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Reductions in circulating endocannabinoid 2-arachidonoylglycerol levels in healthy human subjects exposed to chronic stressors

Buqing Yi [a,*], Igor Nichiporuk [b], Michel Nicolas [c], Stefan Schneider [d,e], Matthias Feuerecker [a], Galina Vassilieva [b], Detlef Thieme [f], Gustav Schelling [a,*], Alexander Choukèr [a,*]

[a] Hospital of the University of Munich (LMU), Marchioninistrasse15, D-81377 Munich, Germany
[b] Institute of Biomedical Problems, 123007 Moscow, Russian Federation
[c] University of Burgundy, SPMS (EA 4180), 21000 Dijon, France
[d] Institute of Movement and Neurosciences, German Sport University Cologne, 50933 Cologne, Germany
[e] Faculty of Science, Health, Education and Engineering, University of the Sunshine Coast, Maroochydore, Queensland, Australia
[f] Institute of Doping Analysis and Sports Biochemistry, D-01731 Dresden, Germany

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ABSTRACT

Increasing evidence indicates that chronic stress, such as social isolation, plays an important role in the development of a variety of psychiatric and somatic disorders. Meanwhile, chronic stress imposed by prolonged isolation and confinement in the spacecraft is also one of the major concerns for the health of future interplanetary space travelers. Preclinical studies suggest that the peripheral endocannabinoid (eCB) system is involved in the regulation of the stress response and eCB signaling is implicated in the pathogenesis of stress-related diseases. However, there are only few human studies addressing this topic, of which most focusing on patients who have already developed a certain type of disorder. It remains unknown whether chronic stress may affect eCB signaling in healthy humans. A 520-d isolation and confinement study simulating a flight to Mars provided an extraordinary chance to study the effects of prolonged stress in healthy humans. During the study period, the participants lived in confinement and could not meet their families, friends, or strangers for more than 500 days. We examined the impact of chronic exposure to isolation and confinement through monitoring their psychological state, brain cortical activity, sympathetic adrenal–medullary system response and eCB signaling response. We observed reduced positive emotion ratings, decreased brain cortical activities and high levels of catecholamine release, indicating that prolonged exposure to isolation and confinement stressors may bring about changes both psychologically and physiologically. Importantly, for eCB signaling response, blood concentrations of eCB 2-arachidonoylglycerol (2-AG), but not anandamide (AEA), were significantly reduced ($p < 0.001$), suggesting that dysregulation of 2-AG signaling might be specifically implicated in the response to chronic stressors.

1. Introduction

Mounting evidence has indicated that chronic stressors could activate the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic–adrenomedullary (SAM) system, leading to release of glucocorticoids such as cortisol (Lopez et al., 1999; Chrousos et al., 2009; Yi et al., 2014) and secretion of catecholamines (Lopez et al., 1999; Choudhury et al., 2009), which may contribute to the development of a variety of health problems including both psychiatric and somatic disorders (Cohen et al., 2007, 2012; McEwen, 1998, 2008; Miller et al., 2007; Reiche et al., 2004). Animal experiments indicate that stress also mobilizes the endocannabinoid (eCB) system in both the brain and the periphery and that the eCB system can modulate behavioral and endocrine responses to stress (Hill et al., 2010; Ramikie and Patel, 2012; Patel and Hillard, 2008). However, the effects of chronic stress in healthy humans are still not well understood, mainly owing to the difficulty of finding a proper study model to track healthy humans living under prolonged stress with strictly controlled environmental factors. In particular, it is unclear how the evolutionarily conserved neuromodulatory eCB system responds to chronic stress in healthy humans.

Chronic stress is not only a health concern in our everyday lives, but also one major concern for preparing future interplanetary space exportation, such as going to Mars, because an interplanetary space traveler has to be isolated and confined in the spacecraft for a long period. A 520-d isolation and confinement study simulating a flight to Mars (Mars520) was performed recently, providing an extraordinary opportunity for investigating the effects of prolonged stress. With a mid-mission landing on a simulated Mars surface,

* Corresponding authors.
E-mail addresses: bootsing.yi@gmail.com (B. Yi), Gustav.Schelling@med.uni-muenchen.de (G. Schelling), alexander.chouker@med.uni-muenchen.de (A. Choukèr).

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the simulation took place in a spaceship-like enclosed habitat under strictly controlled environmental condition. In this study, six healthy male participants lived in confinement and could not meet their families, friends, or strangers. This condition lasted for 520 days. Especially the simulation of returning flight (the period of 320th to 520th day) offered a suitable opportunity to investigate the impact of prolonged chronic stress in view of the fact that almost no acute provoking stimuli during this time. Therefore, we have chosen this time as our study period.

Psychological evaluation of the Mars520 participants was performed independently by several studies with different focus. It has been reported that the participants showed a tendency of 'assigning positive ratings to negative pictures', and the results suggest that psychological stress was increased with time into the mission (Wang et al., 2014). Physiologically, persistent high-level cortisol was observed during the prolonged isolation period, indicating changes of HPA axis activity (Yi et al., 2014). Since these previously reported results indicated that the Mars520 subjects lived under the influence of chronic stress imposed by prolonged isolation, with existing samples or measurements, we performed further analysis on other classical or recently emerged stress markers to get a more comprehensive view of the effects of prolonged isolation and confinement.

In addition to classical stress markers cortisol and catecholamine, the measurement of neocortical dynamic functions by electroencephalography (EEG) is a recently emerged stress indicator. Several studies (e.g. Alonso et al., 2015) suggest that useful stress indexes may be achieved from EEG-based features. Therefore, in this study we have also applied EEG measurements. The main focus of the current study is to investigate the effects of prolonged isolation and confinement on the eCB system in the healthy participants. The eCB system mainly consists of central and peripheral G-protein-coupled receptors (CB1 and CB2) with their corresponding endogenous ligands AEA and 2-AG (Pertwee, 2014). It is known that eCB signaling may regulate the stress response, primarily acting to constrain activation of the HPA axis by means of distributed actions in the limbic and hypothalamic circuits in the brain (Hill and Tasker, 2012; Riebe and Wotjak, 2011). Increasing evidence has established a link between chronic stress and eCB signaling response. Several preclinical studies indicate that repeated restrain stress may increase eCB 2-AG levels (Patel and Hillard, 2008; Patel et al., 2005; Rademacher et al., 2008), and multiple studies in humans also have revealed changes in circulating levels of eCBs in individuals diagnosed with depression or PTSD (Neumeister et al., 2013; Hill et al., 2008a, 2009a, 2013; Hauer et al., 2013), but the nature and direction of eCB signaling response often differ from one study population to the other.

In the current study, psychological state, brain activity, SAM system response and eCB signaling response were analyzed by self-report ratings of positive and negative affect, EEG signals, catecholamine secretion and circulating levels of eCBs, respectively. In addition, we have run correlation tests among these parameters. We hypothesized that the chronic stressors of prolonged isolation and confinement may affect circulating levels of eCBs, and there might be correlation existing among these stress indicators.

2. Methods

2.1. Participants and procedures

This isolation and confinement study simulating a flight to Mars was conducted at the Institute of Biomedical Problems (IBMP) in Moscow and approved by several ethical boards of the Russian Federation and European Space Association authorities. Based on modified astronaut selection criteria (please see details in the supplementary material), six healthy male volunteers (mean ± SD; age (y): 33 ± 6; size (m): 1.76 ± 0.04; weight (kg): 81 ± 5; BMI: 26 ± 2) were selected following a detailed medical history and clinical examination. They provided written informed consent after due approval to spend 520 days in an enclosed habitat. Like a real Mars voyage, the participants lived under an extreme condition of social isolation (no chance of meeting family, friends or strangers) and confinement. Environmental factors were maintained constant throughout the study period. More details about the background of the participants, the experimental procedure, the environmental control and medical control during the study period are described in the supplementary material. All studies were done as outlined in the Declaration of Helsinki.

2.2. Psychological state evaluation

Once monthly, the participants completed the Positive Affect Negative Affect Schedule (PANAS) (Watson et al., 1988). PANAS is a valid and reliable measure of affective states categorized into higher-order dimensions of affective experience called positive and negative affect (Watson et al., 1988). This self-report adjective checklist consists of two 10-item subscales designed to measure positive (i.e., active, alert, attentive, determined, enthusiastic, excited, inspired, interested, proud, and strong) and negative affect (i.e., afraid, ashamed, distressed, guilty, hostile, irritated, jittery, nervous, scared, and upset) experienced during a given time frame. In the current study, we instructed the participants to rate the extent to which they experienced each affective state during the previous three days. Individuals responded to each item on the following scale: (1) very slightly or not at all, (2) a little, (3) moderately, (4) quite a bit, and (5) extremely. Negative affect represents a general dimension of subjective distress subsuming a variety of states such as anger and anxiety. Positive affect represents the "extent to which individuals feel enthusiastic, active, and alert" (Watson et al., 1988). Its adequate psychometric properties have contributed to the widespread use of the PANAS (Watson and Clark, 1997; Crawford and Henry, 2004). Baseline data were collected 21 days before the isolation started.

2.3. Electroencephalographic measurement

Once every two weeks, electroencephalographic measurement was performed before routine exercise. The EEG method was chosen in order to allow – unlike PET- or MRT-scanning – measurements on site and within reasonable time. EEG data collection took place in a sitting position for 3 min. Participants were seated in a relaxed position, with eyes closed. Data were recorded using Brain Vision Amplifier and RecView software (Brain Products GmbH, Munich, Germany) at 500 Hz. A 16-channel active EEG system (Brain Products GmbH, Munich, Germany) with electrodes on sites Fp1, Fp2, F7, F3, F4, F8, T7, T8, C3, C4, P7, P3, P4, P8, O1 and O2) was placed according to the international 10–20 system (Klem et al., 1999). To facilitate signal transduction, electrode gel (SuperVisc, EasyCap GmbH, Herrsching, Germany) was applied on each electrode position via a syringe with a blunt-top needle. EEG recordings were edited with Brain Vision Analyzer 2.0 software (Brain Products, Munich, Germany). The EEG-data were filtered with phase shift free Butterworth filters (low cut-off at 2 Hz, high cut-off at 40 Hz, time constant at 0.0265 s, 48 dB slope) including a Notch-filter at 50 Hz. EEG-channels with impedance above 10 K Ω were excluded from further analysis. The remaining recordings were divided into 4-second segments each allowing a 10% overlap. An automatic artifact correction algorithm was applied, which allowed a maximum voltage step of 50 μV per data sample and an amplitude range of −200 to 200 μV. Identified artifacts were marked and removed from further analysis. Fast Fourier Transformation (FFT) was used defining the raw sum of global field power (GFP, μV^2) for the frequency bands of alpha (7.5–12 Hz) and beta (12.5–35 Hz). Finally all remaining segments were averaged and GFP of the pooled channels was exported via area information export for the specific wave bands. Owing to the limited number of channels it was decided to not further differentiate