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BEHAVIORAL AND [F-18] FLUORODEOXYGLUCOSE MICRO POSITRON EMISSION TOMOGRAPHY IMAGING STUDY IN A RAT CHRONIC MILD STRESS MODEL OF DEPRESSION

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Abstract—We investigated changes in behavior and brain glucose metabolism in a rat chronic mild stress (CMS) model of depression. The CMS model has been used to mimic depression in humans by using various chronic mild stressors in a 4 weeks period. In the present study, we have developed a combination of tests examining behavior (open field test) and hedonic measure (sucrose preference test) after exposure to CMS, and compared this to control non-stressed rats. We found that CMS induced behavioral changes, including decreased central and rearing activity, increased grooming and defecation, reduced body weight, and reduced relative sucrose intake. Moreover, our study suggests that CMS administered for 4 weeks activated left auditory cortex, while left piriform cortex, left inferior colliculus, septal nuclei and periaqueductal gray were deactivated. These changes in region of interest are left–right asymmetrical and lateralized in the left hemisphere. And activity deficits of depression are related with changes of brain activity in all brain regions showing significant changes by CMS in glucose metabolism. There are significant correlations for relative sucrose intake in left piriform cortex, left inferior colliculus and left auditory cortex, and for anxiety-related behavioral measures in septal nuclei and periaqueductal gray. There are lack of significant effects in the mean glucose metabolism of both hemispheres in hippocampus and amygdala induced by CMS possibly because of various reasons. Changes in glucose metabolism support the view that these significant brain regions are involved in chronic stress and depressive mood regulation. The results of this study might contribute to the awareness of changes in behavior and brain activity of depression induced by CMS.

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Abbreviations: ACC, anterior cingulated cortex; Au, auditory cortex; CMS, chronic mild stress; CON, control; DLPFC, dorsolateral prefrontal cortex; FDG, fluorodeoxyglucose; Ic, inferior colliculus; L, left; LH, learned helplessness; LSN, lateral septal nucleus; MRI, magnetic resonance imaging; PAG, periaqueductal gray; PET, positron emission tomography; PFC, prefrontal cortex; R, right; ROI, region of interest; SD, Sprague–Dawley; Se, septal nuclei; SPM, statistical parametric mapping; SSRI, selective serotonin reuptake inhibitor.

Key words: chronic mild stress, depression, sucrose preference, open field behavior, [F-18] FDG micro PET, glucose metabolism.

A direct or indirect long-term exposure to multiple stressors has been suggested to play a role in the development of human depression (Anisman and Zacharko, 1982; Brown, 1993). To obtain a better understanding of the phenomena existing in depression, different animal models have been developed to mimic these conditions. The chronic mild stress (CMS) procedure is an animal model of depression, based on earlier observations by Katz et al. in the late 1980s along with a unique combination of predictive validity (pharmacological profile), face validity (symptom profile) and construct validity (theoretical rationale) (Willner, 1997). This model attempts to analyze putative stressors that contribute to the onset of depression and produce depressive symptoms (Kendler et al., 1995). Most importantly, CMS can elicit the anhedonia, which is considered to be the hallmark of depression (Willner et al., 1987; Pothion et al., 2004). In DSM-IV (American Psychiatric Association, 1994), anhedonia is defined as the loss of interest or pleasure in some things that usually would be enjoyed. Further insight into the development of human depression can be gained by studying the underlying etiological mechanisms of the symptoms in depressive animals induced by CMS.

A large number of studies have found that CMS causes behavioral changes in rodents that parallel symptoms of depression (Willner, 2005). In this model, rats are exposed to a chronic period (greater than 3 weeks, usually 4 weeks) of unpredictable and mild stressors, including water deprivation, continuous lighting, cage tilt, strobe light white noise and others. These depression inducing conditions in the CMS model are more realistic and ethical than other models, however the exact methodology of the model differs between investigations (Matthews et al., 1995; Willner, 1997; Nielsen et al., 2000; Bielajew et al., 2002). The procedure adopted in our study was that of Grippo (2009).

Furthermore, many studies have employed CMS as a model for studying the relationship between depression and immunologic (Edgar et al., 2002), neuroendocrine (Grippo et al., 2005), neurobiological (Bachis et al., 2008), cardiovascular (Grippo et al., 2003) and physiological (Grippo et al., 2002) alterations. All of these findings have contributed to a better understanding of depression.

As the field of neuroscience advances, more and more investigations have been undertaken to study the brain

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areas that are associated with depression and depressive symptoms. Many studies have documented the involvement of the limbic system: a complex of structures that includes the amygdala, hippocampus, insula, parts of the anterior cingulated cortex (ACC), piriform cortex, septal nucleus, and several other brain regions (Davidson et al., 2002). Elevated baseline amygdala activity in depression is positively correlated with the severity of the depression (Drevets et al., 2002). Pause et al. (2003) found that the piriform cortex was responsible for alterations in the processing of emotion in depressed patients. Moreover, there are abnormalities in cerebral cortex, in particular the dorsolateral prefrontal cortex (DLPFC), along with hypometabolism in the ACC (Gonul et al., 2004; Mayberg, 2008). Manjarrez-Gutierrez et al. (2009) found that diabetic subjects with depression showed a higher response of the auditory cortex and an enhanced deterioration of brain serotonergic neurotransmission compared to subjects without depression. There are also studies that have identified stress induced changes in brain activity. For example, Pruessner et al. (2008) reported that human subjects exhibited a profound deactivation of the limbic system after being exposed to a psychosocial stressor. Although numerous studies have evaluated the relationship between brain activity and depression, few studies have empirically analyzed the mechanisms of depression induced by chronic unpredictable mild stress. Certainly, there are many ethical and practical limitations to undergoing such a study with human subjects. Therefore, it is necessary and important to identify animal models that are suitable for studying these specific mechanisms.

F-18 fluorodeoxyglucose ([F-18] FDG) micro positron emission tomography (PET) scan is a functional neuroimaging technique that is helpful to visualize brain activity in small rodents. This technique can aid in the exploration of brain activity changes that occur in depression (Mirrione et al., 2006, 2007). In 2009, the Sung group used this neuroimaging technique to investigate changes in brain activity induced by acute stress in rats and found that changes in brain activity differed depending on the duration of the stress (Sung et al., 2009). However, to our knowledge, no research has been performed that details the changes in brain activity that occur in animals exposed to a CMS protocol using [F-18] FDG micro PET. Therefore, if CMS represents an accurate animal model of depression, CMS would mimic the changes in brain activity that have been reported in depression and glucose metabolism changes should be associated with the changes of sucrose preference and locomotor activity.

EXPERIMENTAL PROCEDURES

Animals

This study was approved by the Animal Research Committee of Fudan University, School of Medicine and all procedures were conducted in accordance with the National Institutes of Health Guidelines for Animal Research (Guide for the Care and Use of Laboratory Animals). The study was carried out using 20 healthy adult male Sprague–Dawley (SD) rats weighing 200–300 g (about 3–4 months of age). All animals were randomly divided into two main groups and were housed singly, at an average room temperature of 22±1 °C and humidity of 50%–60%, with illumination available from 8 AM to 8 PM daily. Laboratory chow and water were given *ad libitum*, unless otherwise noted. All rats were acclimatized to the laboratory 7 days before the beginning of experiments. In addition, the experimental procedures used in the present study were arranged between the beginning of June and July (Fig. 1).

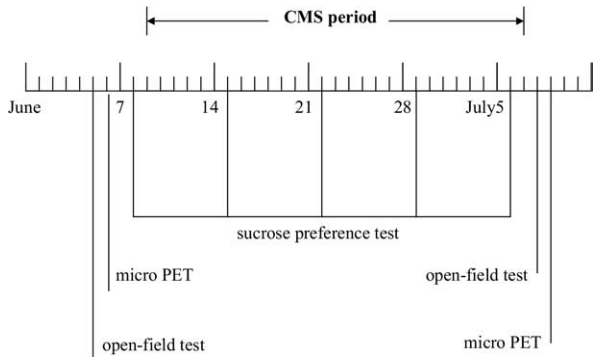

Fig. 1. Time schedule of procedures used in the present study. The numbers indicated calendar dates. Sucrose preference tests were carried out on June 8, 15, 22, 29 and July 6. Open-field tests were conducted on June 5 and July 8. [F-18] FDG micro PET scans were completed on June 6 and July 9.

CMS regimen

According to the most recent CMS research by Willner (2005), it is better if severe stressors (e.g., moderate foot shock) are avoided. Therefore, the CMS procedure of the present study was consistent with methods reported in other studies (Grippo, 2009) and was designed to meet the mild and unpredictable nature of the stressor as closely as possible. All animals were exposed to the following stressors in a random order: grouped housing, continuous overnight illumination, 40° cage tilt along the vertical axis, soiled cage (300 ml of water spilled in the bedding), exposure to an empty water bottle immediately following a period of acute water deprivation, stroboscopic illumination (300 flashes/min), and white noise (Grippo et al., 2006, 2008). According to an admitted typical CMS paradigm reported by Grippo (2009), details of the study procedure, including variety, time, and length of activity are shown in Table 1 (Grippo et al., 2006) and were slightly revised according to our experimental conditions. These procedures were carried out continuously for a total of 4 weeks. Control animals were given ordinary daily care and housed separately in an undisturbed room.

Sucrose preference test and body weight

Fluid intake tests consisted of first depriving animals of food and water for a 20 h period. Animals then had access to two drinking bottles (water and 1% sucrose) positioned side-by-side at the rear of the cage and were allowed to consume the fluids for a period of 1 h. All fluid consumption was recorded by weighing two preweighted bottles before testing and after 1 h. The position of the two bottles (left/right sides of the cages) was varied randomly. In addition, some studies have raised the possibility that the changes in intake of sweet solutions may be related to body weight (Matthews et al., 1995; Harris et al., 1997). Therefore, the fluid intake was expressed in relation to the animals' body weight (mL/kg). Sucrose preference tests were employed to operationally define anhedonia. Specifically, anhedonia was operationally defined as a reduction in sucrose intake compared to the intake values of the control group and baseline values (Willner et al., 1987). Sucrose