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Effects of simulated microgravity on brain plasticity: A startle reflex habituation study

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ABSTRACT

There is limited but increasing evidence that space environment, namely weightless condition, may affect astronauts' cerebral neurotransmitters and cognitive performance. The present experiment hypothesized that learning and brain plasticity are affected by simulated microgravity condition. To this aim, 22 male subjects matching astronauts' characteristics were divided in two groups, Head-Down Bed Rest (HDBR) and Sitting Control. After 3-h bed rest (or sitting condition) subjects started a picture viewing task during which 30 acoustic startle probes (100 dBA loudness), divided into three consecutive blocks, were delivered through headphones while startle reflex amplitude was measured from the EMG of the *orbicularis oculi* muscle. Habituation analysis of the startle reflex showed a normal reflex inhibition across blocks in sitting controls and no habituation in HDBR subjects. Results point to a microgravity-induced lack of startle reflex plasticity in subjects matching astronauts, a learning deficit which may affect the success of long-term space missions.

1. Introduction

A large number of physiological studies has demonstrated the influence of microgravity on osteomuscular, immune and cardiovascular systems [1,2], showing the well known side-effects of decalcification, muscular atrophy, loss of flexibility of the autonomic nervous system [3,4], baroreceptors deconditioning [1], fluid shift from legs to trunk and head, loss of extracellular liquids, orthostatic hypotension [for a review see Ref. 5]. Neurotransmitters relevant for cognitive functioning are among the physiological variables affected by microgravity. Indeed, Blanc et al. [6] have shown that a 17-day spaceflight affect more or less directly neurotransimitter release rats: 5-Hydroxytryptophan (the 5-HT precursor) level was found to be significantly decreased in brainstem, frontal cortex, hypothalamus, thalamus and cerebellum and serotonin (5-HT) was reduced in frontal cortex, thalamus and striatum. Similar findings have also been found in hypergravity paradigms: monoaminergic projections (especially 5-HT) in the ventral horn of spinal cord were significantly reduced in newborn, 15-day and 8-month rats exposed to 1.8 G condition [7]. These results suggest that microgravity might impair neurotransmitters, cognitive processes and learning capacity, possibly as a consequence of the gross physiological, hormonal and cardiovascular changes typically observed in such conditions. As an example, the cardiovascular changes induced by microgravity, namely through an altered baroreceptor reflex, may inhibit the ascending reticular formation activation and cortical frontal functioning and this in turn may impair several neural networks, their related neurotransmitters, and perceptual, motor and cognitive processes [8–11]. Although human studies on real microgravity are lacking on this issue, because of the high costs of experiments in space environment (but also because of the lack of consistent samples of subjects in space missions), microgravity ground simulation studies provided some support to the hypothesis of a weightless-induced cognitive impairment. The most used method to simulate microgravity in humans is the "Head-Down Bed Rest" (HDBR) in which the subject lies on a bed with the head tilted down by 6°. This condition is considered, from cardiovascular, muscular and hormonal points of view, to be very similar to microgravity in space: indeed, the comparison of physiological data collected in space with those obtained from ground simulation has confirmed the validity of bed rest microgravity simulation [5,12]. Other ground studies with human subjects have confirmed the hypothesis that, in addition to the known physiological changes induced by simulated microgravity, also cortical activity is impaired. Indeed, HDBR induced an increased amplitude of low-frequency EEG rhythms, i.e., the delta and theta bands [12,13], indexes known to mark cortical inhibition in the adult brain. This general physiological pattern may unmask the risk of astronauts to develop several specific cognitive deficits in space. One of the critical cognitive functions which is important in long-term space missions is learning, and its neural correlate represented by cortical plasticity. Learning and plasticity can be measured by a variety of probes at different levels of the central nervous system. Habituation represents one of the most elementary and basic form of behavioural plasticity

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and learning [14–16] and reflects a central nervous system gating mechanism aimed at inhibiting the processing of repeated stimuli [17]. Reflexes are automated fast and efficient responses to biologically relevant stimuli: the most investigated in both human and animal literature is the startle reflex, a defensive response under both cortical and subcortical control [18,19], elicited by intense, sudden stimuli, such as a loud shot, which is highly preserved and very similar in all mammals [18,20]. The main hypothesis of the present investigation stated that simulated microgravity impairs learning and brain plasticity as measured by startle reflex habituation. We expected that subjects submitted to 3-h HDBR, compared with sitting controls, would show impaired habituation of the startle reflex.

2. Material and methods

2.1. Participants

Twenty-two healthy males (mean age = 27.05, SD = 2.55) were recruited from engineering and astronomy departments in Padova. Each subject was paid 52€ for his participation. They were randomly assigned to one of two groups of 11 participants each: Bed Rest (BR) and Sitting Control (SC). BR participants (mean age = 26.90, SD = 2.02) were requested to lie down for 4 h in −6° Head-Down Bed Rest position [12], whereas SC (mean age = 27.18, SD = 3.09) were engaged in the same tasks and conditions as BR group, but in the sitting position. Two subjects of the SC group were excluded from statistical analysis due to recording problems (electrode detachment) during the experimental session. BR participants lied down during the whole startle procedure (which occurred at about 3 h since the beginning of bed rest) and the experimenter paid attention that subjects did not fall asleep throughout the whole bed rest period. This was made easier by the fact that most of the time subjects were engaged in instruction administration and in some tests. The experiment was carried out in accordance to the Declaration of Helsinki, and all procedures were carried out with the adequate understanding and written consent of the subjects. The experiment was approved by the local Ethics Committee.

2.2. Procedure

Participants started the experimental procedure at 9:00 a.m. and left the laboratory at 2:00 p.m. They were requested not to drink coffee or tea and not to smoke cigarettes after 8:00 a.m. (1 h before the experimental session). After electrode attachment, the participants were randomly assigned to BR or SC condition. Startle reflex was elicited by an unexpected 100 dBA burst of white noise of 50 ms duration (next referred in the text as to "startle probe") and administered through closed stereo headphones. The EMG startle response was measured from the left *orbicularis oculi* by applying two 5 mm Ag/AgCl electrodes below the lower eyelid at a distance of 10 mm from each other. The additional ground electrode was placed on the left *subclavicle fossa*. The raw EMG signal was amplified with a gain of 10.000 and filtered with a 16 Hz high pass and a 340 Hz 2nd order low pass filter. The signal was also rectified and integrated with a 100-ms time constant integrator. Activity from the *orbicularis oculi* was sampled at 250 Hz, 0.5 s before and 1 s after the startle probe [18,19].

To avoid problems related to unpredictable subject's strategies when left without a task (some subjects close their eyes, others practice active relaxation or active imagery, etc.) startle probe was delivered during a standard picture viewing task. After 3 h of bed rest (or sitting control condition), at around 12:00 a.m., the task started: 75 standardized photographic slides selected from the International Affective Picture System (IAPS) [21] were presented to participants. In order to measure reflex habituation across time, slides for purpose analysis only (subjects were not aware of the blocks as they watched all slides without interruptions) were divided into three consecutive blocks of 25 slides each, with no pause or interruption between the blocks. Slides were projected for 6 s with an inter-trial interval randomly varying between 11 and 14 s, in a pseudo-random sequence for probe presentation. Since the emotional content of the slides may affect startle reflex, the three blocks included the same number and kind of emotional slides to avoid the influence of this variable. Their sequence had to satisfy the following criteria: slides with an acoustic startle probe had to be equally distributed in the three blocks of 25 slides; no more than two slides with or without acoustic probe had to be shown consecutively. Startle probes were presented 4 s after picture onset in 40% of the trials (overall 30 slides had the startle probe, 10 slides in each block).

2.3. Data acquisition and statistical analysis

Analysis of the startle reflex habituation was performed by dividing pictures with startle probe into three blocks of ten slides each. For each subject, raw startle data (measured in μV) were visually inspected to detect and reject rare artifacts and, after averaging all accepted trials, the latency of the greatest peak found in the 20–100 ms time interval was used to set a 20-ms window centred on the peak [for analysis, see also Ref. 18]. Data acquisition and analyses were performed with LabVIEW software (National Instruments) according to Angrilli [22].Within the 20-ms peak-adjusted time window, the mean value of the integrated startle entered ANOVA analysis with one between-subjects variable, Group (Bed Rest vs. Sitting Controls), and one within-subjects factor Block (First vs. Second vs. Third). Newman–Keuls test was used for post-hoc analysis.

3. Results

3.1. Startle reflex habituation

ANOVA of startle reflex habituation revealed a significant Block Main Effect ($F_{(2, 36)} = 8.84$, $p < 0.0008$), overall participants showed a reduced startle reflex in the second and third blocks compared to the first one (Post-Hoc Newman–Keuls: First vs. Second, $p < 0.03$; First vs. Third, $p < 0.002$).

Furthermore, ANOVA revealed a significant Block × Group interaction ($F_{(2, 36)} = 3.41$, $p < 0.05$). Post-Hoc Newman–Keuls comparisons showed in the SC group a significant startle reflex amplitude reduction in the First vs. Second Block ($p < 0.02$) and First vs. Third Block ($p < 0.0007$), while the BR group did not show any startle reflex habituation across stimuli presentation[1] (Fig. 1). Post-hoc analysis did not reveal any significant differences between groups at any block (all ps > 0.20), even in the first block (see Fig. 1 and error bars). Further analysis based on effect size showed Cohen's d of 0.326, 0.192, and 0.353 between groups at Blocks 1, 2 and 3 respectively. Within the sitting control group, Cohen's d between the 1st and 3rd blocks was 0.803 (large effect) but within the BR group it was 0.266 (small effect).

4. Discussion

In the present experiment we used startle reflex habituation as a probe to test the hypothesis that 3 h of simulated microgravity leads to a learning impairment in astronauts. Results showed a normal habituation/inhibition of the startle reflex across the three blocks of startle probes in sitting controls, and an unaffected reflex amplitude in the BR group. Startle reflex reduction across blocks is an important index of learning and plasticity [15]. Habituation represents one of

[1] Further ANOVA analysis carried out on 6 blocks of five startles each, in BR participants, did not reveal any significant effect across blocks, thus the lack of habituation was evident also in a more fine distribution of blocks.