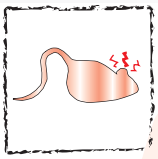

# Role of the Posterior Paraventricular Thalamus in HPA Axis Function and Habituation

Kavita Bhavsar
Mentor: Seema Bhatnagar

The main objective of our project was to gain a better understanding of the effects of chronic stress upon the Hypothalamic-Pituitary-Adrenal (HPA) Axis. Stress in this context may be defined as any threat to homeostasis, or internal environmental balance, of the body [1]. It is important to distinguish the effect of chronic and acute stress upon HPA axis function. Stress can be short-term (acute) or long-term (chronic). Acute stress is the reaction to an immediate threat and is commonly known as the "fight or flight" response [1]. The threat can be any situation that is experienced as a danger. Common stressors include noise, crowding, isolation, illness, hunger, danger and infection. Such stress signals cause the sympathetic nervous system to increase blood pressure and temperature along with adrenalin levels [2].

In contrast, chronic stress includes ongoing disturbances such as psychological pressures, loneliness, physical illness, and financial worries. The consequences of acute stress upon HPA axis activity have been widely studied through many diverse experiments. However, detailed information regarding the function of the HPA axis during periods of chronic stress is still lacking. Further, chronic stress has been associated with specific disorders such as depression and post-traumatic stress disorder [1]. It has been found that HPA axis performance is disrupted during depression; the adrenal gland is considerably enlarged and many patients exhibit high levels of cortisol in the blood stream [3]. Further, excessive levels of corticosteriods over a protracted period are damaging to the immune and nervous systems. In addition, it is interesting to note that aging can be associated with a series of events that excessively stimulate the HPA system while simultaneously weakening the strength of negative feedback responses which terminate corticosteroid generation [4]. Therefore, our study of high levels of chronic stress in the brains of lab rats is extremely significant.

Since chronic stressors have been observed to change the response characteristics of the HPA axis [5], it is crucial to understand how the system works. If the system is operating optimally, there is a prompt shutdown of the HPA axis at the termination

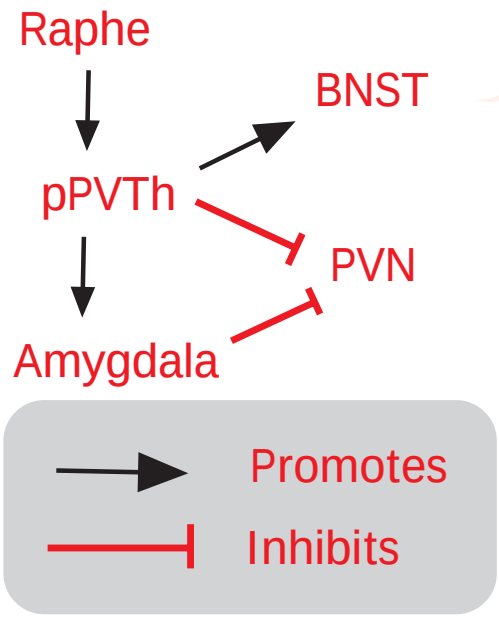


**Figure 1: Stress Neuronal Circuit** The figure above shows possible pathways through which the pPVTh works in order to affect the PVN and ultimately the response to stress. It is believed that the pPVTh functions by inhibiting the amygdala, which normally acts to stimulate the PVN. However, the pPVTh may work through other extensions as well in addition to its output to the amygdala. The pPVTh may directly send an output to the PVN, or may extend to the BNST. The inhibitory effect on the PVN may be achieved by the amygdala working through the BNST to reach the PVN instead of directly extending to the PVN. The raphe nucleus and its secretion of serotonin may also have some important implications.

| Mice Number and Type | Stress Type and Trial # | Staining Results |
|---|---|---|
| 7 (DH2) Mice- with sham lesions | Chronic- Trial 1 | All mice exhibited little staining |
| 12 (DH2) Mice- with actual lesions | Chronic- Trial 1 | 10/12 mice exhibited heavy staining |
| 6 (DH3) Mice- with sham lesions | Acute- Trial 1 | All mice exhibited little staining |
| 14 (DH3) Mice- with actual lesions | Acute- Trial 1 | 14/15 mice exhibited little staining |

Table 1: Animals in the study group DH2 were chronically stressed only. DH2 animals underwent sham and pPVTh lesioning. DH3 animals, however, were only acutely stressed, but also underwent both types of lesioning.

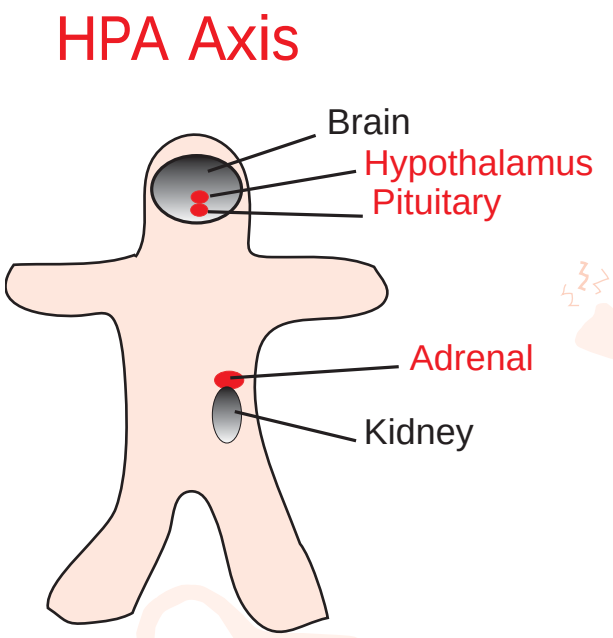


**Figure 2: Hypothalamic-Pituitary-Adrenal Axis** The HPA Axis is a complex network of feedback inhibition utilizing corticosteroids.

of a stress signal. This indicates that the HPA axis behaves as a negative feedback system. It also appears to be the major brain pathway regulating neuroendocrine, autonomic, and immune responses to shifts in homeostasis as a result of stress [6]. The HPA axis ultimately regulates stress responses through the management of glucocorticoid secretion in the outer layer of the adrenal gland, or the adrenal cortex [3]. These manufactured corticosteriods are essential throughout the body in order to promote the conversion of proteins and lipids to carbohydrates, but in the context of stress, they serve as signals of negative feedback [5].

Upon close inspection, it is evident that the HPA axis consists of a complex arrangement of particular nuclei and neuronal structures which act in synchrony (Figure 2). Stress signals trigger the Paraventricular Hypothalamic Nucleus (PVN), which in turn mediates secretion of the proteins Corticotropin-releasing Hormone (CRH) and Arginine Vasopressin [4]. These proteins potentiate the production of another 'messenger' substance termed Adrenocorticotropin (ACTH), which acts upon the receptors in the adrenal cortex, increasing the secretion of glucocorticoids (corticosterone or cortisol) released into the bloodstream [7]. Corticosteroids serve as signals of negative feedback because an increase in levels of cortisol and corticosterone leads to decreased sythesis of ACTH.

Next, our particular study investigates in particular the function of the Paraventricular Thalamus (pPVTh) in the HPA axis. Data obtained from previous studies has shown that pPVTh lesions enhance facilitation of stress response and lesions of this area block habituation. The pPVTh is thought to function, more importantly, only at the onset of chronic stress. Our research continued this line of study by examining which brain regions are changed by pPVTh lesions in acute vs. chronically stressed rats. It was hypothesized that the pPVTh functions to inhibit the PVN directly or though various neuronal circuits involving the pPVTh (Figure 1). The pPVTh recieves input from the dorsal raphe and sends an output to the basolateral, basomedial, and central nuclei of the Amygdala which also extends to the PVN. The dorsal raphe is a key brain structure which is responsible for the production of the pleasure-stimulating neurotransmitter, serotonin. The amygdala similarly controls emotions, but covers a broad range of feelings including anger and fear. It is believed that the pPVTh normally functions to inhibit the amygdala, which acts to stimulate the PVN and hence stress response. However, the pPVTh may also extend to the bed nucleus of the stria terminalis (BNST), which contains central and medial extended amygdala elements (Figure 1).

In our animal studies, we utilized Sprague-Dawley male rats. Our first study was randomly termed DH2. In this study, seven rats were sham lesioned and twelve rats underwent actual pPVTh lesions. Sham lesions are superficial lesions and were

performed for both the DH2 and DH3 studies in order to ensure that the results obtained were due to the actual destruction of the tissue, not the process of delving into the brain. Both DH2 sham and pPVTh lesioned animals were chronically stressed by being placed in restraint cages for thirty minute intervals per day over a period of seven days. On day eight, the animals were euthanized and after one hour the brains were perfused.

The DH3 study, in contrast, utilized acute stressors. Six rats were sham lesioned and fourteen rats underwent pPVTh lesions. The 20 rats were placed in restraint cages for thirty minutes on day one, and euthanized (also on day one). Following death, the rat brains were perfused in parallel to the DH2 study.

Next, brains obtained from both DH2 and DH3 studies were surgically removed and preserved in formalin so that they could be sliced. Brains were sliced at 30 microns. These slices of brain tissue then underwent a staining procedure coined Fos-Immunocytochemistry (Fos ICC), in which the protein secretions of activated neurons are visually marked. Following Fos ICC, each slice of tissue was mounted on a slide, and the slides were analyzed under a high power microscope to evaluate which brain regions were activated during stress.

It was anticipated that the HPA axis would be highly activated and exhibit heavy Fos staining when the pPVTh was lesioned in chronically stressed rats only. Acutely stressed rats with pPVTh lesions were expected to exhibit less Fos staining than chronically stressed rats with pPVTh lesions. Moreover, acutely stressed rats with pPVTh and sham lesions were expected to show equivalent amounts of Fos staining. Sham lesioning of chronically stressed rats should have no effect on normal HPA acis operation, and the inhibitory role of the pPVTh should remain functional and undisrupted. Thus, chronically stressed rats with sham lesions were also expected to show little Fos staining. Specific brain regions that were expected to show activation during the chronic stressing of the pPVTh lesioned animals included the amygdala, BNST, and the PVN.

Preliminary results indicate that only pPVTh lesioned animals exhibited high Fos staining during chronic stressing, implying that the normal function of the pPVTh is indeed to inhibit the PVN.

Future studies will continue this line of research by lesioning areas other than the pPVTh, such as the amygdala and the BNST. If the amygdala is destroyed, will the pPVTh still be able to function normally through the BNST or a direct extension to the PVN? Or is the amygdala necessary for the inhibitory effect of the pPVTh on the PVN? More research is necessary to clarify the pathways of response to chronic stress.

## Acknowledgements

I would like to thank Professor Seema Bhatnagar who is our lab's principal investigator and an excellent mentor. She has assisted me every step of the way in my individual research project.

## Citations

1. Wolfgang, Walz. The Neuronal Environment: Brain Homeostasis in Health and Disease. Humana Press (2002).
2. Bear, M.; Connors, B.; and Paradiso, M. Neuroscience: Exploring the Brain. Lippincott Williams & Wilkins Publishing (2002).
3. James, Vivian H.T. The Endocrine Function of the Human Adrenal Cortex. Academic Press Incorporated (1998).
4. Rose, Clifford F. Control of the Hypothalamo-Pituitary-Adrenocortical Axis. International Universities Press (1989).
5. Toates, Frederick. *Biological Psychology*. Prentice Hall Publishing. Essex, England (2001).
6. Dallman, M.; Bhatnagar, S.; and Viau, V. The HPA (hypothalamic-pituitary- adrenal) Axis. *Encylopedia of Stress*. Academic Press (2002).
7. Nelson, Don Harry. Adrenal Cortex: Physiological Function and Disease. Saunders W B Co (1990).

## Additional References

1. Jasmin and Ohara Neuroscience Lab. Gabriella Janni. University of California, San Francisco. 2/16/02. http://anatomy.ucsf.edu/ohara/index.html
2. Schatzberg, A. and Nemeroff, C. Hypothalamic-Pituitary-Adrenal AXIS: Physiology, Pathophysiology, and Psychiatric Implications. Lippincott Williams & Wilkins (1998).
3. Shepherd, Gordon M. *Neurobiology*. Oxford University Press (1994).

## About the Author

Kavita Bhavsar is a third year undergraduate in Biopyschology and Cognitive Science at the University of Michigan-Ann Arbor. In addition to her neuroendocrinology lab, she is also a research assistant for Dr. Mark Russell in Pediatric Cardiology which deals primarily with gene therapy. She is the official treasurer for University Students Against Cancer Executive Board and secretary of the AMSA-Premedical Executive Board.