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A change in hippocampal protocadherin gamma expression in a learned helpless rat☆

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ABSTRACT

Depression is a disease with a complex etiology, that is only beginning to be studied from a genetic perspective. A selectively bred line of rats susceptible to learned helplessness, a model of depression, presents an opportunity to search for genes affecting the depressive symptoms found in the helpless model which may provide clues toward understanding the human disease. A microarray study revealed a small set of genes with altered expression in the hippocampus of the congenitally helpless rats. We selected one of these genes, a member of the γ-protocadherins for further study to determine the basis for the change in expression. Helpless animals demonstrated an increased expression primarily in CA1 neurons. Protocadherins have been implicated in synapse development and these results suggest they may play a role related to the reduced neuroplasticity observed in depression. Additionally, a number of genes linked to pathways known or suspected to be involved in depression were also detected, these will require further verification and study.

Abbreviations: LH, learned helpless; NLH, non-learned helpless(LH resistant); cLH, congenitally helpless; cNLH, congenitally non-learned helpless; GWAS, genome-wide association study; GFAP, glial fibrilary acidic protein; MAP-2, microtubule associated protein 2; Pcdhga11, protocadherin gamma A11; Kmo, kynurenine 3-hydroxylase; Gsbs, G-substrate; Slc35d3, solute carrier family 35 member D3; LTP, long term potentiation; LTD, long term depression; cGMP, cyclic guanadine monophosphate; qPCR, quantitative polymerase chain reaction; NAD, nicotinamide adenine dinucleotide; NMDA, N-methyl-D-aspartate; NO, nitric oxide; PKG, cGMP-dependent protein kinase; DRN, dorsal raphe nuclei; CpG, cytosine-guanidine dinucleotide; EC, entorhinal cortex; CTD, C-terminal domain; VCD, variable cytoplasmic domain; PSD, post-synaptic density; RNAi, RNA interference; PSD-95, post-synaptic density protein of 95 kD; CaMKII-α, calcium-calmodulin dependent kinase 2 alpha subunit; CaMKII-γ, calcium-calmodulin dependent kinase 2 gamma subunit; SNIP, SNAP-25 interacting protein; pcdh, protocadherin; LAS AF, Leica Application Suite Advanced Fluorescence; FBS, fetal bovine serum; NDS, normal donkey serum; RMA, robust Multi-array Averaging; CA1, cornu ammonis area1; CA3, cornu ammonis area 3; BNST, bed nucleus of the stria terminalis; LHb, lateral habenula

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1. Introduction

Learned helplessness is a model for depression first introduced in 1967 by Overmier and Seligman (1967). It mimics many of the symptoms of depression, including anhedonia (Vollmayr et al., 2004) sleep disturbances (Adrien et al., 1991) an increased response to stress, and reversal with antidepressant treatment. Additionally, learned helpless animals exhibit a reduced ability to suppress corticosterone in response to dexamethasone treatment with no differences observed between naïve and non-helpless rats (Greenberg et al., 1989). This closely mirrors melancholic depression, where a majority of patients are dexamethasone non-responders (Roy, 1988).

Based on learned helplessness, we began breeding rats based on outcomes in the learned helplessness test, grouping the most vulnerable and the most resistant to acquiring the behavior into two groups for breeding (Henn and Vollmayr, 2005). The rats were subjected to a training session in which they received and unpredictable and uncontrollable stressor in the form of foot shock, and are tested a day later for the ability to acquire escape behavior by pressing a lever to terminate shocks. Helpless and non-helpless animals from these tests were selectively bred to create lines that would be a more consistent source of helpless or helpless resistant animals. Following the procedure the described (Vollmayr and Henn, 2001) two lines of rats resulted: one congenitally helpless(cLH) and the other helpless resistant(cNLH). After five generations of selective breeding (Henn and Vollmayr, 2005) the cLH rats have over a 90% frequency of scoring as learned helpless (LH) in the original test that developed the line; while the cNLH rats are resistant to developing learned helplessness.

These lines have been subjected to many studies to compare the model with depression. Tests examining behaviors indicative of anhedonia and anhedonia versus motivation (Vengeliene et al., 2005; Vollmayr et al., 2004; Enkel and Spanagel, et al., 2010). Tests ruling out deficits in memory and learning, and documenting increased fear response and fear extinction (Shumake et al., 2005; Schulz et al., 2009) all show responses consistent with a depressive phenotype. There are also some intrinsic alterations in metabolic activity of several brain regions in the cLH rats demonstrated by cytochrome oxidase in situs (Shumake et al., 2001, 2004, 2003, 2002). This work demonstrated the central role of the lateral habenula (LHb) in helplessness (Li et al., 2011, 2013).

The use of selective breeding has translated a behavioral model into a genetic model. Determining which genes are involved in these animals response to stress may yield insights into how a the brain responds to stress. More importantly, it may highlight how the responses differ between a stress sensitive and stress resilient brain. Considering that twin studies have estimated a heritability between 25% and 64% (Kendler and Prescott, 1999; Kendler et al., 1995; Ørstavik et al., 2007) in depression, it is likely that an individual's genetic makeup plays some role in determining whether an individual will develop depression. Unfortunately, attempts to identify genes through genome-wide association studies (GWAS) have produced disappointingly low numbers of genes with at least seven studies collecting data and at least three meta analyses yielding two genes one each from the papers which had the smallest sample sizes (Muglia et al., 2010; Sullivan et al., 2009; Shi et al., 2011; Shyn et al., 2011; Wray et al., 2012; Kohli et al., 2011; Aragam et al., 2011; GWAS Consortium, 2012). Such difficulties likely result from diverse etiologies, environmental effects, and a large number of genes that could be affecting susceptibility to developing depression. The reduced genetic and environmental diversity of the cLH and cNLH rats allow us to sidestep these problems in searching for genes which may play a role in the disorder.

While many brain regions have been studied in relation to depression, one of the most extensively studied is the hippocampus. Evidence for a role in depression range from decreased hippocampal volume in depressed patients (Videbech and Ravnkilde, 2004), to the effects of antidepressants on animal models of depression (Zink et al., 2005), to changes in cell morphology related to neuronal plasticity (Morales-Medina et al., 2009; Hajszan et al., 2009), to PET imaging of cerebral blood flow that links hippocampal blood flow to Hamilton Depression scale scores (Videbech et al., 2002). The cLH rats also show behavioral correlates of depression such as a negative bias in ambiguous cue response tests (Enkel and Gholizadeh et al., 2010), mimicking the pessimistic biases and interpretations exhibited by depressed patients. The model has been examined for the presence of a possible link between neurogenesis and depression measure (Gould et al., 1998; Henn and Vollmayr, 2004) and it demonstrates reduced long term potentiation in learned helpless animals (Ryan et al., 2010). Blocking hippocampal output to multiple structures important in the regulation of emotion as demonstrated via lesions of the fornix blocks the development of learned helplessness (Leshner and Segal, 1979). We therefore judged the hippocampus to be a good first target for expression studies, and focused our work on the changes in expression within the hippocampus of the congenitally helpless rats.

Measuring variations in gene expression in the hippocampus, allows one to identify genes with altered expression regardless of whether it is a result of genetic differences, epigenetic changes, or an alteration in the numbers of certain cell populations. Yet we found only a single gene that was verifiably altered: a protocadherin, a member of a remarkable family of genes primarily expressed within the central nervous system. The protocadherins make up the largest family within the cadherin superfamily, with most of them within three clusters. These genes are thought to be involved in synaptogenesis and as a means of assigning a distinct molecular identity to individual neurons or cell surfaces.

2. Results

2.1. *Microarray of cLH and cNLH hippocampal tissue*

Congenitally Helpless rats and congenitally helpless resistant rats were trained and tested as described in Section 4.1 which is based on previously published techniques (Henn and Vollmayr, 2005). The hippocampi from cLH ($n=5$) and cNLH