Running head: HB-GAM KNOCK-OUTS

Neuroanatomical Characteristics of Mice Deficient in Heparin Binding Growth Associated Molecule (HB-GAM)

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Abstract

Pervasive Developmental Disorder (PDD) is a categorical term used to classify disorders in which the patient suffers deficits in three main domains: language, behavior and social skills. Heparin Binding Growth Associated Molecule (HB-GAM) is an extracellular protein involved in neurite outgrowth and radial migration. Mice deficient in HB-GAM demonstrate deficits in cognition, behavior and social skill similar to what is seen in PDD patients. Furthermore, PDD patients have been found to have brain abnormalities in the limbic system; amygdala, hippocampus, entorhinal cortex and cerebellum. Based on the idea that brain abnormalities are highly correlated with abnormal behavior, the present study focused on the neuroanatomy of the entorhinal cortex of mice deficient in HB-GAM. It was observed that mice deficient in HB-GAM show abnormal neuroanatomy similar to that seen in human PDD brains: tightly packed small neurons in entorhinal cortex layer IV. From these findings, we propose that HB-GAM Knock-outs may be a good mouse model for human PDD.

Neuroanatomical Characteristics of Mice Deficient in Heparin Binding Growth Associated Molecule (HB-GAM)

Pervasive Developmental Disorder (PDD) is a categorical term used to characterize disorders affecting three main domains: language, behavioral diversity and social skills (American Psychiatric Association, 2000). Due to the rapid increase in incidence of PDDs, it is of growing interest to find a nonhuman model that can allow further studies in search for treatment or cure of these disorders. It has been consistently reported that patients suffering of PDDs show neuroanatomical abnormalities in the cerebellum and the limbic system, specially the amygdala, hippocampus and the entorhinal cortex (Bauman & Kemper 2005, Palmen et. al., 2004, Dicicco-Bloom et al., 2006, Polleux & Lauder 2004 and Salmond et al., 2005). These alterations in neuronal anatomy may account for the different behavioral, cognitive and social skill deficits observed in PDD patients.

Based on the fact that PDD patients demonstrate abnormal behavior, high anxiety levels and cognition deficits, previous research has focused on areas of the brain that are implicated in these different tasks and behaviors. Using technological advances such as fMRI, EEGs, and neurophysiology, previous studies have found that one of the main brain regions involved in mediating social skill, anxiety levels and memory are the limbic cortex and to a lesser extent, the cerebellum ((Bauman & Kemper 2005, Palmen et. al., 2004, Dicicco-Bloom et al., 2006, and Polleux & Lauder 2004). Similarly, it has been shown that lesion to the limbic system; specifically the entorhinal cortex results in increase defensive behavior and memory difficulties. (Meunier et al., 2006). These conclusions are further supported by the findings that the entorhinal cortex relay the

information into the hippocampus which is known to be involved in memory (Kahn et al., 2008). Studies looking at brains of human autistic patients have consistently reported abnormal patterns in the limbic system, specially in the hippocampus, amygdala and entorhinal cortex, the reports suggest a decrease in cell size and an increase in cell density in these areas. (Casanova et al., 2006, Bauman & Kemper 2005, Palmen et. al., 2004, Dicicco-Bloom et al., 2006 and Saitoh et al., 2001).

Previous research in our lab has suggested that behavioral, social and cognitive deficits similar to those experience by PDD patients is observed in mice deficient in Heparin Binding Growth Associated molecule (HB-GAM). HB-GAM is an extracellular protein involved in neurite outgrowth and associated with hippocampal synaptic plasticity (Pavlov et el., 2002). In addition, HB-GAM has been found to play a major role in neuron radial migration (Heniola et al., 2006). Since behavioral and social deficits similar to those appreciated in PDDs were observed and the brain regions involved have been identified, the purpose of this experiment is to analyze the entorhinal cortex neuroanatomy of HB-GAM Knock-outs and observe if any abnormalities correlate with those seen in PDD human brains (i.e. cell size and density). In order to do this, neuron cell body area in entorhinal cortex layers IV and V will be calculated and inter-neuron distances will be measured. It is hypothesized that like in human PDD cases, mice deficient in HB-GAM will have a significant decrease in cortical (entorhinal layers IV, V) neuron size and an increase in cell density.

Method

Mouse specifics

A total of nineteen mice were used for this study. Ten wild type and nine HB-GAM knock-out mice on a mixed 129Xc57b1/6 background strain (Amet el al., 2007).

Tissue Preparation

The animals had been sacrificed and perfused. The brain tissues had been sectioned, mounted and stained using cresyl violet.

Tissue Selection and Analysis

Only tissues containing the descending hippocampus were chosen for analysis. Two sections were chosen per animals. The selected tissue was placed under an Olympus BX51 Microscope containing a camera that projected the image onto a computer screen. A total of 20-40 neurons from the Entorhinal Cortex layers IV and V were randomly chosen and the somas were manually traced using the image analysis software Neurolucida (version 8.001, MicroBrightfield BioSciences, Williston, VT) the files were then saved onto the computer. The image analysis software NeuroExplorer (version 8.001, MicroBrightfield BioSciences, Williston, VT) was used to compute the cell body area in squared micrometers. The software Microsoft Excel (Microsoft 200) was used to calculate the mean cell body area for each animal. The Image J software (National Institutes of Health, Bethesda, MD) was sued to calculate the inter-neuron distances defined as the distance between the center of the closer two cell bodies in the same plane. To balance for experimenter bias, the genotypes of the mice were not revealed to the experimenters until the end of the study.

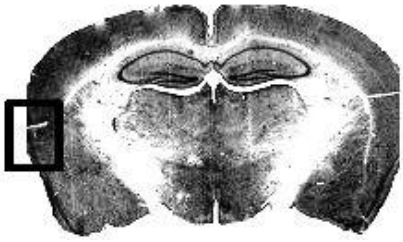

Figure 1. Nissl stain section with entorhinal cortex highlighted.

Statistical Analysis

The software SPSS for Windows was used to conduct all the statistical analysis for this project. An Independent Samples t-Test for Equality of the Means was conducted for neuron cell area in Entorhinal layers IV and V. In addition, a Pearson Correlation analysis was conducted between the individual layer's mean cell area and the respective layer's inter-neuron distances.

Results

Neuronal Area

Neuronal area in layer IV of the entorhinal cortex for HB-GAM Knock-outs was smaller than in wild type mice. However, neuronal area in layer V did not differ between HB-GAM Knock-outs and wild type mice. Figure 1 displays the mean cell area for HB-GAM Knock-outs and wild type mice in layers IV and V of the entorhinal cortex. The data shows that the mean cell area in entorhinal cortex layer IV was smaller for HB-GAM Knock-outs than for wild type mice and that the mean cell areas in layer V were close to equal. Mean cell area in entorhinal cortex layer IV was found to be significant, (t (16) = 3.519, p = .003). Mean cell area in entorhinal cortex layer V was not found to be significant, (t (17) = .125, p = .902).

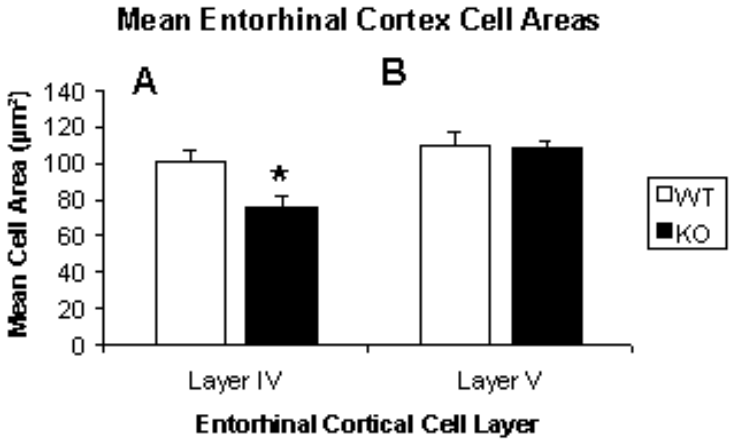

Figure 2. Mean cell area A: entorhinal layer IV and B: entorhinal layer V.

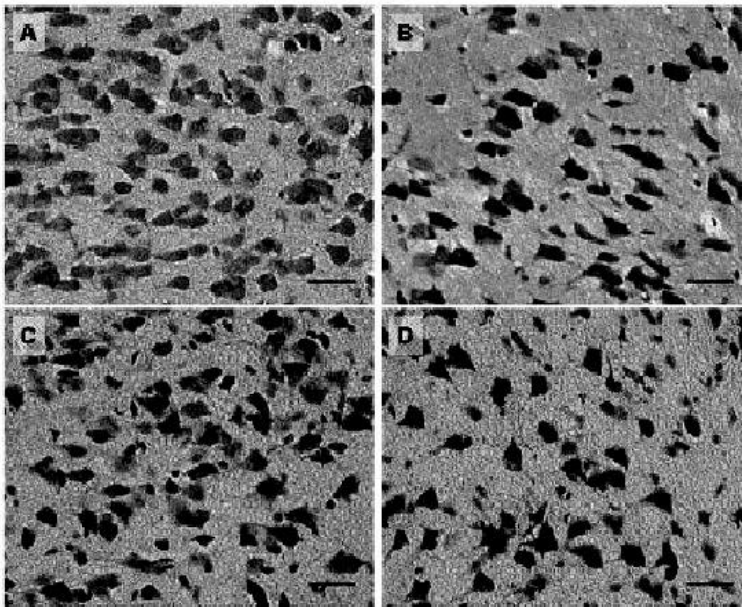

Figure 3. Nissl stained entorhinal cortex layer IV A: HB-GAM Knock-out, B: wild type and layer V C: HB-GAM Knock-out, D: wild type.

Inter-neuron Distance and Mean Cell Area Correlations

Entorhinal cortex layer IV Inter-neuron distances and mean cell areas were highly correlated. However, entorhinal cortex layer V inter-neuron distances and mean cell areas

were not highly correlated. Figure 4 displays the correlation graph between inter-neuron distance and mean cell area in entorhinal cortex layer IV. The data suggests a strong correlation between the size of the neuron and the distance between cells. This correlation was found to be statistically significant, (r (19) = .653, p< .01).

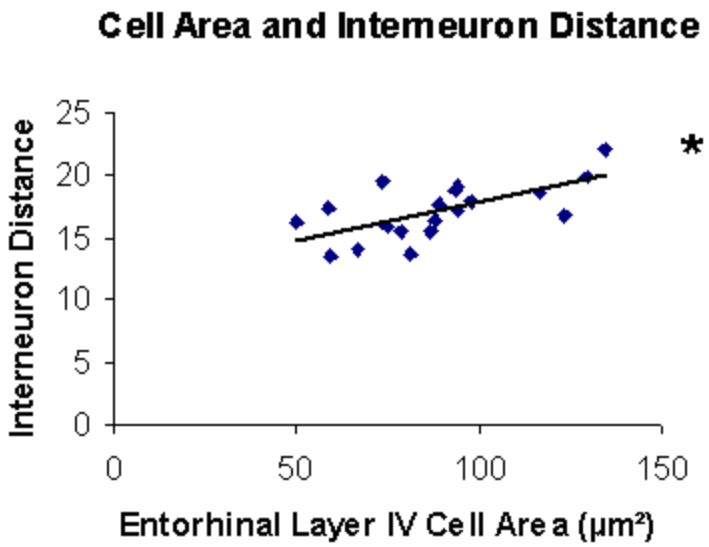

Figure 4. Pearson correlation between entorhinal cortex layer IV mean cell area and inter-neuron distance.

Discussion

Deficiency of Heparin Binding Growth Associated Molecule causes neuroanatomical abnormalities in the entorhinal cortex layer IV but not in layer V. Mice deficient in HB-GAM have smaller and more tightly packed neurons in entorhinal cortex layer IV but neuron size and inter-neuron distance did not vary between knock-outs and wild type in layer V. These findings suggest that the presence of normal levels of HB-GAM is crucial for a normal anatomical brain development since the lack of it causes limbic system brain abnormalities. Hence it follows, that as hypothesized; as a result of

deficiency of HB-GAM, smaller neurons that are more tightly packed are observed in entorhinal cortex layer IV but no significant abnormalities are observed in layer V.

No previous literature was found in which the neuroanatomy of HB-GAM Knock-outs was studied. However, the findings from this experiment offer the possibility of HB-GAM Knock-outs to be consider as a mouse model for human PDDs. These mice seem to be a good model for human PDDs because they express behavioral, cognitive and social deficits similar to those observed in human PDDs. Furthermore, the neuroanatomical findings here presented are in strong agreement with the neuroanatomical findings seen in human PDD brains. Human PDD brains have been shown to have limbic system anatomical abnormalities consisting of higher densities of smaller neurons (Bauman & Kemper 2005, Palmen et. al., 2004, Dicicco-Bloom et al., 2006, Polleux & Lauder 2004 and Salmond et al., 2005) which are the same findings observed in HB-GAM Knock-outs. Due to the strong similarities in both behavior and neuroanatomy between HB-GAM Knock-outs and human PDDs, it is proposed that HB-GAM Knock-outs may be a good model for human PDDs.

One of the greatest limitations in this experiment was the lack of time which prevented the anatomical analysis of other brain areas involved in PDD. In this experiments we analyzed the entorhinal cortex layers IV and V only, ideally we would have liked to analyze other brain regions such as the thalamus, amygdala, cerebellum and hippocampus. In addition, although significant difference between HB-GAM Knock-outs and wild type was found in layer IV for neuron size and inter-neuron distance, the small sample size may be a limiting factor as to why no significant inter-neuron distance was

observed in layer V. Lastly, the tissue stain chosen may have limited this experiment in that cresyl violet stains all types of cells as opposed to specific cell types.

Since it was found that neurons in entorhinal cortex layer IV are smaller and more tightly packed, further research may want to analyze the plasticity of the systems involved in the signaling pathway with the entorhinal cortex. For example, neurophysiology studies may want to look at the changes that cells in the thalamus have to undergo to compensate for the fact that more and smaller cells have to be targeted in entorhinal cortex layer IV. That is, do cells in the thalamus have more branches to target the greater number of cells in layer IV or is more neurotransmitter being released at the synapses to excite more than once cell at a time. In addition, it would be of great interest to analyze the neuroanatomy of the cerebellum to see if like in human PDD, there is a decrease in cell size and an increase in cell density.

References

Amet, L.E., Lauri, S.E., Hienola, A., Croll, S.D., Lu, Y., Levorse, J.M., *et al.* (2001). Enhanced hippocampal long-term potentiation in mice lacking heparin-binding growth-associated molecule. *Molecular and Cellular Neuroscience, 17*, 1014 – 1024.

Bauman, Margaret L., Kemper, Thomas L. (2005). Neuroanatomic observations of the brain in autism: a review and future directions. *International Journal of Developmental Neuroscience, 23,* 183-187.

Casanova, M.F., van Kooten, I.A., Switala, A.E., van Engeland, H., Heinsen, H., Steinbusch, H.W., *et al.* (2006). Minicolumnar abnormalities in autism. *Acta Neuropathologica, 112* (3), 287 – 303.

Courchesne, E. (2002) Anatomy and Neurobiology of Autism: Abnormal early brain development in autism. *Molecualr Psychiatry, 7,* S21-S23.

Deuel, Thomas F., Zhang, Nan, Yeh, Hsui-Jen. Silos-Santiago, Inmaculada, & and Wang Zhao-Yi. (2002). *Archives of biochemistry and Biophysics,* Vol. 397, No. 2, 15, 162–171.

DiCicco-Bloom, E., Lord, C., Zwaigenbaum, L., Courchesne, E., Dager, S.R., Schmitz, C., *et al.* (2006). The developmental neurobiology of autism spectrum disorder. *Journal of Neuroscience, 26* (26), 6897 – 6906.

Haznedar, M.H., Buchsbaum, M.S., Wei, Tse-Chung., Hof, P.R., Cartwright, C.,Hollander, E., Bienstock, C. (2000). Limbic Circuitry in Patients With Autism Spectrum Disorders Studies With Positron Emission Tomography and Magnetic Resonance Imaging. *The American Journal of Psychiatry, 157* (7), 1994 – 2001.

Hienola, A., Tumova, S., Kulesskiy, E. & Rauvala, H. (2006). N-syndecan deficiency impairs neural migration in brain. *Journal of Cell Biology, 174* (4), 569 – 580.

Kahn, Itamar, Andrews-Hanna, Jessica R., Vincent, Justin L., Snyder, Abraham Z., & Buckner, Randy L. (2008). Distinct Cortical Anatomy Linked to Subregions of the Medial Temporal Lobe Revealed by Intrinsic Functional Connectivity. *Journal of Neurophysiology,* 1-43.

Kemper, Thomas L., Bauman, Margaret L. (2002) Anatomy and Neurobiology of Autism: Neuropathology of infantile autism. *Molecualr Psychiatry, 7,* S12-S13.

Kerr, Kristin M., Agster Kara L., Furtak Sharon C., & Burwell Rebecca D. (2007). Functional Neuroanatomy of the Parahippocampal Region: The Lateral and Medial Entorhinal Areas. *Hippocampus, 17,* 697–708.

Lavenex, Pierre & Amaral David G. (2000). Hippocampal-Neocortical Interaction: A Hierarchy of Associativity. *Hippocampus, 10,* 420–430.

Meunier, M., Cirilli, L. & Bachevalier, J. (2006). Responses to affective stimuli in monkeys with entorhinal or perirhinal coprtex lesions. *The Journal of Neuroscience, 26* (29), 7718 – 7722.

Pavlov, I., Voikar, V., Kaksonen, M., Lauri, S.E., Hienola, A., Taira, T., *et al.* (2002). Role of heparin-binding growth-associated molecule (HB-GAM) in hippocampal LTP and spatial learning revealed by studies on overexpressing and knockout mice. *Molecular and Cellular Neuroscience, 20* (2), 330 – 342.

Palmen, Saskia J.M.C., Engeland, H.V. Hof, P.R., & Schmitz, C. (2004). Neuropathological findings in autism. *Brain. 127,* 2572-2583.

Polleux, F. & Lauder, J.M. (2004). Toward a developmental neurobiology of autism.

Mental Retardation and Developmental Disabilities Research Reviews, 10, 303 – 317.

Ramirez , Julio J., Campbell, David, Poulton, Nona, Barton , Cole, Swails, Jennifer, Geghman, Kindiya, Courchesne, Stephanie L., & Wentworth, Sean. (2007). Bilateral Entorhinal Cortex Lesions Impair Acquisition of Delayed Spatial Alternation in Rats. *Neurobiology of Learning and Memory, 87* (2), 264–268.

Saitoh, Osamu, Karns, C.M., Courchesne E. (2001). Development of the hippocampal formation from 2 to 42 years: MRI evidence of smaller are dentate in autism. *Brain*, *124*, 1317-1324.

Salmond, C.H., Ashburner, J., Connelly, A., Friston, K.J., Gadian, D.G. & Vargha-Khadem, F. (2005). The role of the medial temporal lobe in autism spectrum disorders. *European Journal of Neuroscience, 22* (3), 764 – 772.