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Abstract

Modernization and a need for efficiency in our everyday lives has lead to increased consumption of packaged and processed products, pesticides, drugs, etc. Though convenient, these products periodically include substances damaging to both living organisms and the environment in a variety of fashions, including disrupting the endocrine system. Endocrine Disrupting Compounds, or EDCs, are particularly damaging as the endocrine system is quintessential in the development of any organism. Lack of regulation in developing countries as well as a nascent understanding of EDCs in both their formation and effects may already be disturbing human and animal health worldwide--some EDCs have been shown to act similarly to xenoestrogens, and it is suspected that they play a role in the earlier onset of puberty in developing countries, provoking the question of what other effects EDCs may have on a system as delicate and paramount as the endocrine system.

Over the summer, I will be imaging the neurons of zebrafish at 3, 5, 7, 9, 11, 13, 15, 17, and 19 days old to understand and establish the baseline response pattern of zebrafish neurons

involved in the reproductive endocrine system in a non-hazardous environment. Once I establish baseline neuroendocrine activity in zebrafish with respect to neurons involved in GnRH secretion, I will introduce zebrafish to environmental toxins such as Bisphenol A (BPA) and hopefully understand both the short and long-term effects of these toxins on zebrafish development, to which parallels of human development (with respect to neuroendocrine behavior and ultimately crucial developmental checkpoints common to both species) may be drawn.

Biological Sketch

I am a sophomore in the Class of 2019 majoring in Biological Sciences with a concentration in General Biology and a minor in East Asian Studies. I was born in Atlanta, Georgia and moved to Northern Virginia at the age of 3, where I stayed for the rest of my life. My interest in biology started with DK's *The Human Body* for children, where colorful organs and diagrams enticed me to flip to the next page. The only show I watched at the age of two, according to my parents, with full attention was *Bill Nye: The Science Guy* and even nearing twenty I can affirm that my eagerness has not been diluted. Later in elementary and middle school, I was awed by science documentaries on the Science Channel and Discovery Channel, particularly marveled by the researchers interviewed and wishing I might be like them one day. These aspirations lead me to the Cancer Research Training Award at the National Institutes of Health, where I was a research intern in the Laboratory of Biochemistry and Molecular Biology in the National Cancer Institute the summer before my senior year of high school. My work there suggested a link between early replication and tumorigenesis in fission yeast, leading me to both a Research Report Badge and a Semifinalist Badge in the 2015 Intel Science Talent Search,

which strongly encouraged me to continue along the path of research in college. A few months later I was accepted as a Tanner Dean's Scholar to Cornell, and it wasn't long until I started discussing research prospects with Colleen M. Kearns, my (awesome) faculty advisor. In September of 2016 I started working in Motoko Mukai's lab and fell in love with my interdisciplinary work on neuroendocrinology and environmental toxins, so much so that I decided to spend my remaining two college summers in the lab. Aside from scientific research, I have been studying Japanese language and culture for seven consecutive years and will finally be going to Japan in the beginning of this summer for 2-3 weeks to meditate and experience art in various temples as a part of my "Zen Buddhism" class. Lastly, safe to say, I do watch a lot of anime.

Shreya Nandi, Class of 2019

Biological Sciences Major, East Asian Studies Minor

BPA and you: Influence of Environmental Endocrine Disruptors on Reproductive Neuroendocrine Pathways in Zebrafish

The goal of my project is to understand whether reproductive health and development is impacted by the presence of synthetic molecules and substances that are known to function as endocrine disruptors such as Bisphenol A (BPA). In industrialized countries, BPA is widely used to create plastics, lining of canned beverages, dental sealants, and thermal printed receipts[1]. So pervasive in our daily lives, greater than 90% of Americans carry detectable levels of BPA[2]. As a synthetic and recently introduced compound, one wonders whether BPA induces any ill effects among the general population. Indeed, BPA has been linked to both female and male reproductive dysfunctionalities such as polycystic ovarian syndrome (PCOS)[7,9], anorexia nervosa, amenorrhea[5,10], recurrent miscarriage[8], lower sperm count (Terasawa et. al), and decreased libido in males[4]. In female rhesus monkeys, direct doses of BPA (2ng/mL) falling within the range of human exposure (0.3-4ng/mL) resulted in suppression of GnRH and the hormone kisspeptin, which has the ability to exert influence over GnRH activity[3]. Thus, we should take a quick look at the reproductive cycle to see where endocrine disrupting compounds (EDCs) may exert an influence.

GnRH is first released by the hypothalamus, prompting the release of FSH and LH from the anterior pituitary, which waltz with other hormones in the gonads--such as estradiol in females and testosterone in males--to temporally regulate ovulation and sperm production, which are vital in reproduction.

In females, the cycle is as follows: After the anterior pituitary releases FSH and LH, FSH (aided by LH) will stimulate follicular growth. Cells of the growing follicle will start to produce estradiol. These low levels of estradiol will inhibit the anterior pituitary from producing more FSH and LH (negative feedback) until estradiol concentration reaches a certain threshold, wherein estradiol will then positively regulate LH production, sending LH levels soaring as well. This results in the final maturation of the follicle and the end of the follicular phase. The follicle and adjacent wall will rupture, freeing the secondary oocyte (egg) which will now make its way into the fallopian tube. Next, the luteal phase begins, where the high concentration of LH stimulates the remaining follicular tissue to form the corpus luteum, which secretes progesterone and estradiol. Progesterone and estradiol promote the thickening of the uterine wall as well as growth of arteries and endometrial glands that allow for the secretion of nutrient-rich fluid to early embryos. The combination of progesterone and estradiol exerts negative feedback on the hypothalamus, resulting in the falling of FSH and LH levels. This also prevents a second egg from maturing while the first egg reaches the extent of its stay in the uterus. Because FSH and LH hormone secretion resulted in the formation and sustenance of the corpus luteum, their falling levels result in the disintegration of the corpus luteum, resulting in a sharp decline in progesterone and estradiol levels. As progesterone and estradiol fall and the corpus luteum disintegrates, arteries constrict, and a lack of oxygen causes the uterine lining to disintegrate, which is promoted by prostaglandin. Menstruation occurs, while the hypothalamus and pituitary gland "take a break" until LH is again secreted by the anterior pituitary and the cycle starts again. In the female menstrual cycle, we see many chances for EDCs such as BPA to interfere with normal progression of the cycle. It is no small feat for the body to coordinate such a massive

system of hormones and specialized cell types to promote reproduction in a strict, monthly manner, and thus, disruption on the primary level--the GnRH-secreting neurons--will likely have the most discernible impact on the cycle. One may consider the following musical analogy: during the tense silence that precedes the start of a concert, every musician has their eyes trained on the conductor for the gesture that will start the melody. It is not the case that *every* instrument will be heard at the first signal--perhaps the flutes will start, and after 8 beats, the trombones and saxophones will kick in, and after 16 beats, the timpani will finally make its debut as other instruments wax and wane with the melody. Though the conductor will try their best to keep the symphony on a steady rhythm, a late start by the flutes may confuse every other player in the symphony, turning classical into a cacophony.

Beyond the reproductive cycles themselves, exposure to EDCs before the reproductive systems have fully matured may have a lasting impact on the reproductive system, as Fernandez et. al found that neonatal *in vivo* BPA in female rats results in the disruption of GnRH activity, acceleration of puberty onset, permanent changes in the estrous cycle, and $ERK_{1/2}$ activation, which is a protein kinase that mediates cell proliferation and death[6].

Thus, we may conclude that disruption to GnRH activity has the potential to corrupt reproductive success as a whole if not proper development of organisms, ringing alarm bells among all Darwinian-competitive organisms, or really any individual organism that intends to develop and reproduce as usual.

I am particularly eager to be engaged in this type of research as I've never been involved in anything so incredibly disciplinary nor have I worked with organisms larger than fission yeast in the past. In addition, my status as a pre-med student leads me to appreciate the biomedical

implications of my work, where the recent phenomenon of EDCs impacts you and me on an incredibly personal level, and I am profoundly excited to be researching at the forefront of this field.

Still in the nascent stages of my project, my work this summer will be that of establishing the baseline activity of GnRH-secreting neurons. Subsequent to the establishment and analysis of a baseline, we may add BPA in different concentrations to the environment of the zebrafish to determine whether BPA at certain concentrations affects GnRH activity, and thus reproductive activity, in any manner.

This research will be carried out in Stocking Hall under the Mukai Lab. I will be using a strain of zebrafish modified for transparency, a large microscope for neuronal imaging, and a smaller microscope to assist in the embedding and freeing of zebrafish from the imaging platform, which consists of a small, 1-inch diameter glass plate, agar, and the preferred environment of the zebrafish: Hank's solution.

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