

# Resting State Brain Activity During Long-Term Dry Immersion

Ivan E. Lazarev; Elena S. Tomilovskaya; Inesa B. Kozlovskaya

- BACKGROUND:** The purpose of this work was to investigate the brain's rhythmic activity during a simulated microgravity condition (namely dry immersion).
- METHODS:** During dry immersion, which lasted for 5 d, nine subjects (healthy men, 20 to 29 yr of age) were individually placed in a tub ( $2.2 \times 1.1 \times 0.85$  m) filled with water (temperature was kept constant at about 33°C). Subject floated in the tub without bodily support in the supine horizontal position, but isolated from the water by waterproof material. Resting state EEGs were registered at the fourth or fifth day of dry immersion. Under the control conditions, resting state EEGs were registered while subjects laid in a supine position on a couch.
- RESULTS:** Compared to the control condition, EEG power in the alpha range (8–13 Hz) was greater in dry immersion; this effect was distributed across the whole scalp. No effects of dry immersion were found for the beta, delta, or theta frequency bands.
- CONCLUSION:** The results of the study, similar to those obtained in a real spaceflight, indicate that support withdrawal is an important contributor to brain activity alterations in weightlessness.
- KEYWORDS:** EEG, weightlessness, support withdrawal, dry immersion.

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Investigations of human performance and brain activity under microgravity conditions has expanded significantly in recent years. There are considerable data depicting various ways in which cosmonauts and astronauts fail to perform goal-directed actions or movement control.<sup>3,17,21</sup> Meanwhile, studies of brain activity involving alterations or impairment in space show similar observed phenomena but are not very numerous.

Schneider and colleagues<sup>25</sup> reported that in relatively short periods of weightlessness during parabolic flight, electroencephalographic (EEG) changes are observed in the beta2 (18–35 Hz) frequency range in the brain's right frontal lobe; power density was decreased in the 0-g (weightlessness) condition compared to the 1-g in-flight condition. According to Schneider, this result is due to changes in the emotional state of the subjects during the switch to weightlessness. The presence of avoidance motivation (such as fear and uncertainty) is known to elicit greater activation in the right frontal lobe compared to the left frontal lobe, although this notion is mostly supported by the change in alpha range EEG.<sup>1,6,13</sup> Although emotional states were not measured directly, the probable change in activation

levels in the right frontal lobe (as indexed by diminished beta) may point to the experience of pleasant feelings during the exposure to weightlessness. In much longer periods of weightlessness (from 4 to 155 d), during stays at the International Space Station (ISS), Cheron<sup>4</sup> found that EEG power is increased in the alpha frequency range (near 10 Hz). Supposedly, this increase is connected to the lower amount of gravity-related signals under microgravity conditions. According to Klimesch and Pfurtscheller,<sup>14,23</sup> a pronounced alpha rhythm may be related to lower neural activity or may be a sign of active inhibition of irrelevant activity, the decreased gravity-related signal may lead to the lower activity of relevant sensory systems, and

From the School of Psychology, National Research University Higher School of Economics, Moscow, Russia, and the SSC RF Institute of Biomedical Problems, Russian Academy of Sciences, Moscow, Russia.

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Address correspondence to: Ivan E. Lazarev, Ph.D., School of Psychology, National Research University Higher School of Economics, Myasnitskaya st. 20, Moscow 101000, Russia; verazali@gmail.com.

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thus to more alpha oscillations in the EEG. No alpha EEG asymmetry was noted in the spaceflight experiment, although Cheron<sup>4</sup> mentions cosmonaut reports about weightlessness not being an unpleasant state.

Since experiments in weightlessness are hindered for obvious organizational reasons, various simulative techniques are used. Models of microgravity on the ground are widely used for these studies. One of the most popular models has been head-down tilt bed rest (HDBR). This procedure is known to elicit some changes in body fluid redistribution, similar to the ones observed in weightlessness.<sup>2</sup>

Vaitl<sup>28</sup> found increased delta (0.50–3.75 Hz), theta (4–7.75 Hz), and, to a lesser degree, alpha (8–13.75 Hz) frequency band power during HDBR compared to head-up bed rest. It is worth noting that HDBR did not last more than 1 d, so (as in the study of Schneider) rather earlier effects were registered. Han and colleagues<sup>12</sup> generally confirmed the notion that EEG spectral power increases in a broad range of frequencies: delta, theta, and alpha in HDBR. Power increase in the lower part of the EEG spectrum is explained by cardiovascular effects: fluids are redistributed during HDBR, so the cardiovascular system tries to maintain homeostasis between circulation and brain activity. The changes in brain activity registered by means of EEG do not necessarily have their source at the cortex level: in a functional magnetic resonance imaging (fMRI) investigation, it was shown that metabolic activity in the brain during HDBR was altered at the level of the thalamus (more specifically, in the left part of it<sup>19,20</sup>).

Dry immersion (DI) is another alternative microgravity simulative technique, which some researchers believe has advantages over HDBR.<sup>15,16,22</sup> The main distinctive feature of DI is the reduced amount of support afferentation, due to the loss of bodily support (see Methods for more details). A short term DI model has been used to investigate alterations in brain activity by means of EEG. It gave contradictory results to the ones obtained by Vaitl and Hun: in general, there was a decrease in EEG power in higher frequency bands, particularly in the alpha range<sup>18</sup> at the second day of DI. Interestingly, alterations in brain hemisphere activation were also found: slow presaccadic negative EEG potentials were more pronounced in the right-sided areas during 6 d of DI.<sup>27</sup> Still, since in this case slow potential measures were taken outside of the tub, this result may be more related to the adaptation processes connected to environmental changes (the switch from DI) than to processes related to the loss of support.

In our opinion, there is one question which has not been clearly addressed in the above mentioned studies: are the changes in brain activity related to the short period of adaptation to the new environment, or to the period when adaptation is already finished? Obviously, the difference in results reported by Schneider and Cheron may be explained by the amount of time their study participants spent in weightlessness. The same question applies to the modeling studies involving HDBR or DI.

The current study is dedicated to the investigation of the brain's rhythmic activity during simulated microgravity

conditions (namely dry immersion). The study is different from the one performed by Kuznetsova and colleagues<sup>18</sup> since here we report changes that occurred at the fourth and fifth days of dry immersion, in an attempt to separate the effects related to adaptation processes with ones related to the withdrawal of bodily support. Thus the purpose of the study was to investigate probable changes in brain activity related to the period when adaptation to the new environment is already finished. More specifically, we investigated oscillatory brain activity in the alpha, theta, delta, and beta ranges. In order to control for possible brain activity changes related to emotional states, subjective emotional state reports and alpha asymmetry indices were also evaluated.

## METHODS

### Subjects

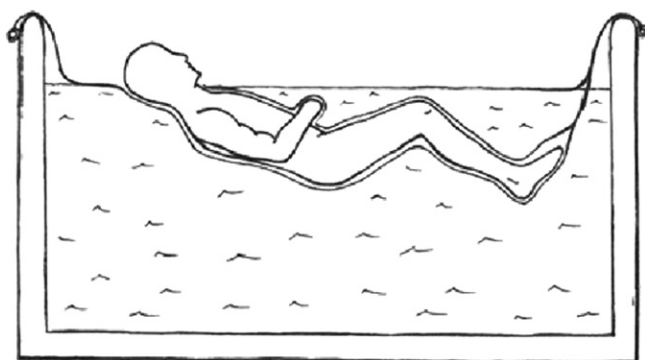
In our study, 10 healthy volunteers were paid to serve as subjects. Informed consent was obtained from all individual subjects. The experimental protocol was reviewed and approved by the RF SSC-IBMP Bioethics Board. All procedures performed in studies involving human subjects are conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All of the volunteers were men between 20 and 29 yr of age. Later, one individual was excluded from the analysis because he did not feel well during the process of dry immersion. He developed a gastroenteric upset and was unable to sleep at nights, so his arousal level during EEG registration times obviously differed from those of the other subjects.

### Equipment

The experiment was performed in a chamber with standard ceiling lighting. The EEG was recorded from 64 electrodes using a Quickcap (Compumedics™, Abbotsford, Victoria, Australia) electrode cap. The ground electrode was placed near the Fz electrode, whereas the reference electrode was placed near the vertex area. Electrodes were adjusted to obtain the impedance's lowest possible values and impedance was kept below 20 kΩ. The EEG was amplified using a Neuroscan2 (Compumedics™) system, band-pass filtered (0.15–400 Hz), and digitized at 1000 Hz.

### Procedure

The procedure of dry immersion is described in detail elsewhere (see Navasiolava *et al.*<sup>22</sup>). The participant was placed in a tub (2.2 × 1.1 × 0.85 m) filled with water kept at a constant temperature of about 33°C. Since the subject was isolated from the water by free floating waterproof material, the immersion was 'dry' (see Fig. 1). In our study, the subject was floated in the tub in a supine horizontal position for 5 d; he was allowed to leave the tub for 15 min each day for hygiene procedures. The experiment consisted of two sessions: one before the dry immersion procedure and the next one at the fourth day of DI (for five



**Fig. 1.** Dry immersion procedure. The subject was placed in the tub and separated from the water with waterproof fabric.

subjects) or at the fifth day of DI (for four subjects). We have pooled the fourth and fifth days' data together because it is known that at this time the effects of DI change rather quantitatively and thus in DI studies it is a common practice to merge data from days 3 to 7.<sup>22</sup>

Both sessions were performed at about 1800. During the first (control) session, subjects laid in a supine position on the couch, to keep their posture similar to the one experienced during dry immersion. During the second session, they floated in the dry immersion tub, again in a supine position. Each session consisted of about 2 min of EEG recording during which subjects were asked to close their eyes and relax. After the EEG recording, the subjective emotional state of subjects were assessed by means of a standardized questionnaire.<sup>8</sup>

### Statistical Analysis

During the offline processing, the EEG was re-referenced to averaged mastoids. After that, it was divided in 4-s length epochs (50% overlap), visually inspected for artifacts, and submitted to Fast Fourier transformation. The EEG was analyzed within EEGLAB<sup>7</sup> software.

EEG absolute power was calculated in the delta (1–3 Hz), theta (4–7 Hz), alpha (8–13 Hz), and beta (14–19 Hz) frequency bands. After that, relative power values were obtained as a proportion of total power in the 1–19 Hz range. Relative power values were submitted for the statistical analysis.

An analysis of variance (ANOVA) with repeated measures was used to analyze the EEG. The repeated measure factors were: 'immersion' (2 levels for paired comparisons of 2 sessions before and during dry immersion) and 'electrode' (62 levels).

The EEG alpha power asymmetry index was calculated as  $(R-L)/(R+L)$  where R is the alpha (8–13 Hz) mean power index across the frontal right electrodes (F4, F6, and F8) and L is the mean power index in the frontal left electrodes (F3, F5, and F7, accordingly). Thus each subject was characterized by two asymmetry indices: in the DI session and in the control condition. Indices were submitted for the nonparametric Wilcoxon signed ranks test. The values of subjective emotional states obtained in control sessions and in dry immersion sessions were also submitted for the nonparametric Wilcoxon

signed ranks test. All statistical tests were performed by means of the SPSS 13 package.

## RESULTS

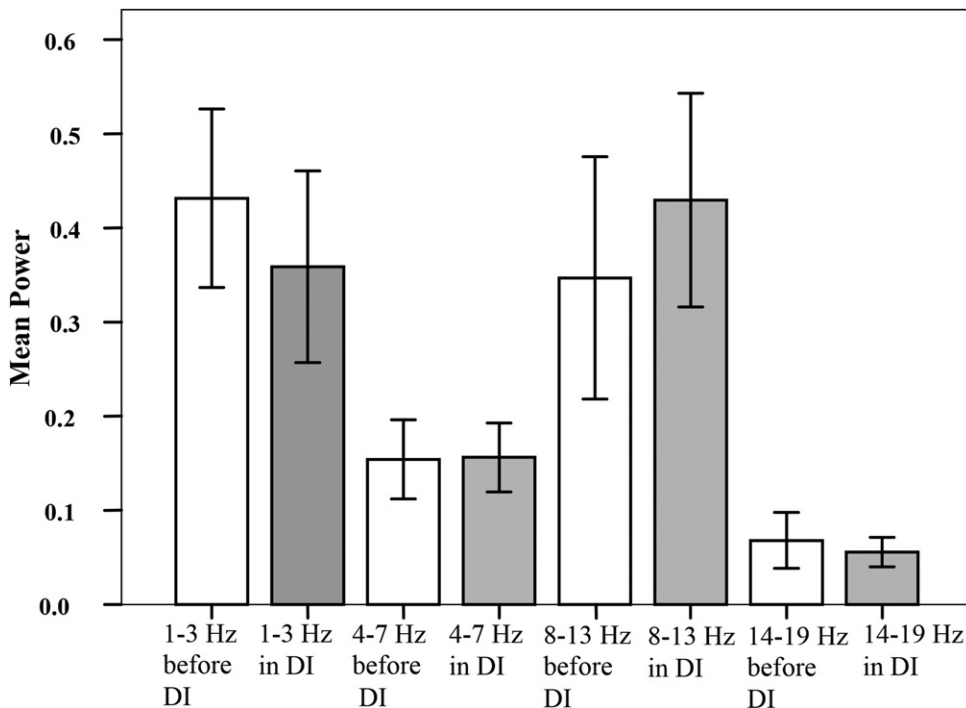
The comparison of emotional states before and during dry immersion did not reveal any significant changes ( $Z = -1.826$ ,  $P = 0.07$ ), although subjects generally ranked their mood as higher during the dry immersion. Alpha asymmetry indexes did not differ across conditions ( $Z = -0.059$ ,  $P = 0.95$ ). There were also no correlations found between subjective emotional state ranks and alpha asymmetry indices in the dry immersion or the control condition.

Absolute total power of EEG in the range 1–19 Hz did not change significantly across sessions ['Immersion' factor:  $F(1,8) = 3.608$ ,  $P = 0.09$ ; 'Immersion'  $\times$  'Electrode' factor:  $F(61,488) = 1.448$ ,  $P = 0.07$ ]. Compared to the control condition, total delta (1–3 Hz) power was slightly lower during dry immersion, but this effect was not significant ['Immersion':  $F(1,8) = 4.428$ ,  $P = 0.07$ ; 'Immersion'  $\times$  'Electrode':  $F(61,488) = 1.452$ ,  $P = 0.23$ ]. No significant differences were found in the theta (4–7 Hz) range in the control condition or dry immersion ['Immersion':  $F(1,8) = 1.768$ ,  $P = 0.22$ ; 'Immersion'  $\times$  'Electrode':  $F(61,488) = 1.033$ ,  $P = 0.41$ ]. Compared to the control condition, total alpha (8–13 Hz) power was greater during dry immersion (**Fig. 2**, **Fig. 3**) and this effect was distributed across the whole scalp ['Immersion':  $F(1,4) = 6.106$ ,  $P < 0.05$ ,  $\eta_p^2 = 0.433$ ; 'Immersion'  $\times$  'Electrode':  $F(61,488) = 1.507$ ,  $P = 0.22$ ]. There were no differences in the beta (14–19 Hz) frequency range across sessions ['Immersion':  $F(1,8) = 2.409$ ,  $P = 0.16$ ; 'Immersion'  $\times$  'Electrode':  $F(61,488) = 1.255$ ,  $P = 0.31$ ].

## DISCUSSION

In this study we observed a significant increase of the alpha range oscillation power (8–13 Hz) in the resting state EEG at the fourth and fifth days of dry immersion; the size of this effect indicates that dry immersion's influence was rather salient. No significant changes were found in the theta (4–7 Hz), beta (14–19 Hz), or delta (1–3 Hz) ranges. Overall EEG absolute power did not change significantly, indicating that changes in relative power happened due to changes in the general pattern of neural activity rather than a local independent increase of absolute power in the alpha range.

These changes were not connected to the changes in the subjects' emotional states because there were no relationships found between mood and frontal alpha asymmetry (which is considered to be sensitive to the emotional state<sup>1</sup>), and there was also no significant change of the emotional state itself from the control to dry immersion sessions. This contradiction to Schneider's results is not too surprising, since dry immersion and short-term weightlessness obviously differ in their potential to elicit a change of emotional state.



**Fig. 2.** Mean power values in the delta (1–3 Hz), theta (4–7 Hz), alpha (8–13 Hz), and beta (14–19 Hz) ranges in the vertex (Cz) area (mean  $\pm$  SE).

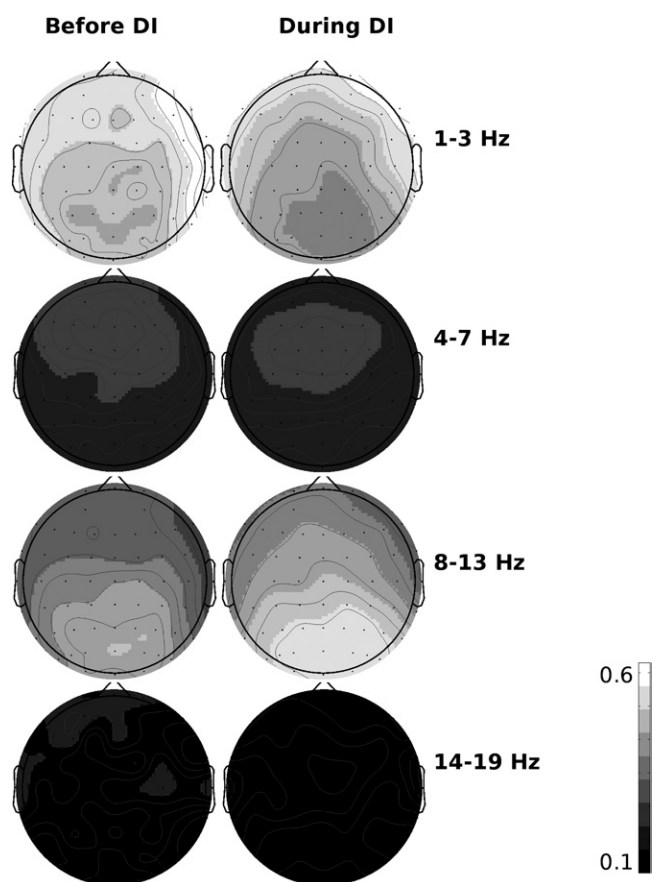
Potential stress does not seem to play a role either: even if we do not consider the absence of significant mood changes, there was no change in theta EEG activity, which is expected when a stressful event takes place.<sup>9,10,26</sup> No other factors related to cognitive load or task performance seem to play a role in this effect; in both cases the EEG was recorded during the resting state and subjects were explicitly asked to relax. Thus the main finding of the study seems to be related to the specific prolonged posture change experienced during dry immersion. Dry immersion can cause a lot of interconnected effects in almost all physiological systems, including the neuromuscular, skeletal, cardiovascular, and immune systems, and it can also affect metabolism, respiration, and thermoregulation.<sup>22</sup> There is a possibility that a single variation in modulation of a particular system's functioning may cause the observed increase of alpha rhythm power. We nonetheless expect that most of the effect does not change the level of cortical activation directly, since there is a general opinion that the cortex is not involved directly in the regulation of physiological systems' functioning, like that of the immune and cardiovascular systems. In our opinion, the alpha rhythm power increase occurred not because of changes in any particular physiological system, but rather from the diminution of afferent signals. A main result of this study seems to contradict the data obtained in other spaceflight simulation experiments, most of which found an increase in the low-frequency (theta and delta) bands. It is worth noting, however, that a decrease in EEG power was always found in the first hours or initial 2 d of the experimental manipulation (change of posture during bed rest or in the water immersion).

In our study, this effect was observed at the fourth and fifth days of the dry immersion and thus the length of manipulation

was significantly longer. According to Vaitl,<sup>28</sup> changes in the circulatory system's performance are the main source of brain activity modulation during posture changes. In our opinion, circulatory system performance changes do not directly modulate brain activity, but might act by means of a mediator. As has been shown earlier in animal studies, stress can elicit a significant increase in the power of theta and delta EEG activity.<sup>26</sup> It is also known that during dry immersion the production of the stress hormone cortisol increases only during the first day of the immersion and not later.<sup>22</sup> Back pain experienced during dry immersion mostly disappears at the second or third day.<sup>22</sup> As for head-down bed rest, there is no difference in perceived negative or positive affects nor in the perceived stress level during

HDBR.<sup>5</sup> It is also known that the cortisol level (which may point to the possible experience of stress) remains stable or even slightly decreases during HDBR.<sup>5,24</sup> It is worth noting that in the aforementioned HDBR studies, measures were taken no earlier than the 13<sup>th</sup> day of the bed rest. In cases of shorter bed rest (10 d long), cortisol levels remain higher compared to the control condition, pointing to the initial stress response.<sup>11</sup> Thus the higher level of alpha depression observed at the second day of DI by Kuznetsova,<sup>18</sup> as well as the increase in low-frequency (delta and theta) oscillations observed during head-down bed rest by Vaitl, may be due to a pronounced stress response accompanying the first hours of a posture change. The difference in results obtained in the current study and in the one by Kuznetsova<sup>18</sup> can probably be explained by the ongoing process of adaptation to the new environment during the first 2 d of posture changes and at the end of this process up to the fourth day of immersion. If this is true and our results are unrelated to the short-time adaptation process, the increase in alpha power may be due to the main effect of dry immersion: a decrease in the input of support afferentation. Still, the number of subjects in our study is rather small and further studies are needed to generalize this notion.

Interestingly, our results are similar to the ones obtained during a real spaceflight in which EEG recordings were performed on or after the fourth day of the flight.<sup>4</sup> Cheron explains the observed increase of alpha oscillations during a spaceflight as being a reflection of vestibular network activity modulation due to a reduction of gravity-related signals. Since gravity-related signals are reduced, the vestibular network, which includes the parietal cortex, becomes inactive as indexed by more pronounced alpha oscillations. In DI, gravity-related signals remain unchanged, but both DI experiment participants and cosmonauts/astronauts



**Fig. 3.** Mean power spectrum value topographic maps in the delta (1–3 Hz), theta (4–7 Hz), alpha (8–13 Hz), and beta (14–19 Hz) ranges. Left: before dry immersion (DI); right: at the fourth/fifth day of dry immersion.

experience a reduction of support related afferent information input. Thus we assume that the reduction of support and the absence of gravity-related signals, rather than absence of gravity-related signals alone, may be the main factors responsible for the modulation of resting state brain activity during weightlessness when the initial adaptation process has ended.

## ACKNOWLEDGMENTS

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*Authors and affiliations:* Ivan E. Lazarev, Ph.D., School of Psychology, National Research University Higher School of Economics, Moscow, Russia; and Ivan E. Lazarev, Ph.D., Inesa B. Kozlovskaya, M.D., Ph.D., and Elena S. Tomilovskaya, Ph.D., Institute of Biomedical Problems RAS, Moscow, Russia.

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