulation, both rotated and control subjects showing a dramatic NGF elevation in the parietal and frontal cortex. Ontogenetical data on brain function during adolescence suggest that prominent neural change occurs in this period in regions such as the frontal cortex not only in humans but also across mammalian species ranging from rodents to non-human primates (Spear and Brake, 1983; Primus and Kellogg, 1990; Laviola et al., 1999; Spear, 2000). Therefore, manipulation *per se* (in the present study consisting, in the non rotated controls, in the daily experimental procedure

for kaolin adaptation and consumption evaluation) at adolescence seems relevant to the long-lasting changes in NGF levels observed at adulthood, such an effect potentially overwhelming any possible changes due to the exposure to rotational stimuli. Indeed, there is evidence that specific neuronal response and survival might be associated to individual neurotrophic factor released either by target-innervated tissues or through paracrine or autocrine mechanism (Canals et al., 2001). This neurotrophic dependency of the neurons in the central nervous system can be altered not only by surgical or chemical manipulation, but also by behavioural responding. It is therefore possible that the different response in neurotrophins distribution observed in our studies is part of this homeodynamic mechanism. However, these and other questions arising by the present findings need to be addressed with future studies. Likewise the influence of NGF on peripheral responsive cells, the effect on immunocompetent cells, the effect on undifferentiated stem cells localized in the brain ventricles, olfactory bulbs and in the hippocampal formation (McEwen and Sapolsky, 1995) remains to be explored. In addition, the possible involvement of NGF in the adaptation of the vestibular system to changes of gravitational field needs to be investigated in the future.

Finally, animals rotated as Y-A showed marked elevation in levels of NGF and BDNF in the hypothalamus. Elevations in NGF levels in this area has already been related to stressful stimuli (Alleva et al., 1996; Cirulli et al., 2000), this peptide being a key element in controlling neuroendocrine responses (Levi-Montalcini, 1987), and increased levels of BDNF in the hypothalamus have been reported in animals subjected to acute or repeated stress (such as immobilization; Smith et al., 1995), this latter neurotrophic factor overlapping distributions and functions in several brain areas with NGF (Lindsay, 1993). Thus, such NGF and BDNF increase in animals exposed as Y-A appear to parallel behavioural observations indicating that older animals experience a higher level of stress upon centrifugation when compared to younger ones.

## 4.4 Final remarks

Exposure to 2*g* hypergravity induces subtly but relevant behavioural and neurobiological changes in both adult and developing CD-1 mice. In general, results from this series of studies confirm and extend previous findings on the neurobehavioural response of rodents to hypergravitational stimuli. Indeed, stress related to rotation *per se*, as well as coping and relative plastic changes in selected brain areas, besides alterations of the gravito-inertial vector, might significantly contribute for the observed behavioural changes. Thus, mice represent a particularly suitable model for future space-biology research aimed at understanding neurobiological and behavioural consequences of adaptation and re-adaptation to different gravity environments. Further ground-based studies using prolonged and/or earlier exposure of this small-sized mammalian species should be developed in view of future spaceflights and prolonged permanence of both humans and animals outside Earth's gravitational field. Data coming from these studies could result useful to setting up adequate countermeasures to protect human health during space missions. For example, pica behaviour as well as open eyes resting behaviour, which have been characterized as two functional behavioural indexes of MS in our mice model, could be utilized in pharmacology researches to test drugs' effectiveness in the prevention and treatment of astronauts' space adaptation syndrome (Reschke et al., 1986; Homick et al., 1997), the latter representing a related disorder to MS, which may highly debilitate astronauts compromising the success of the missions.

Studying the effects of exposure to altered gravitational environments on nervous system, besides acquisition of knowledge relevant for spaceflights and prolonged permanence of both humans and animals in space, offers a unique opportunity to gain insight in the developing nervous system. Indeed, exposure to a "new" gravity condition – such as hyper-, microgravity or weightlessness, likely representing stressing and extreme environments – during an early stage of development induces functional

rearrangements and long-lasting neurobehavioural adaptations, representing a very powerful tool for developmental neurobiology investigation.

Moreover, further understanding of basic processes underlying the development of the nervous system, with a special emphasis on brain plasticity, might be relevant for the prevention and treatment of psychopathologies and neurodegenerative diseases. Data coming from these studies could result useful for the prevention and treatment of those reversible forms of mood disorders and neurodegenerative syndromes related to a rapid ageing process experienced by astronauts during space missions, while could integrate data concerning the "accelerated ageing processes" and the "reversible ageing processes" displayed by astronauts during and after spaceflight respectively (Clément and Reschke, 1996; Wassersug, 1999; Strollo, 2000).

# References

Alleva E, Aloe L, Bigi S. An update role for nerve growth facror in neurobehavioural regulation of adult vertebrates. Rev Neurosci, 1993; 4: 41-62.

Alleva E, Francia N. Psychiatric vulnerability: suggestions from animal models and role of neurotrophins. Neurosci Biobehav Rev, 2008, Epub ahead of print.

Alleva E, Petruzzi S, Cirulli F, Aloe L. NGF regulatory role in stress and coping of rodents an humans. Pharmacol Biochem Behav, 1996; 1: 65-72.

Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci Biobehav Rev, 2003; 27: 3-18.

Andersen SL, Teicher MH. Stress, sensitive periods and maturational events in adolescent depression. Trends Neurosci, 2008; 31: 183-191.

Archer J. Rodent sex differences in emotional and related behavior. Behav Biol, 1975; 14: 451-479.

Baer LA, Li GH, Ronca AE, Wade CE. Early and late effects of perinatal hyper-gravity exposure on developing CNS. Grav Space Biol Bull, 2000; 14: 23.

Berthoz A (1996) How does the cerebral cortex process and utilize vestibular signals? In: Disorder of the vestibular system (Baloh RW, Halmagyu GM, eds), pp 113-125. New York: Oxford UP.

Blanc S, Somody L, Gharib A, Gauquelin G, Gharib C, Sarda N. Counteraction of spaceflight-induced changes in the rat central serotonergic system by adrenalectomy and corticosteroid replacement. Neurochem Int, 1998; 33: 375-382.

Borison HL. Area postrema: chemoreceptor circumventricular organ of the medulla oblongata. Prog Neurobiol, 1989; 32: 351-390.

Bouët V, Gahery Y, Lacour M. Behavioural changes induced by early and long-term gravito-inertial force modification in the rat. Behav Brain Res, 2003; 139: 97-104.

Bouët V, Wubbels RJ, de Jong HA, Gramsbergen A. Behavioural consequences of hypergravity in developing rats. Brain Res Dev Brain Res. 2004; 153: 69-78.

Branchi I, Santucci D, Vitale A, Alleva E. Ultrasonic vocalizations by infant laboratory mice: a preliminary spectrographic characterization under different conditions. Dev Psychobiol, 1998; 33: 249-256.

Burton RR. Can centrifugation at increased G be used to predict results of 0G? J Gravit Physiol, 1999; 6: 1-4.

Bruce LL. Adaptations of the vestibular system to short and long-term exposures to altered gravity. Adv Space Res, 2003; 32: 1533-1539.

Canals JM, Checa N, Marco S, Akerud P, Michels A, Perez-Navarro E, Tolosa E, Arenas E, Alberch J. Expression of brain-derived neurotrophic factor in cortical neurons is regulated by striatal target area. J Neurosci, 2001; 21: 117-124.

Castren E, Zafra F, Thoenen H, Lindholm D. Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. Proc Natl Acad Sci USA, 1992; 89: 9444–9448.

Chabbert C, Brugeaud A, Lennan G, Lehouelleur J, Sans A. Electrophysiological properties of the utricular primary transducer are modified during development under hypergravity. Eur J Neurosci, 2003; 17: 2497-2500.

Chinn HI, Smith PK. Motion sickness. Pharmacol Rev, 1955; 7: 33-82.

Cirulli F, Alleva E, Antonelli A, Aloe L. NGF expression in the developing rat brain: effects of maternal separation. Brain Res Dev Brain Res, 2000; 123: 129-134.

Cirulli F, Berry A, Alleva E. Early disruption of the mother-infant relationship: effects on brain plasticity and implications for psychopathology. Neurosci Biobehav Rev, 2003; 27: 73-82.

Cirulli F, Francia N, Berry A, Aloe L, Alleva E, Suomi SJ. Early life stress as a risk factor for mental health: Role of neurotrophins from rodents to non-human primates. Neurosci Biobehav Rev, 2008; Epub ahead of print.

Cirulli F, Francia N, Branchi I, Antonucci MT, Aloe L, Suomi SJ, Alleva E. Changes in plasma levels of BDNF and NGF reveal a gender-selective vulnerability to early adversity in rhesus macaques. Psychoneuroendocrinology, 2008; Epub ahead of print.

Clément G, Reschke MF (1996) Neurosensory and sensory-motor functions. In: Biological and medical research in space (Moore D, Bie P, Oser H, eds), pp 178-258. Heidelberg: Springer Verlag.

Connor B, Dragunow M. The role of neuronal growth factors in neurodegenerative disorders of the human brain. Brain Res Brain Res Rev, 1998; 27: 1-39.

de Cabo de la Vega C, Pujol A, Paz Viveros M. Neonatally administered naltrexone affects several behavioral responses in adult rats of both genders. Pharmacol Biochem Behav, 1995; 50: 277-286.

DeFelipe J, Arellano JI, Merchan-Perez A, Gonzalez-Albo MC, Walton K, Llinas R. Spaceflight induces changes in the synaptic circuitry of the postnatal developing neocortex. Cereb Cortex, 2002; 12: 883-891.

Dit Duflo GS, Gestreau C, Lacour M. Fos expression in the rat brain after exposure to gravito-inertial force changes. Brain Res, 2000; 861: 333-344.

Dobie T, McBride D, Dobie T Jr, May J. The effects of age and sex on susceptibility to motion sickness. Aviat Space Environ Med, 2001; 72: 13-20.

Eskin A, Riccio D. The effects of vestibular stimulation on spontaneous activity in the rat. Psychol Rec, 1966; 16: 523-527.

Fox RA, Daunton NG, Corcoran ML. Study of adaptation to altered gravity through systems analysis of motor control. Adv Space Res, 1998; 22: 245-253.

Fox WM. Reflex-ontogeny and behavioural development of the mouse. Anim Behav, 1965; 13: 234-241.

Francia N, Santucci D, Chiarotti F, Alleva E. Cognitive and emotional alterations in periadolescent mice exposed to 2 g hypergravity field. Physiol Behav, 2004; 83: 383-394.

Gaboyard S, Sans A, Lehouelleur J. Differential impact of hypergravity on maturating innervation in vestibular epithelia during rat development. Dev Brain Res, 2003; 143: 15-23.

Gazenko OG, Ilyin EA. Investigations onboard the biosatellite Cosmos-1667. Adv Space Res, 1986; 6:101-106.

Geisler HC, van der Fits IB, Gramsbergen A. The effects of early vestibular deprivation on the motor development in the rat. Behav Brain Res, 1997; 86: 89-96.

Giménez y Ribotta M, Sandillon F, Privat A. Influence of hypergravity on the development of monoaminergic systems in the rat spinal cord. Brain Res Dev Brain Res, 1998; 111: 147-157.

Gorski JA, Balogh SA, Wehner JM, Jones KR. Learning deficits in forebrain-restricted brain-derived neurotrophic factor mutant mice. Neuroscience, 2003; 121: 341-354.

Goy RW, McEwen BS (1980). Sex differences in behavior: rodents, birds, and primates. In: Sexual differentiation of the brain (Goy RW, McEwen BS, eds), pp 13-73. Cambridge: The MIT Press.

Gray JA. Sex differences in emotional behaviour in mammals including man: endocrine bases. Acta Psychol, 1971; 35: 29-46.

Graybiel A, Kennedy RS, Knoblock EC, Guedry FE, Jr., Mertz W, McLeod ME, Colehour JK, Miller EF, 2nd, Fregly AR. Effects of Exposure to a Rotating Environment (10 Rpm) on Four Aviators for a Period of Twelve Days. Aerosp Med, 1965; 36: 733-754.

Grigoriev AI, Egorov AD, Nichiporuk IA. Neurohumoral mechanism of space motion sickness. Acta Astronaut, 1988; 17: 167-172.

Grusser OJ. Information theory and signal processing in sense organs and the central nervous system. Naturwissenschaften, 1972; 59: 436-447.

Highstein SM, Cohen B. Neurolab mission. Curr Opin Neurobiol, 1999; 9: 495-499.

Homick JL, Reschke MF, Miller EF (1997) The effects of prolonged exposure to weightlessness on postural equilibrium. In: Biomedical results from Skylab (Johnston RS, Dietlein LF, eds), pp 104-112. Washington DC: Scientific and Technical Information Office, NASA.

Hubel DH, Wiesel TN. The period of susceptibility to the physiological effects of unilateral eye closure in kittens. J Physiol; 1970; 206: 419-436.

Huot RL, Plotsky PM, Lenox RH, McNamara RK. Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats. Brain Res, 2002; 950: 52-63.

Lindsay RM (1993) Brain-derived neurotrophic factor: an NGF-related neurotrophin. In: Neurotrophic factors (Loughlin SE, Fallon JH, eds), pp 257-284. San Diego (CA): Academic Press.

Inglis EM, Zuckerman KE, Roberts M, Kalb RG. Altered dendritic morphology in a population of motoneurons in rats reared in microgravity. Abstr Soc Neurosci, 1999; 91: 9.

Javid FA, Naylor RJ. Variables of movement amplitude and frequency in the development of motion sickness in Suncus murinus. Pharmacol Biochem Behav, 1999; 64: 115-122.

Jokerst MD, Gatto M, Fazio R, Gianaros PJ, Stern RM, Koch KL. Effects of gender of subjects and experimenter on susceptibility to motion sickness. Aviat Space Environ Med, 1999; 70: 962-965.

Kalb R, Solomon D. Space exploration, Mars, and the nervous system. Arch Neurol, 2007; 64: 485-490.

Laviola G, Adriani W, Terranova ML, Gerra G. Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models. Neurosci Biobehav Rev, 1999; 23: 993-1010.

Le Bourg E. A review of the effects of microgravity and of hypergravity on aging and longevity. Exp Gerontol, 1999; 34: 319-336.

Levi-Montalcini R. The nerve growth factor 35 years later. Science, 1987; 237: 1154-1162.

Levi-Montalcini R, Aloe L, Alleva E. A role for nerve growth factor in nervous, endocrine and immune systems. Prog NeuroEndocrinIm, 1990; 3: 1-10.

Lewin GR, Barde YA. Physiology of the neurotrophins. Annu Rev Neurosci, 1996; 19: 289–317.

Lim DJ, Stith JA, Stockwell CW, Oyama J. Observations on saccules of rats exposed to long-term hypergravity. Aerosp Med, 1974; 45: 705-710.

Loizzo A, Capasso, Galietta G, Severini C, Campana G, Spampinato S. Vas deferens response to selective opioid receptor agonists in adult mice is impaired following postnatal repeated mild stress. Eur J Pharmacol, 2003; 458: 201-205.

Luneburg U, Flohr H. Effects of melanocortins on vestibular compensation. Prog Brain Res, 1988; 76: 421-429.

Lyons TJ. Women in the fast jet cockpit--aeromedical considerations. Aviat Space Environ Med, 1992; 63: 809-818.

McCaffrey RJ, Graham G. Age-related differences for motion sickness in the rat. Exp Aging Res, 1980; 6: 555-561.

McEwen BS, Sapolsky RM. Stress and cognitive function. Curr Opin Neurobiol, 1995; 5: 205-216.

Miller AD, Rowley HA, Roberts TP, Kucharczyk J. Human cortical activity during vestibular- and drug-induced nausea detected using MSI. Ann N Y Acad Sci, 1996; 781: 670-672.

Mitchell D, Krusemark ML, Hafner D. Pica: a species relevant behavioral assay of motion sickness in the rat. Physiol Behav, 1977; 18: 125-130.

Mitchell D, Laycock JD, Stephens WF. Motion sickness-induced pica in the rat. Am J Clin Nutr, 1977; 30: 47-150.

Mobley WC, Rutkowski JL, Tennekoon GI, Gemski J, Buchanan K, Johnston MV. Nerve growth factor increases choline acetyltransferase activity in developing basal forebrain neurons. Brain Res, 1986; 387: 53-62.

Money KE. Motion sickness. Physiol Rev, 1970; 50: 1-39.

Morey-Halton ER, Hill EL, Souza KA. Animals and spaceflight: from survival to understanding. J Musculoskelet Neuronal Interact, 2007; 7: 17-25.

Nasello AG, Machado C, Bastos JF, Felicio LF. Sudden darkness induces a high activity-low anxiety state in male and female rats. Physiol Behav, 1998; 63: 451-454.

Nguon K, Ladd B, Sajdel-Sulkowska EM. Exposure to altered gravity during specific developmental periods differentially affects growth, development, the cerebellum and motor functions in male and female rats. Adv Space Res, 2006; 38: 1138-1147.

Ortiz RM, Wang TJ, Wade C E. Influence of centrifugation and hindlimb suspension on testosterone and corticosterone excretion in rats. Aviat Space Environ Med, 1999; 70: 499-504.

Ossenkopp KP, Frisken NL. Defecation as an index of motion-sickness in the rat. Physiol Psychol, 1982; 10: 355-360.

Ossenkopp KP, Ossenkopp MD. Animal models of motion sickness: are nonemetic species an appropriate choice? Physiologist, 1985; 28: 61-62.

Ossenkopp KP, Rabi YJ, Eckel LA, Hargreaves EL. Reductions in body temperature and spontaneous activity in rats exposed to horizontal rotation: abolition following chemical labyrinthectomy. Physiol Behav, 1994; 56: 319-324.

Otten U, Baumann JB, Girard J. Stimulation of the pituitary-adrenocortical axis by nerve growth factor. Nature, 1979; 282: 413-414.

Oyama J, Platt WT. Reproduction and growth of mice and rats under conditions of simulated increased gravity. Am J Physiol, 1967; 212: 164-166.

Palanza P. Animal models of anxiety and depression: how are females different? Neurosci Biobehav Rev, 2001; 25: 219-233.

Park AH, Hu S. Gender differences in motion sickness history and susceptibility to optokinetic rotation-induced motion sickness. Aviat Space Environ Med, 1999; 70: 1077-1080.

Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav, 1986; 24: 525-529.

Primus RJ, Kellogg CK. Developmental influence of gonadal function on the anxiolytic effect of diazepam on environment-related social interaction in the male rat. Behav Pharmacol, 1990; 1: 437-446.

Rage F, Givalois L, Marmigere F, Tapia-Arancibia L, Arancibia S. Immobilization stress rapidly modulates BDNF mRNA expression in the hypothalamus of adult male rats. Neuroscience, 2002; 112: 309-318.

Reschke MF, Anderson DJ, Homick JL. Vestibulo-spinal modification as determined with the H-reflex during Spacelab-1 flight. Exp Brain Res, 1986; 64: 367-379.

Rodgers RJ, Johnson NJ. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol Biochem Behav, 1995; 52: 297-303.

Ronca AE. Mammalian development in space. Adv Space Biol Med, 2003; 9: 217-251.

Ronca AE, Alberts JR. Effects of prenatal spaceflight on vestibular responses in neonatal rats. J Appl Physiol, 2000; 89: 2318-2324.

Russo-Neustadt A, Ha T, Ramirez R, Kesslak JP. Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. Behav Brain Res, 2001; 120: 87–95.

Sajdel-Sulkowska EM. Brain development, environment and sex: what can we learn from studying graviperception, gravitransduction and the gravireaction of the developing CNS to altered gravity? Cerebellum, 2007; 6: 1-17.

Sajdel-Sulkowska EM, Li GH, Ronca AE, Baer LA, Sulkowsky GM, Koibuchi N, Wade CE. Effect of hypergravity exposure on the developing central nervous system: possible involvement of thyroid hormone. Exper Biol Med, 2001; 226: 790-798.

Sajdel-Sulkowska EM, Nguon K, Sulkowski ZL, Rosen GD, Baxter MG. Purkinje cell loss accompanies motor impairment in rats developing at altered gravity. Neuroreport, 2005; 16: 2037-2040.

Santucci D, Corazzi G, Francia N, Antonelli A, Aloe L, Alleva E. Neurobehavioural effects of hypergravity conditions in the adult mouse. Neuroreport, 2000; 11: 3353-3356.

Singer W. Neurobiology. Striving for coherence. Nature, 1999; 397: 391, 393.

Smith G. Animal models of Alzheimer's disease: experimental cholinergic denervation. Brain Res, 1988; 472: 103-118.

Smith MA. Hippocampal vulnerability to stress and aging: possible role of neurotrophic factors. Behav Brain Res, 1996; 78: 25-36.

Smith MA, Makino S, Kim SY, Kvetnansky R. Stress increases brain-derived neurotropic factor messenger ribonucleic acid in the hypothalamus and pituitary. Endocrinology, 1995; 136: 3743-3750.

Snetkova E, Chelnaya N, Serova L, Saveliev S, Cherdanzova E, Pronych S, Wassersug R. Effects of space flight on Xenopus laevis larval development. J Exp Zool, 1995; 273: 21-32.

Sondag HN, de Jong HA, Oosterveld WJ. Altered behaviour in hamsters conceived and born in hypergravity. Brain Res Bull, 1997; 43: 289-294.

Sondag HN, de Jong HA, Oosterveld WJ. Behaviour of adult hamsters subjected to hypergravity. J Vestib Res, 1999; 9: 13-18.

Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev, 2000; 24: 417-463.

Spear LP, Brake SC. Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. Dev Psychobiol, 1983; 16: 83-109.

Spruijt BM, van Hooff JA, Gispen WH. Ethology and neurobiology of grooming behavior. Physiol Rev, 1992; 72: 825-852.

Sternberg WF, Ridgway CG. Effects of gestational stress and neonatal handling on pain, analgesia, and stress behavior of adult mice. Physiol Behav, 2003; 78: 375-383.

Strollo F. Adaptation of the human endocrine system to microgravity in the context of integrative physiology and ageing. Pflugers Arch, 2000; 441: 85-90.

Takeda N, Hasegawa S, Morita M, Matsunaga T. Pica in rats is analogous to emesis: an animal model in emesis research. Pharmacol Biochem Behav, 1993; 45: 817-821.

Temple MD, Kosik KS, Steward O. Spatial learning and memory is preserved in rats after early development in a microfravity environment. Neurobiol Learn Mem, 2002; 78: 199-216.

Thoenen H. Neurotrophins and neuronal plasticity. Science, 1995; 270: 593-598.

Tomko DL, Barbaro NM, Ali FN. Effect of body tilt on receptive field orientation of simple visual cortical neurons in unanesthetized cats. Exp Brain Res, 1981; 43: 309-314.

Tyler DB, Bard P. Motion sickness. Physiol Rev, 1949; 29: 311-369.

Wall PM, Messier C. Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. Neurosci Biobehav Rev, 2001; 25: 275-286.

Tyler WJ, Petzold GC, Pal SK, Murthy VN. Experience-dependent modification of primary sensory synapses in the mammalian olfactory bulb. J Neurosci, 2007; 27: 9427-9438.

Walton K. Postnatal development under conditions of simulated weightlessness and space flight. Brain Res Rev, 1998; 28: 25-34.

Walton K, Heffernan C, Sulica D, Benavides L. Chances in gravity influence rat postnatal motor system development: from simulation to space flight. Grav Space Biol Bull, 1997; 10: 111-118.

Wassersug RJ. Life without gravity. Nature, 1999; 401: 758.

West JB. Physiology in microgravity. J Appl Physiol, 2000; 89: 379-384.

Wilpizeski CR, Lowry LD, Contrucci RB, Green SJ, Goldman WS. Effects of head and body restraint on experimental motion-induced sickness in squirrel monkeys. Aviat Space Environ Med, 1985; 56: 1070-1073.

Wright LL, Bell RW. Interactive effects of parity and early pup stress on the open field behavior of laboratory rats. Dev Psychobiol, 1978; 11: 413-418.

Wubbels RJ, de Jong HA. Vestibular-induced behaviour of rats born and raised in hypergravity. Brain Res Bull, 2000; 52: 349-356.

Wubbels RJ, van Marle J, Sondag HN, de Jong HA. Effects of hypergravity on the morphological properties of the vestibular sensory epithelium. II. Life-long exposure of rats including embryogenesis. Brain Res Bull, 2002; 58: 575-580.

Yates BJ, Miller AD, Lucot JB. Physiological basis and pharmacology of motion sickness: an update. Brain Res Bull, 1998; 47: 395-406.

Zhang TY, Parent C, Weaver I, Meaney MJ. Maternal programming of individual differences in defensive responses in the rat- Ann NYAS, 2004; 1032: 85-103.

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**Pubblicazioni nell'ambito del progetto di ricerca tema del dottorato**

1. N. Francia, G. Corazzi, S. Petruzzi, D. Santucci, E. Alleva "Behavioural responses to hypergravity in the CD-1 mouse". *Acta Astronautica*, 58: 401-410, 2006.

2. N. Francia, M. Simeoni, S. Petruzzi, D. Santucci, L. Aloe, E. Alleva "Repeated acute exposures to hypergravity during early development subtly affect CD-1 mouse neurobehavioural profile". *Brain Research Bulletin*, 69: 560-572, 2006.

3. D. Santucci, N. Francia, V. Trincia, F. Chiarotti, L. Aloe, E. Alleva "A mouse model of neurobehavioural response to altered gravity conditions: An ontogenetical study". *Behavioural Brain Research*, 2008, *Epub ahead of print*.

**Pubblicazioni nell'ambito di progetti di ricerca a latere**

4. N. Francia, F. Cirulli, F. Chiarotti, A. Antonelli, L. Aloe, E. Alleva "Spatial memory deficits in middle-aged mice correlate with lower exploratory activity and a subordinate status: role of hippocampal neurotrophins". *European Journal of Neuroscience*, 23: 711-728, 2006.

5. E. Alleva, N. Francia, M. Pandolfi, A.M. De Marinis, F. Chiarotti, D. Santucci "Organochlorine and heavy-metal contaminants in wild mammals and birds of the Urbino-Pesaro province, Italy: an analytic overview for potential bioindicators". *Archives of Environmental Contamination and Toxicology*, 51: 123-134, 2006.

6. D. Santucci, A. Sorace, N. Francia, L. Aloe, E. Alleva "Prolonged prenatal exposure to low-level ozone affects aggressive behaviour as well as NGF and BDNF levels in the central nervous system of CD-1 mice". *Behavioural Brain Research*, 166: 124-130, 2006.

7. F. Cirulli, N. Francia, L. Aloe, S.J. Suomi, E. Alleva "Early risk factors for neuropsychiatric diseases. Comparative approaches to investigate interactions between genes and environment". In: ISS-NIH Collaboration Programme 2006 Progress Report Meeting: Istituto Superiore di Sanità, Rome, July 4-6, 2006. *Rapporti ISTISAN* 06/50: 101-108, 2006.

8. E. Alleva, N. Francia "Psychiatric vulnerability: Suggestions from animal models and role of neurotrophins". *Neuroscience and Biobehavioral Reviews*, 2008, *Epub ahead of print.*

9. F. Cirulli, N. Francia, A. Berry, L. Aloe, E. Alleva, S.J. Suomi, "Early life stress as a risk factor for mental health: Role of neurotrophins from rodents

to non-human primates". *Neuroscience and Biobehavioral Reviews*, 2008, *Epub ahead of print.*

10. F. Cirulli, N. Francia, I. Branchi, M.T. Antonucci, L. Aloe, S.J. Suomi, E. Alleva, "Changes in plasma levels of BDNF and NGF reveal a gender-selective vulnerability to early adversity in rhesus macaques". *Psychoneuroendocrinology*, 2008, *Epub ahead of print*.

**Presentazioni a congressi**

*Presentazione del poster "Changes in NGF salivary levels during short-term space flight"*. Nerve Growth Factor and related Neurotrophic Factors: from laboratory to clinic. NGF 2006. International Congress Center. Lyon (Francia), 25-29 maggio 2006. (Abstract, p. 173).

*Presentazione del poster "Age-related changes in social behaviour: a study on agonistic interactions of 26-months-old CD-1 mice"*. 5th Forum of European Neuroscience. FENS Forum 2006. Vienna (Austria), 8-12 luglio 2006. (Abstract, http://fens2006.neurosciences.asso.fr/4DLINK7/4DCGI/Resum.corin.5033513620).

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*Relazione "Early risk factors for neuropsychiatric diseases: comparative approaches to investigate interactions between genes and environment"*. 16th Annual Meeting of International Behavioral Neuroscience Society. Rio de Janeiro, Brazil, June 12-16, 2007.

*Presentazione del poster "Communal nesting as a strategy to improve mother-infant relationship in developing mice under altered gravitational environment"*. Biennal International Symposium of ELGRA – European Low Gravity Research Association. Florence, Italy, September 4-7, 2007.

*Presentazione del poster "Early adversity affects nerve growth factor and brain-derived neurotrophic factor and disrupts neurobehavioral development in macaques"*. 39th Annual General Meeting of European Brain and Behaviour Society. Trieste, Italy, September 15-19 2007.

*Relazione "Salivary NGF, cortisol and ACTH levels in an astronaut during the Eneide mission"*. 4th European Congress "Medicine in space and in extreme environments achievements for health care on Earth", Patronized by Berlin Government, Berlin, Germany, October 24-26, 2007.

*Presentazione del poster "Early experiences affect brain plasticity and behavioral development: data from rodents and non-human primates"*. 37th Annual Meeting of the Society for Neuroscience, Neuroscience 2007. San Diego, CA, November 3-7, 2007.

*Relazione "Ground-based paradigms to investigate neurobehavioural effect of alterated gravitational environment in mammals"*. Technology for Artificial Gravity and Microgravity Simulation, Technical Directorate of the European Space Agency (ESA). ESTEC, Noordwijk, The Netherlands, December 10-12, 2007.

**Partecipazione a Corsi**

Frequenza al Ciclo di Seminari settimanali su tematiche di Fisiologia Generale e di Biologia del Comportamento presso il Reparto di Neuroscienze comportamentali del Dipartimento di Biologia cellulare e Neuroscienze dell'Istituto Superiore di Sanità, 2006-2008.

"*On-line Training Courses*" per lo staff scientifico, richiesti ai fini dell'ammissione alle attività sperimentali presso i laboratori dell'NIH (*National Institute of Health*), USA, aprile 2006:

*"Introduction to Laboratory Safety" della Division of Safety, O.R.S., dell'NIH (sito web: http://www.ors.od.nih.gov/labsafety/).*

*"Protection of Human Research Subjects" dell'NIH Office of Human Subjects Research (sito web: http://ohrs.od.nih.gov/cbt/cbt.html).*

*"Technology Transfer Online Training" dell'NIH (sito web: http://tttraining.od.nih.gov).*

*"NIH Research Ethics Orientation" dell'NIH (sito web: http://ethicsorientation.nih.gov).*

*"NIH Computer Security Awareness" del Department of Health & Human Services dell'NIH (sito web: http://irtsectraining.nih.gov).*

*"Prevention of Sexual Harassment Training" dell'NIH Equal Employment Opportunity (sito web: http://eeotraining.nih.gov).*

*"Disability Awareness Training" dell'NIH Equal Employment Opportunity (sito web: http://eeotraining.nih.gov).*

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Corso: "Complicazioni ostetriche e outcome neuropsicologico in età evolutiva: aspetti clinici e sperimentali", organizzato dall'Istituto Superiore di Sanità. Roma, 4-5 dicembre 2007.

## ALTRE ATTIVITA'

### Pubblicazioni a carattere istituzionale e/o divulgativo

D. Santucci, N. Francia, E. Alleva (a cura di) "Montagna e Salute - Atti del Workshop". Collana: Quaderni della Montagna, Serie: Acta, N. 1, Istituto Nazionale della Montagna, Bonomia University Press, 2006, 121 p.

N. Francia, I. Pistella, D. Santucci, M. Pandolfi, E. Alleva "Popolazioni sentinella (uccelli e mammiferi) e inquinamento ambientale nella provincia di Pesaro-Urbino". In: "Montagna e Salute - Atti del Workshop", (D. Santucci, N. Francia, E. Alleva, a cura di). Collana: Quaderni della Montagna, Serie: Acta, N. 1, Istituto Nazionale della Montagna, Bonomia University Press, 2006. Capitolo 4, pp. 35-53.

N. Francia, E. Alleva "La realtà e le prospettive della Legge 6/2000". In: Atti del Convegno "Genetica ed evoluzione umana: fra divulgazione, formazione e ricerca". (O. Leone, A. Saladino, a cura di). Università della Calabria, Rende (CS), Italy, 2006.

N. Francia, E. Alleva "Humans-dogs (and dogs-humans) relationship: emerging problems in urban areas". In: Human and non-human animals interaction: contextual, normative and applicative aspects. Rapporti ISTISAN, 07/40: 58-67, 2007.

E. Alleva, N. Francia "Hunting, fishing, and trapping: falcons, hawks, and nocturnal birds of prey". In: Encyclopaedia of Human-Animal Relationships (M. Bekoff Ed.). Greenwood Press, Colorado, USA, Vol. 3, pp. 961-964, 2007.

E. Alleva, N. Francia "Zoos and acquariums: amphibians and zookeepers". In: Encyclopaedia of Human-Animal Relationships (M. Bekoff Ed.). Greenwood Press, Colorado, USA, Vol. 4, pp. 1341-1342, 2007.

E. Alleva, N. Francia "Zoos and acquariums: reptiles and zookeepers". In: Encyclopaedia of Human-Animal Relationships (M. Bekoff Ed.). Greenwood Press, Colorado, USA, Vol. 4, pp. 1360-1362, 2007.

E. Alleva, N. Francia "Prefazione". In: Aristotele. Vita, attività e carattere degli animali. *Historia animalium – Libri VIII-IX* (A.L. Carbone, a cura di). Duepunti edizioni, Palermo, pp. i-vii, 2008.

E. Alleva, N. Francia, G. Bignami "Convegno: A trent'anni dalla legge 180: la psichiatria prima e dopo Franco Basaglia". Notiziario dell'Istituto Superiore di Sanità, 21: 7-9, 2008.

E. Alleva, F. Cirulli, N. Francia "Rapporti uomini e cani, e cani e uomini, nel Terzo Millennio: problemi emergenti". In: L'uso e l'abuso degli animali: spunti per un'azione didattica (M.C. Barbaro, A.M. Rossi, C. Bedetti, a cura di). Dispense per la scuola, Istituto Superiore di Sanità, 2008 (in corso di stampa).

**Attività di divulgazione/formazione**

Supervisione e cura della sezione "Etologia Applicata" del sito WEB *http://www.iss.it/neco*, del Reparto di Neuroscienze comportamentali dell'Istituto Superiore di Sanità, con Giovanni Laviola e Augusto Vitale.

Relazione: "Dal lupo al cane dal punto di vista dell'Etologia", nell'ambito del Convegno "Il cane: origini, evoluzione, cultura", organizzato dal Comune di Verona e Associazione Gruppo Cinofilo DLF di Verona, Palazzo Gran Guardia, Verona, 6 maggio 2007.

Relazione: "Etologia dell'aggressività", nell'ambito del Convegno "L'aggressività. Quello che c'è di bene nel cosiddetto male", organizzato dal Comune di Verona e Gruppo Cinofilo DLF - Verona, Palazzo Gran Guardia, Verona, 4 maggio 2008.

**Attività didattica**

Lezione su: "Rapporti uomini e cani, e cani e uomini: problemi emergenti" nell'ambito del Corso di Formazione "Uomini e animali, differenti contesti e modalità di interazione: aspetti applicativi e normativi", destinato a operatori del Sistema Sanitario Nazionale in ambito veterinario, sanità pubblica e di istituti pubblici di ricerca (10 crediti formativi ECM per la figura del medico veterinario), organizzato dal Reparto di Neuroscienze comportamentali, Dipartimento di Biologia cellulare e Neuroscienze, dell'Istituto Superiore di Sanità (Direttori del corso: Giovanni Laviola e Augusto Vitale), 18 e 19 dicembre 2006.

Lezione su: "La Legge 6/2000 per la promozione delle attività di divulgazione scientifica" nell'ambito del Master in Comunicazione della Scienza e della Tecnologia, presso la Facoltà di Scienze MM. FF. NN. dell'Università degli Studi di Roma "Tor Vergata (Direttore: A. Novelletto), 4 dicembre 2007.

Lezione su: "Interpretare il comportamento canino: il linguaggio posturale del cane" nell'ambito del Corso di Formazione "Terapie e Attività Assistite in Italia: attualità, prospettive e proposta di linee guida", destinato a operatori del Sistema Sanitario Nazionale in ambito veterinario e sanità pubblica, ed educatori cinofili (con crediti formativi ECM), organizzato dal Reparto di Neuroscienze comportamentali, Dipartimento di Biologia cellulare e

Neuroscienze, dell'Istituto Superiore di Sanità (Direttori del corso: Enrico Alleva, Francesca Cirulli e Daniela Santucci), 4 e 5 dicembre 2008.

**Organizzazione di Workshop e Convegni**

Membro della Segreteria scientifica del Workshop internazionale: "Risk factor for mental health: Translational models from behavioural neuroscience", organizzato nell'ambito del Programma di Collaborazione Istituto Superiore di Sanità - *National Institute of Health* dal Reparto di Neuroscienze comportamentali, Dipartimento di Biologia cellulare e Neuroscienze, dell'Istituto Superiore di Sanità (Progetti: "Early risk factors for neuropsychiatric diseases. Comparative approaches to investigate interactions between genes and environment", Responsabili scientifici: Francesca Cirulli ed Enrico Alleva; "Levels of autoantibodies to neuro-receptor fragments in etiology of compulsive behavior and drug addiction", Responsabile scientifico: Giovanni Laviola; "Neurobehavioral phenotyping of genetically modified mouse models of mental retardation", Responsabile scientifico: Laura Ricceri). Roma 3 e 4 luglio 2006.

Membro della Segreteria scientifica del Convegno: "A trent'anni dalla legge 180: la psichiatria prima e dopo Franco Basaglia", organizzato dal Reparto di Neuroscienze comportamentali, Dipartimento di Biologia cellulare e Neuroscienze, dell'Istituto Superiore di Sanità (Direttori scientifici: Enrico Alleva e Giorgio Bignami). Roma, 24 settembre 2008.

Membro della Segreteria scientifica del Convegno: "Interferenti endocrini: valutazione e prevenzione dei possibili rischi per la salute umana", organizzato dai Dipartimenti di Sanità Pubblica Veterinaria e Sicurezza Alimentare e Biologia cellulare e Neuroscienze dell'Istituto Superiore di Sanità (Responsabili scientifici: Alberto Mantovani e Gemma Calamandrei). Roma, 15 ottobre 2008.

**Organizzazione di Corsi**

Membro della Segreteria tecnica del Corso di Formazione "Nozioni di strategia individuale per la stesura di un lavoro scientifico internazionale. Come, dove, quando" (con crediti formativi ECM); 10 e 17 febbraio 2007, Dipartimento di Medicina Sperimentale, Università degli Studi di Roma "La Sapienza". Il corso è stato organizzato dall'Istituto Superiore di Sanità di Roma (Direttori del corso: Paola De Castro ed Enrico Alleva).

Membro della Segreteria tecnica della seconda edizione del Corso di Formazione "Nozioni di strategia individuale per la stesura di un lavoro scientifico internazionale. Come, dove, quando" (con crediti formativi ECM); 7 e 8 novembre 2008, Dipartimento di Medicina Sperimentale, Università

degli Studi di Roma “Sapienza”. Il corso è stato organizzato dall’Istituto Superiore di Sanità di Roma (Direttori del corso: Paola De Castro ed Enrico Alleva).

Membro della Segreteria scientifica del Corso di Formazione “Terapie e Attività Assistite in Italia: attualità, prospettive e proposta di linee guida” (con crediti formativi ECM), destinato a operatori del Sistema Sanitario Nazionale in ambito veterinario e sanità pubblica ed educatori cinofili; 4 e 5 dicembre 2008, Istituto Superiore di Sanità. Il corso è stato organizzato dal Reparto di Neuroscienze comportamentali, Dipartimento di Biologia cellulare e Neuroscienze, dell’Istituto Superiore di Sanità di Roma (Direttori del corso: Enrico Alleva, Francesca Cirulli e Daniela Santucci).