The Effects of an Altered Dopaminergic System on Behavior, Development and Physiology in *Drosophila melanogaster*

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Introduction

Neuromodulators play a vital role in development and controlling the Central Nervous System (CNS) but all of their attributes to whole body development and behavior are not completely understood. One neuromodulator, dopamine (DA), has been seen to have devastating effects to whole body function if the concentrations are altered. For example, cocaine a major drug of abuse results in excess amounts of dopamine to occur in the synapse. Cocaine used during pregnancy causes subtle molecular and behavioral effects on fetal brain tissue. In postnatal life these effects are manifested in decreased IQ scores and learning deficiencies [Morrow 2006]. Also a decrease in dopamine levels, within a particular location within the CNS, is known to be associated with Parkinson's disease, and has effects on motor control [Contreras-Vidal and Stelmach 1995].

In insects, neuromodulators and hormones are known to effect and regulate development of whole animal as well as their nervous system. It is well established that hormones such as ecdysone and juvenile hormone alter neural development and differentiation [Garen et al, 1977; Pak and Gilbert, 1987; Truman, 1996]. The surge of ecdysone in the pupal stage of Drosophila likely plays a key role in inducing gross alterations in neural circuitry [Kraft et al, 1998; Thummel, 1996; Truman and Reiss, 1988] and motor unit function [Li and Cooper, 2001; Li et al, 2001]. However, the lack of or over expression of neuromodulators has not been as intensely investigated in the development of the Drosophila CNS as the molt regulating hormone ecdysone.

It is well established that sensory input early in life dictates the formation of central circuits which after a critical period the circuits become hard wired. Historical examples are known in the visual system in cats and monkeys which lead to the Noble Prize for Hubel and Wiesel [Hubel and Wiesel, 1963a, b,1968, 1970]. Their work was seminal since it had direct implications for humans. We now refer to such bridging of bench science to clinical implications as translational research. Since neuromodulators can alter the activity of whole circuits or parts of a circuit, modulation can then have an impact on the formation and maintenance in neuronal communication within the CNS. Examining the effects on sensory integration within the CNS to motor commands by neuromodulators, such as with DA, may be better approached in relatively less complex organisms so that detailed understanding can be obtained. The hope is that analogies can be made for more complex animal brains such as the human from simpler systems to aid in translational research.

In order to understand the role of the dopaminergic system and the manifestations for health related treatment one needs to understand what role the system has on development and acute functions. This is possible by examining simpler systems with fewer wiring diagrams and fast developmental times, allowing one to examine the effects of perturbing systems easier and quicker. Responses from manipulations in Drosophila can later be re-examined with cruder techniques in more complex animals, but one will better know what to look for in the complex system. Using a simpler model with a shorter life span,
like *Drosophila*, will provide answers and an understanding the roles of dopamine rapidly.

The role of DA in *Drosophila* has not fully been investigated; however research has been conducted on larval exposure to Tyrosine Hydroxalase inhibiting drugs for 24 hours and demonstrated that the exposure did not alter behavior but delayed development [Neckameyer 1996]. My preliminary data, as does that of Pendleton et al. (2004), contradicts these findings, although we each used slightly different methods.

In order to examine the effects of reduced DA in the CNS researchers have used primarily two approaches. One approach by genetically engineering fly strains that lack the enzyme to synthesis DA, and a decreased locomotive behavior was detected with these mutants [Pendleton et al. 2002]. Such a line is called the “pale strain” since the flies, as adults, lack pigmentation. The second approach is to pharmacologically block the synthesis of DA. One drug is AMVT which targets tyrosine hydroxalase, the rate limiting enzyme to make dopamine. The feeding of larvae AMVT for an extended interval and examining the effects of stress, cardiac functions and sensitivity of the CNS to exogenous applications of DA has not yet been established. The acute effects of AMVT on larvae have been shown by Neckameyer, (1996) as mentioned earlier. Also female adults that were fed AMVT show altered courtship behavior and as well as reduced ovarian maturation [Neckameyer 1998].

In addition, neuromodulators provide a rapid way in which animals can tune up or down activity within a neural circuit and may be responsible for rapid changes in behavior, as recently examined for aggressive behavior in *Drosophila* [Baier et al., 2002]. It has already been established that alterations DA has an effect on sexual behavior [Neckameyer, 1998] and promotes an increased movement in adult flies [Friggi-Grelin et al., 2003] but can depress synaptic transmission directly at the NMJ in larval *Drosophila* [Cooper and Neckameyer, 1999]. Even in other insects, such as bees, the whole animal behavior is affected by DA [Taylor et al., 1992]. The results of the courtship studies showed phenomena of altered behavior but lacked the experimental design to demonstrate which sensory modalities are responsible for the altered behavior. In general, these older studies stimulated people to examine the location of DA circuits and other neuromodulators in the *Drosophila* brain to obtain a better understanding what circuits might be influenced by neuromodulators [Baier et al., 2002; Blenau and Baumann, 2001; Monastirioti, 1999]. One particular pathway that DA is associated with, which is of interest to me, is neural control of olfaction [Margulies et al., 2005]. My studies are different then what has previously been addressed since I am examining not only behaviors in larvae and adults but examining if DA receptor sensitivity maybe altered in the CNS and heart when endogenous levels of DA are lowered.

One aspect of my proposed research is examining the effects of dopamine and the influence on the neuronal circuit formation involved with olfaction and taste perception in larvae and adults. This aspect of the project can have direct implications in understanding if DA impacts the development of these
neuronal circuits as well as acute function of already established circuits. I do plan to conduct associative learning projects related to this topic.

One purpose of these studies is to deplete DA during various stages of development and test the effects on larval olfaction and learning. Also I plan to examine adults separately in acute and chronic studies for the impact on the lack of DA with olfaction function. These studies will bridge the physiological measures of the sensory-CNS-motor circuits, for general as well as specific effects of DA on neuronal circuits.

This project is not a continuation of any previous research in Dr. Cooper's lab. The initial idea came from the HPLC results of my last project on focusing on serotonin and its role in Drosophila larvae, which was combined with the work of a graduate student and published in the European Journal of Neuroscience. This past study involved a drug that prevented serotonin synthesis. HPLC analysis revealed that dopamine, along with serotonin (5-HT), was decreased by an enzyme blocker that was supposed to be specific to 5-HT. I became interested in investigating to see if the effects observed in this study were entirely due to reduced 5-HT or if the reduced DA had an effect. As I furthered my reading and analyzed the results obtained, the project has evolved to the potential of a grant submission and future publications.

**Specific Aims:**
1. To determine the effects of AMVT on larval and pupal development.
2. To determine if adult flies being administered AMVT have an altered stress tolerance.
3. To determine the impact of AMVT on locomotive behavior, body wall movements and larval eating behavior. This lays the groundwork for later associative learning projects and CNS function.
4. To determine, by electrophysiological recordings of CNS circuits, if larvae fed AMVT have an altered sensitivity to dopamine exposure. Also part of this aim is to examine if stimulated sensory paths or heightened or depressed when larva are deprived of DA synthesis.
5. Investigate effects of AMVT on the sensitivity of the larval heart to DA.
6. Determine the impact of AMVT to adult fly to a immune stressor.
7. To determine by HPLC analysis if exposure to AMVT has an alteration on 5-HT levels within the larval and adult brains.