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CONSTRUCT AND FACE VALIDITY OF A NEW MODEL FOR THE THREE-HIT THEORY OF DEPRESSION USING PACAP MUTANT MICE ON CD1 BACKGROUND

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Abstract—Major depression is a common cause of chronic disability. Despite decades of efforts, no equivocally accepted animal model is available for studying depression. We tested the validity of a new model based on the three-hit concept of vulnerability and resilience. Genetic predisposition (hit 1, mutation of pituitary adenylate cyclase-activating polypeptide, PACAP gene), early-life adversity (hit 2, 180-min maternal deprivation, MD180) and chronic variable mild stress (hit 3, CVMS) were combined. Physical, endocrinological, behavioral and functional morphological tools were used to validate the model. Body- and adrenal weight changes as well as corticosterone titers proved that CVMS was effective. Forced swim test indicated increased depression in CVMS PACAP heterozygous (Hz) mice with MD180 history, accompanied by elevated anxiety level in marble burying test. Corticotropin-releasing factor neurons in the oval division of the bed nucleus of the stria terminalis showed increased FosB expression, which was refractive to CVMS exposure in wild-type and Hz mice. Urocortin1 neurons became over-active in CMVS-exposed PACAP knock out (KO) mice with MD180 history, suggesting the contribution of centrally projecting Edinger–Westphal nucleus to the reduced depression and anxiety level of stressed KO mice. Serotoninergic neurons of the dorsal raphe nucleus lost their adaptation ability to CVMS in MD180 mice. In conclusion, the construct and face validity criteria suggest that MD180 PACAP HZ mice on CD1 background upon CVMS may be used as a reliable model for the three-hit theory.

Key words: maternal deprivation, chronic variable mild stress, CRF, Urocortin1, serotonin, FosB.

INTRODUCTION

Major depression is a mood disorder affecting 350 million people globally. This stress-related mental condition has a substantial impairing effect on various fields of the patient's life resulting sometimes in suicide (WHO, 2016). Despite the severity we still lack a deeper understanding of etiology, pathomechanism and a reliable therapy. For instance, opposing clinical phenomena (i.e. weight loss vs. gain, insomnia vs. hypersomnia) (for details see reference: Diagnostic and Statistical Manual of Mental Disorders-5 2013) support the diagnosis of human major depressive disorder. This suggests that the entity of major depressive disorder harbors conditions of heterogeneous neurobiological mechanisms (for review see Kormos and Gaszner, 2013; Farkas et al., 2016). At therapeutical level, anti-depressant drugs, acting on monoaminergic systems, proved to be ineffective in about 30% of the cases (Catena-Dell Osso, 2013). These observations demonstrate that beyond monoaminergic systems other neural circuits are also involved and the discovery of new, more reliable animal models is imperative. Extended studies on multiple stress adaptation centers would also accelerate the search for potential drug targets (Kormos and Gaszner, 2013; Farkas et al., 2016).

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Abbreviations: 5-HT, serotonin; ACTH, adrenocorticotropin; AFR, animal facility rearing; BSTov, oval division of bed nucleus of stria terminalis; CeA, central nucleus of amygdala; CORT, corticosterone; cpEW, centrally projecting Edinger–Westphal nucleus; CRF, corticotropin-releasing factor; CTRL, control; CVMS, chronic variable mild stress; DR, dorsal raphe nucleus; EDTA, ethylene-diamine tetra-acetic acid; FST, forced swim test; HPA, hypothalamus–pituitary–adrenal; HZ, heterozygous; KO, knock out; LDB, lightdark box; MANOVA, multifactorial analysis of variance; MBT, marble burying test; MD180, maternal deprivation (180 min long); MS15, maternal separation (15 min long); NDS, normal donkey serum; PACAP, pituitary adenylate cyclase-activating polypeptide; PBS, phosphate-buffered saline; PD, postnatal day; PVN, paraventricular nucleus; SSD, specific signal density; TST, tail suspension test; Ucn1, urocortin 1; WT, wild type.

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In the last decades numerous models and theories (for instance monoaminergic, neurotrophin, and glucocorticoid theories) have been elaborated to explain the neurobiological alterations in stress-related mood disorders (Kormos and Gaszner, 2013; Farkas et al., 2016). More recently the three-hit concept of resilience and vulnerability became more widely accepted: genetic factors (hit 1), early-life environmental challenges (hit 2) and late-life environmental stress (hit 3) increase the risk of depression (de Kloet et al., 2007; Daskalakis et al., 2013,).

Stressful stimuli are conveyed to the limbic system. Such effects exert a higher order control on stress response, which is primarily orchestrated by the hypotha lamus–pituitary–adrenal (HPA) axis. Corticotropin releasing factor (CRF) produced by the parvocellular division of the paraventricular nucleus of the hypothalamus (PVN) controls the release of adrenocorticotropin (ACTH) from the anterior lobe of pituitary. ACTH regulates the glucocorticoid response [i.e. corticosterone, (CORT) in rodents] from the adrenal cortex (for review see: Silverman and Sternberg, 2012). The HPA axis reactivity is often affected in mood disorders at CRF level (Nemeroff et al., 1984; Hartline et al., 1996). Early-life events (i.e. maternal deprivation, MD) may cause long-lasting changes in the activity of CRF neurons (Plotsky et al., 2005; Desbonnet et al., 2008). Such alterations are accompanied by elevated ACTH and CORT levels (Plotsky et al., 2005) and cognitive impairment (Aisa et al., 2007) in the rat.

Pituitary adenylate cyclase-activating polypeptide (PACAP) has regulatory role in stress adaptation response (Norrholm et al., 2005; Agarwal et al., 2005; Stroth et al., 2011, for reviews see: Hammack et al., 2010; Pinhasov et al., 2011; Kormos and Gaszner, 2013; Hammack and May, 2015). Mice lacking the functional PACAP gene (knock out, KO) on various genetic background have been generated and have extensively been studied on their stress adaptation (Hashimoto et al., 2001, 2009; Stroth and Eiden, 2010; Tsukiyama et al., 2011; Gaszner et al., 2012, 2014; Kormos and Gaszner, 2013; Lehmann et al., 2013; Mustafa et al., 2015; Kormos et al., 2016). It has to be pointed out that the effect of PACAP deficiency is also a function of the mouse strain used (Hattori et al., 2012).

PACAP KO mice on CD1 background used also in our laboratory show marked abnormalities in their psychomotor activity, circadian CORT rhythm, hippocampal glucocorticoid receptor mRNA expression and body temperature (Hashimoto et al., 2009). The behavioral assessment on mood status in naive CD1 PACAP KO mice revealed anxiolytic phenotype (Hashimoto et al., 2001, 2009; Girard et al., 2006; Gaszner et al., 2012) associated with depression-like anomalies in forced swim test (FST) (Hashimoto et al., 2001, 2009; Gaszner et al., 2012). Interestingly, the chronic variable mild stress (CVMS) exposure in these mice dramatically reduced the depression-like phenotype (Kormos et al., 2016).

Non-stressed PACAP KO mice on the C57BL/6 J × 129SvEv hybrid background exert also reduced anxiety in the elevated plus maze and slightly reduced depression-like phenotype in the FST (Hattori et al., 2012), however the behavioral phenotype of these mice upon chronic stress is to date not known.

To the contrary, C57BL/6 N PACAP mutants were shown to exert normal anxiety and depression levels if they had not been exposed to stress. Social defeat however caused reduced anxiety and depression levels in C57BL/6 N PACAP KO mice (Lehmann et al., 2013).

Regardless the genetic background PACAP deficiency blunts the function of the HPA axis both at the level of PVN–CRF neurons and CORT release in acute (C57BL/6 N mice: Stroth and Eiden, 2010, CD1 mice: Tsukiyama et al., 2011; Gaszner et al., 2012) and chronic models such as social defeat (C57BL/6 N mice: Lehmann et al., 2013), repeated restraint stress (C57BL/6 N mice: Mustafa et al., 2015) and CVMS (CD 1 mice: Kormos et al., 2016). The altered stress response, and depression-like phenotype found in PACAP mutants on CD1 background suggested that these animals could be used in a new model for depression based on the three-hit theory (for review see also: Farkas et al., 2016).

Extrahypothalamic CRF systems such as the oval nucleus of the bed nucleus of stria terminalis (BSTov) and central nucleus of amygdala (CeA) also play significant roles in stress and depression (for reviews see: Carrasco and de Kar, 2003; Kehne and Cain, 2010; Kovács, 2013; Waters et al., 2015). For instance, lentiviral overexpression of CRF induced depression-like phenotype in the BST in mice (Regev et al., 2011). Interestingly, PACAP containing nerve fibers were found in the BSTov (Hannibal, 2002). More specifically, PACAP fibers innervate CRF neurons in the BSTov (Kozicz et al., 1997). This enables interaction between PACAP and extrahypothalamic CRF through PAC1 receptors with behavioral significance (for reviews see: Hammack et al., 2010; Kormos and Gaszner, 2013).

The CRF neuropeptide family member Urocortin 1 (Ucn1) is expressed mainly in the centrally projecting Edinger–Westphal nucleus (cpEW) (Vaughan et al., 1995; Janssen and Kozicz, 2013). Ucn1 neurons show selective sensitivity to various types of acute stressors (Kozicz et al., 2001; Gaszner et al., 2004), which is blunted by MD in the rat (Gaszner et al., 2009) and in PACAP KO mice (Gaszner et al., 2012). Chronic stress exposure results in a constantly high c-Fos expression in Ucn1 neurons in wild type (WT) mice (Korosi et al., 2005). CVMS induces FosB expression in murine Ucn1 neurons, which is blunted in PACAP KO mice (Kormos et al., 2016). Tree shrews react to chronic stress with a reduction of Ucn1 neuron count (Kozicz et al., 2008a). Interestingly, upregulation of Ucn1 mRNA in female suicide victims was demonstrated (Kozicz et al., 2008b).

Serotonin (5-HT) is a monoamine neurotransmitter expressed mainly in the dorsal raphe nucleus (DR) (for reviews see: Graeff et al., 1996; Carrasco and de Kar, 2003; Holmes, 2008). The 5-HT cells of DR innervate the hippocampus, medial prefrontal cortex, septum, extended amygdala and the basal ganglia (Steinbusch et al., 1981; Ma et al., 1991). 5-HT–DR neurons were found to be sensitive to acute (Bouwknecht et al., 2007)