Russ Patrick Award

GnRH and GnIH in the Rat Hippocampus

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Research Topic:

The hippocampus, a brain structure involved in learning and memory, is vulnerable to destruction during the progression of Alzheimer's Disease (AD). This damage to the hippocampus is predictive of AD, and is correlated with worsening cognitive symptoms; it has been hypothesized that hippocampal atrophy could play a causal role in the characteristic and debilitating memory loss of AD (Dhikav, 2011). In light of gender differences in the epidemiology of AD there has been great interest in the role of sex hormones (such as estrogen and testosterone) in the pathogenesis of the disease. It has been hypothesized that the greater prevalence of AD amongst women is related to the decline of estrogen levels following menopause (Janicki, 2010). Given the potential therapeutic target this offers for the treatment of AD it is crucial to identify the hormonal mechanisms that underlie normal hippocampal functioning.

Gonadotropin-Releasing Hormone (GnRH) and Gonadotropin-Inhibitory Hormone (GnIH) are hormones released by the hypothalamus that control levels of estrogen and testosterone in the body (Sherwood, 1993).

GnRH has long been considered the "master hormone" of reproductive control, but recent evidence is accumulating implicating this powerful hormone in non-reproductive roles. The hippocampus is a dense site of GnRH receptors (Jennes, 1994), and administration of GnRH to hippocampal tissue has been demonstrated to increase connections between hippocampal neurons (Prange-Kiel, 2008), and to increase the excitability of the hippocampus (Lu, 1999). This suggests GnRH acts in the hippocampus to regulate hippocampal function; but despite this suggestive evidence, it is still unknown whether GnRH is naturally present in the hippocampus of mammals. It seems unlikely that there would be such a density of GnRH receptors and such

clear physiological effects of GnRH within the hippocampus if GnRH did not play an important role there in living mammals. It is possible the GnRH could be manufactured by the hippocampus itself, or it is possible that GnRH from the hypothalamus is being transported to the hippocampus by an alternate route.

Gonadotropin-Inhibitory Hormone (GnIH) is a newly identified hormone, which was discovered in quails (Tsutsui, 2000). GnIH has opposing actions to GnRH in the body, inhibiting the release of estrogen and testosterone in the gonads. To date the majority of work on GnIH has been conducted with birds, where GnIH has been demonstrated to regulate breeding in response to stress (Kirby, 2009). Very little work has been done with GnIH in mammalian models. It is possible that it has similar (but opposing) physiological effects within the hippocampus as GnRH, but this has not yet been studied. As with GnRH, it is unknown whether GnIH is present in the hippocampus, and if so whether it is created there or if it brought in from the hypothalamus.

Our study seeks to identify possible sources of GnRH and GnIH to the rat hippocampus, describe the distribution of these hormones within the hippocampus, and determine whether there are any sex differences in these distributions.

My project employs a technique called immunohistochemistry (IHC), which allows us to look at slices of brain tissue and identify any neurons producing GnRH or GnIH. We apply antibodies to the tissue; these antibodies are capable of recognizing and binding to the hormone of interest. Once they are bound several other compounds are added to the tissue, which further enhance the binding of the antibodies. Finally a dye is added, which reacts only with antibodies bound to the hormone in question and turn these molecules a deep brown color. This means that under a microscope any neurons that produce GnRH of GnIH will appear dark brown against a

light tan background. Using a microscopy analysis system we can count the pixels of dark brown stain and compare the total amount of staining between males and females, or between different regions of the hippocampus. This allows us to draw conclusions about where the most GnRH or GnIH is present, which gender has more of the hormones, and how hormone levels change across the reproductive cycle in females.

Value of research

This study is the first to propose a direct method of GnRH transport to the hippocampus, and the first to characterize GnIH distribution within a mammalian model. Furthermore we are the first to provide not only consistent evidence of the presence of GnRH and GnIH within the rat hippocampus, but also a quantified analysis of regional and sex differences therein. This represents an important advance in our understanding of hormonal control of cognition.

Previous research on hormones and cognition has focused largely on estrogen, which has been demonstrated to have multiple neuroprotective effects within the hippocampus (for review see: Brown, 2009). Our study provides an important link for all previous research on estrogen; it is possible that GnRH and GnIH control estrogen production in the hippocampus, as they do in the gonads. GnRH and GnIH could thus prove valuable therapeutic agents in the treatment of neurodegenerative diseases such as Alzheimer's Disease.

Student impact:

Receiving an AURA award allowed me to experience much more than just a job, this project has been an incredible source of personal growth and opportunity.

I began work on this project in the July 2011. During the summer of 2011 and into the academic year I began piloting our IHC protocols, which took the better of 7 months to fully optimize. I received my AURA award from February- May 2012. By this time the piloting of my

antibodies was complete and I began implementing the protocols in my study. I collected and prepared my tissues and conducted all of my IHC protocols. I analyzed my preliminary data and I presented this work at the annual meeting of the Canadian Association for Neuroscience in May 2012. I continued my analysis through the summer of 2012 and I am currently preparing my first manuscript for publication.

As an AURA award recipient I have been given the opportunity to be responsible for a research project from start to finish. I have learned animal husbandry, dissection, tissue collection preservation and histology techniques. I have learned rat neuroanatomy, microscopy procedures and IHC quantification. I now have a detailed understanding of IHC protocols, and am a critical consumer of hormonal research. I have learned about every stage of a research project; from ethical approval to journal submissions and I have presented this research at a major conference.

The process of piloting my IHC protocols taught me the importance of consistency in research and of keeping accurate records. Any minute error in my protocol from one pilot to the next was enough to invalidate any of my pilot results, as I could no longer compare across trials. My lab notebook grew from a perfunctory checklist of protocol procedures to a place to record detailed impressions and motivations for my various actions. I have become a much more aware, conscientious and meticulous researcher.

I have also learned that research can never be conducted in isolation. This study brought me into contact with a number of knowledgeable researchers both within UBC and beyond. Dr. Victor Viau of UBC was kind enough to invite me to his lab and personally looked over my early slides to help me interpret some of my more unexpected findings. I left his lab with gifts of tissue and a new antibody, new ideas in my mind, and practical advice to hone my IHC and histology

techniques. Dr. Jon Epp of the University of Toronto helped me troubleshoot my IHC protocols; his suggestions deepened my understating of the IHC process. Dr. Donal Skinner from the University of Washington similarly consulted with me on unexpected findings, and provided me with a new antibody and a protocol to try. The many consultations and discussions I held with others gave me invaluable new pieces of knowledge, and the successful completion of this project would not have been possible without the generous help that all provided.

Not only has this study given me a large degree of practical training in common neuroendocrinology techniques but it has also revolutionized my way of thinking. Simply put, learning about experimental methods in a classroom was not enough to teach me to think as a scientist. I feel there is a new maturity in my ability to interpret and understand scientific information, and while I have a long way to go before reaching the high levels of expertize of those such as Dr. Soma, I feel ready to begin asking my own questions and exploring them through even more independent research. I feel confident in my abilities to time-manage, to perform complicated scientific procedures and to synthesize and interpret large amounts of data.

In sum this study represents and important stepping stone toward the rest of my career. This was my first research project and working in Dr. Soma's lab has confirmed my desires to pursue graduate studies and given me the necessary skills to make this possible. I thank Dr. Soma, the entire Soma lab and all of my collaborators for giving me their time, their trust and for sharing their knowledge and expertise. I thank AURA UBC for making it possible for students such as myself to explore scientific research and discover a new passion.

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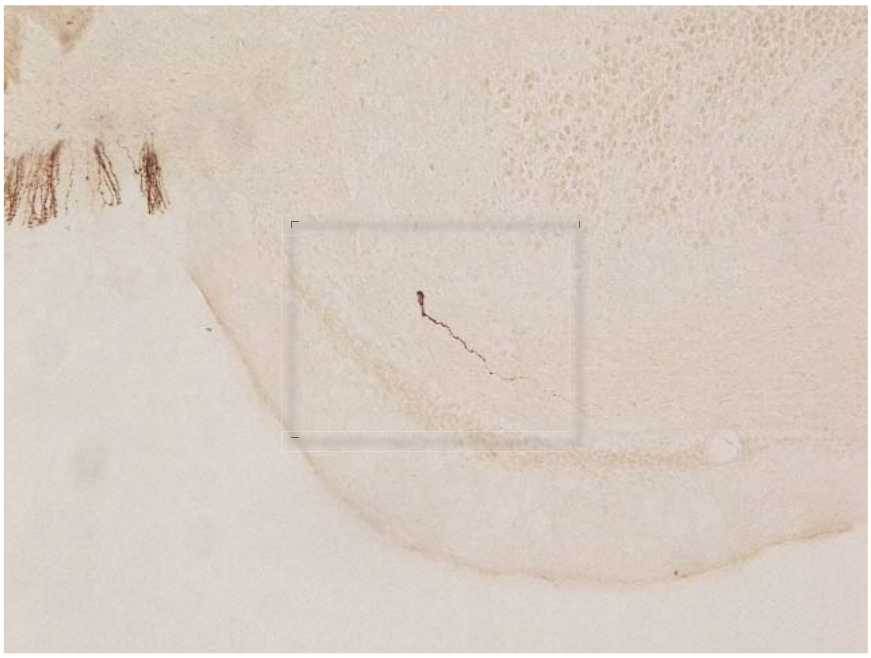
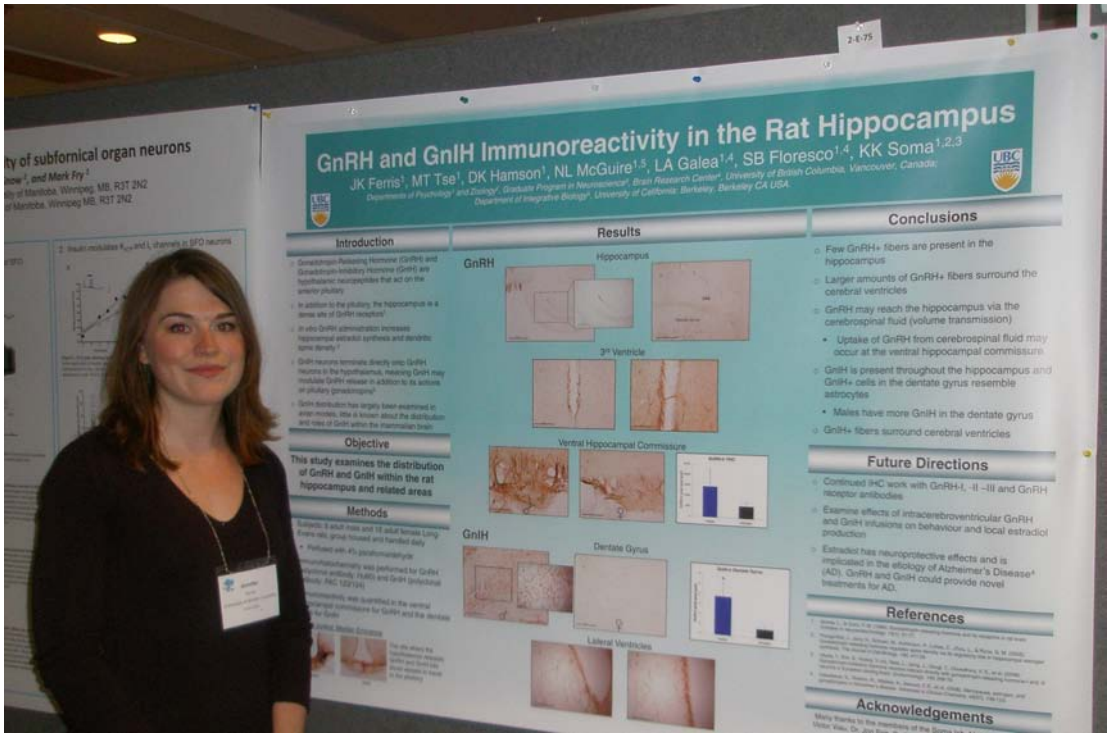


IHC staining of a neuron expressing GnRH in the rat hippocampus



Me, a happy AURA recipient, at the Canadian Association of Neuroscience annual meeting 2012