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Ultrasound of alternating frequencies and variable emotional impact evokes depressive syndrome in mice and rats

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ABSTRACT

Emotional stress is primarily triggered by the cognitive processing of negative input; it is regarded as a serious pathogenetic factor of depression that is challenging to model in animals. While available stress paradigms achieve considerable face and construct validity in modelling depressive disorders, broader use of naturalistic stressors instead of the more prevalent models with artificial challenges inducing physical discomfort or pain may substantially contribute to the development of novel antidepressants. Here, we investigated whether a 3-week exposure of Wistar rats and Balb/c mice to unpredictably alternating frequencies of ultrasound between the ranges of 20–25 and 25–45 kHz, which are known to correspond with an emotionally negative and with a neutral emotional state, respectively, for small rodents in nature, can induce behavioural and molecular depressive-like changes. Both rats and mice displayed decreased sucrose preference, elevated "despair" behaviour in a swim test, reduced locomotion and social exploration. Rats showed an increased expression of SERT and 5-HT2A receptor, a decreased expression of 5-HT1A receptor in the prefrontal cortex and hippocampus, diminished BDNF on gene and protein levels in the hippocampus. Fluoxetine, administered to rats at the dose of 10 mg/kg, largely precluded behavioural depressive-like changes. Thus, the here applied paradigm of emotional stress is generating an experimental depressive state in rodents, which is not related to any physical stressors or pain. In essence, this ultrasound stress model, besides enhancing animal welfare, is likely to provide improved validity in the modelling of clinical depression and may help advance translational research and drug discovery for this disorder.

1. Introduction

Although multiple models of neuropsychiatric disorders have been generated during the last decades and; overall, current depression models achieve face and construct validity to a considerable extent (Maccari and Morley-Fletcher, 2007; Pollak et al., 2010; Overstreet, 2012; Willner and Belzung, 2015), numerous limitations remain unresolved (Sams-Dodd, 2005; McArthur and Borsini, 2006; Munos and Chin, 2009; Bouwknecht, 2015). Therefore, the failure to discover new antidepressants with improved efficacy and concerns over the clinical utility of recently developed drugs has raised important questions regarding the validation of preclinical studies (Pigott et al., 2010; Hoffman, 2013). While other substantial challenges may slow down drug discovery and development in the field of depression, such as the negligence of sex, age and environmental factors in pre-clinical work (Bourin, 2015; Homberg and Kalueff, 2016) and the suboptimal design of clinical trials (Insel and Sahakian, 2012; Millan et al., 2015). These failures, at least in part, stem from how ethologically relevant they are (Dzirasa and Covington, 2012; Borsini, 2012; Hoffman, 2013).

Abbreviations: BDNF, brain-derived nerve factor; cDNA, complementary deoxyribonucleic acid; HT, hydroxytryptamine; PCR, polymerase chain reaction; RNA, ribonucleic acid; SERT, serotonin transporter; US, ultrasound; VEGF, vascular endothelial growth factor.

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Fundamentally, rodents can be insufficient predictors for the identification of human pathophysiology (Insel, 2007; Borsini, 2012). One of the most essential differences between the two species might consist in the features of so-called "emotional", or "psychological"/"mental" stress. This is a state of distress, which is primarily triggered in an individual by the cognitive processing of adverse events, resulting in negative emotions and a physiological stress response (Chrousos and Gold, 1992; Simonov, 1997; Gold, 2015). Cognitive evaluation is considered to be a core species-specific element of emotional stress in humans (Beck et al., 1979; Clark et al., 2009). The term "emotional stress" is commonly used to label human adversities, such as the death of relatives, divorce, humiliation or defeat, deterioration of socio-economic status, which frequently precipitate clinical depression (Shalev et al., 1998; Hankin and Abramson, 2001; Lesch, 2004). A focus on this phenomenon as a separate form of stress which is predominantly determined by cognitive elements originates from the view of Epicurus who emphasized on the "imperturbability of mind" ("*ataraxia*") as a key prerequisite of overcoming the external noxious influences (reviewed in Chrousos and Gold, 1992).

Emotional stress is considered as a distress or anxiety suffered as a response to a sudden, severe, and saddening experience; thus, as a state of distress which is not triggered by any organic or physical disturbances unlike the "classical" state of stress (Selye, 1974; Chrousos and Gold, 1992; Simonov, 1997). While other types of stressors can be associated with negative emotions and elements of emotional stress as well, that is manifested, e.g., as increased signs of anxiety and despair behaviours after physical stress, pain, externally induced increases of inflammatory factors or glucocorticoid levels, they are not triggered by perception/processing of adverse information and do not involve mechanisms of cognitive evaluation in the first place. Obviously, it is challenging to mimic the neurobiology of these stressors in animals in general and in rodents specifically.

Meanwhile, as discussed above, the ethologically relevant mimicking of stress in animal models of depression may be crucial for translational research in this field, since stress can play a major role in the development of a depressive disorder. While depressive disorders are caused by a multi-factorial neuropsychiatric pathology where the occurrence of stress experiences does not necessarily result in clinical depression in an individual, sustained or repetitive stress was shown to increase its incidence in the population (Lesch, 2004; Vergne and Nemeroff, 2006; Jabbi et al., 2007; Maccari and Morley-Fletcher, 2007; Tsuji et al., 2014). Increased susceptibility of an individual to a stress-induced depressive state is viewed as a complex interplay between genetic and environmental factors that trigger maladaptive neurobiological changes (de Kloet et al., 2005; McEwen and Stellar, 1993; Jabbi et al., 2007; Jacobson and Cryan, 2007).

In recent years there has been a move toward animal models that mimic emotional/psychological stress and therefore rely on naturalistic approaches, such as repeated social defeat, social isolation, social fear conditioning, chronic subordinate colony housing, (Buwalda et al., 2005; Nestler and Hyman, 2010; Chaouloff, 2013; Toth and Neumann, 2013), housing in a deficient cage environment (Baram et al., 2012) or conditions of social instability (Mormède et al., 1990), aberrant maternal care or deprivation of it (Baram et al., 2012), and others. Yet, some of these paradigms do implicate pain, inflammation and noxious elements of a physical nature. Moreover, a majority of the most commonly used translational research depression models employ physical stressors such as restraint, foot shock, heat, food and water deprivation, cold, inflammation and others (Willner, 1995, 2005; Strekalova et al., 2011; Harro, 2013; Overstreet, 2012). Together, while the currently available chronic stress depression models effectively induce emotional stress and signs of a depressive state in animals, there is a clear need for the improvement of their etiological relevance and predictability in drug discovery (McArthur and Borsini, 2006; Hoffman, 2013; Willner and Belzung, 2015).

Here, we sought to investigate whether the chronic exposure to the ultrasound of a variable emotional impact, described earlier as a potent stress procedure in rodents (Litvin et al., 2007; Kuraoka and Nakamura, 2007, 2010) which is free from physical/invasive elements typically used with the models of stress-induced depressive state, could induce depressive-like changes in rats and mice.

In the current studies, we have chosen to employ an exposure of mice and rats to ultrasound that unpredictably alternates its frequencies between 20 and 25 kHz, which corresponds to a vocalization of both species to a "negative emotional state", and frequencies of 25–45 kHz that are associated with a "neutral" emotional state in both rats and mice (Kuraoka and Nakamura, 2010). The application of the ultrasound stimulation, using the parameters indicated above, was based on well-established ethological observations. Although the nature of species-specific information transmitted by rodents at the ultrasonic range is not entirely clear, it has been found that, surprisingly, mice and rats display largely overlapping emotional sensitivity to the sounds of the defined ranges of frequencies. As for instance, both mice and rats emit the sounds in a range of 20–25 kHz in life-threatening conditions, such as social defeat, pain and maternal separation (Hahn and Lavooy, 2005; Borta et al., 2006; Portfors, 2007; Takahashi et al., 2010).

The signals of 50 kHz and higher were found to be generated by mice and rats during physiologically positive experiences and are considered as a manifestation of animals' states that are regarded as parallels of "positive emotions". In particular, ultrasounds at this range of frequencies are emitted during mother–pup interactions, mating and positive social interactions (Branchi et al., 1998; Holy and Guo, 2005; Constantini and DAmato, 2006; Panksepp et al., 2007; Okabe et al., 2010). The threshold for upper level frequencies was therefore set to be 45 Hz or lower. Interestingly, while rats and mice are different species, the species–species differences between rats and mice in the emission and perception of the ultrasounds are limited. They were shown mainly to be owing to distinct impacts of such factors, as social structure/interactions and a territorial context and implicate more complex patterns of sound emissions than the here applied ultrasonic radiation (Constantini and DAmato, 2006).

In the present study, young Wistar rats and Balb/c mice were subjected to a 3-week ultrasound alternating in its frequencies in the two above indicated ranges. A cohort of rats has also received fluoxetine at the dose of 10 mg/kg/day. We found that in both rats and mice, the ultrasound exposure has decreased sucrose intake and preference, elevated floating behaviour in the swim test, and diminished social interactions and locomotion. In rats, the analyses of the mRNA levels of several brain areas, including the hippocampus, have revealed increases in the expression of SERT, 5-HT1A and 5-HT2A receptors and a decrease in the expression of BDNF. Immunohistochemical assay demonstrated diminished BDNF protein levels in the hippocampus. Most of the above-indicated behavioural and molecular changes induced by chronic ultrasound exposure of alternating frequencies were rescued by chronic dosing with fluoxetine, suggesting that they manifest a depressive-like state.

2. Materials and methods

2.1. Animals

Experiments were performed on male Wistar rats, that were 14 weeks old, and male Balb/c mice, that were 10 weeks old. All animals were provided by Stolbovaja, RAS, Moscow region via a provider licensed by Charles River (http://www.spf-animals.ru/about/providers/animals). All animals were housed individually in standard plastic cages (42 × 26 × 15 cm for rats and 27 × 22 × 15 cm for mice) and maintained on a 12-hour light/dark cycle, under controllable laboratory conditions (22 ± 1 °C, 55% humidity, room temperature 22 °C), food and water were available ad libitum. Housing conditions and all experimental procedures were set up and