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# Behavioral phenotype of Vang6 mutant *Drosophila melanogaster* pertaining to the Olfactory System

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Behavioral phenotype of Vang6 mutant *Drosophila melanogaster* pertaining to  
the Olfactory System

By

Sarah Lynn Austin

A thesis submitted to the Department of Biology of The College of Brockport:  
State University of New York, in partial fulfillment of the requirements for the  
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**Abstract:**

There are many proteins that aid in the development of the olfactory system, specifically Wnt pathway and planar cell polarity (PCP) pathway proteins. It has been shown that Vang6 mutant flies have distinct olfactory abnormalities, as do Wnt5 mutant flies. In addition, *Drosophila melanogaster* (*Drosophila*) Wnt5 mutants have an improper olfactory response compared to wildtype *Drosophila*. After using a T-maze to explore the behavioral tendencies of Vang6 mutant *Drosophila* and wildtype WT1118 flies, it was shown that there is no significance between wildtype and Vang6 mutant *Drosophila* selecting air (control component) or Carbon Dioxide (CO<sub>2</sub>) (test component).



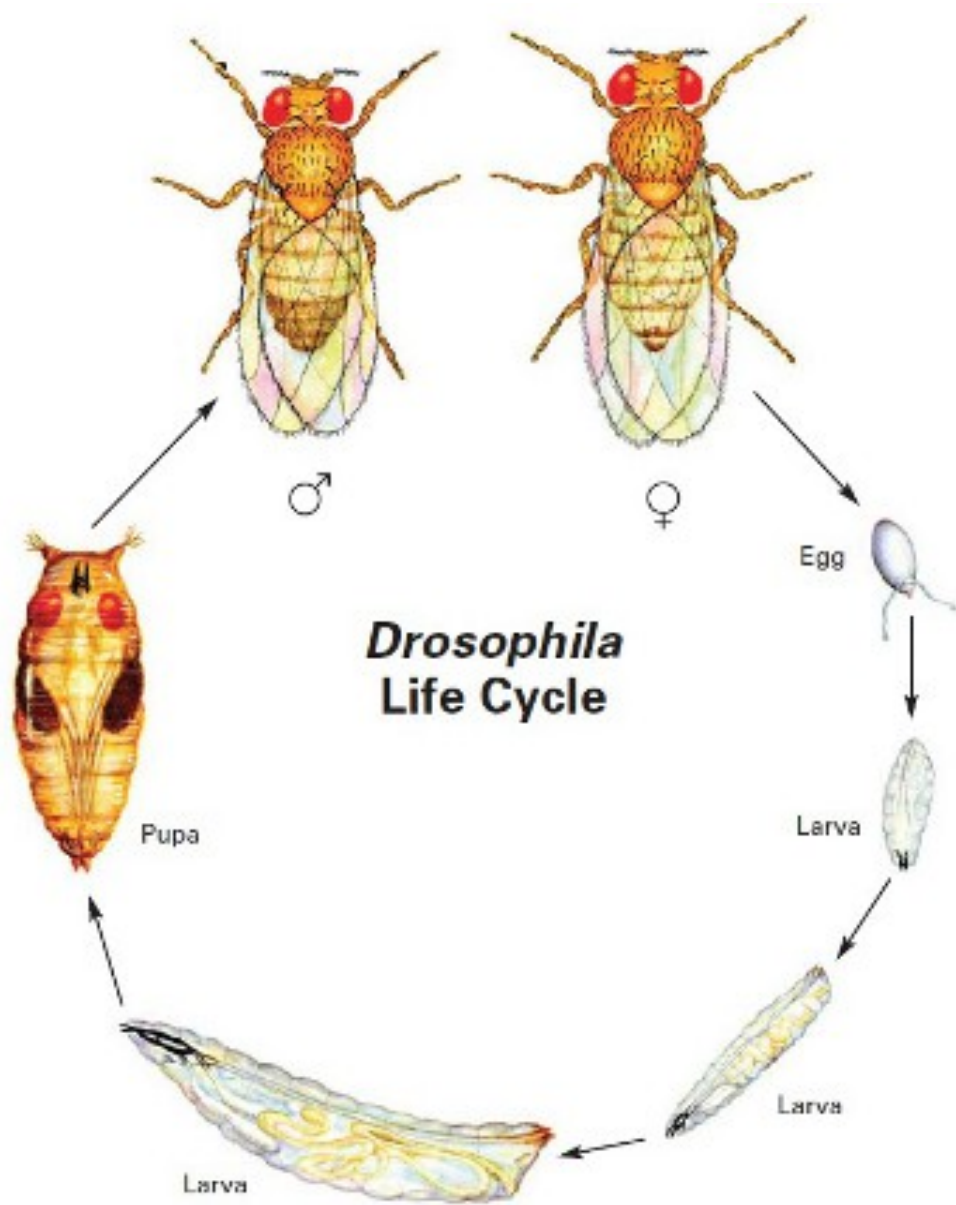
## Chapter 1: Introduction

### ***Drosophila* as a model organism:**

In the scientific world, *Drosophila* is a model organism and essential for experimentation. These organisms have a long history of aiding scientists in discovering information, developing new theories and thus have greatly advanced human knowledge of biology. *Drosophila* as a subject of research has been valuable in many aspects including linkage, sex determination, genetic communication, molecular, biochemical and developmental genetics, chromosomal abnormalities, gene transmission, evolutionary trends, penetrance and expressivity<sup>1</sup>. Characteristics of this organism, such as its short reproductive cycle, ability to generate numerous offspring, simple breeding nature, inexpensive maintenance and diet, and most importantly the similarity of its genome to humans, make *Drosophila* the ideal organism for biology based research.

The *Drosophila*, or “fruit fly,” genome is composed of 13,600 genes and 8 chromosomes as compared to the human genome which has 46 chromosomes and 27,000 genes<sup>2</sup>. The simplicity of the *Drosophila* genome as compared to humans makes this organism excellent for genetic experimentation. In addition, almost 75% of disease identified genes in humans have functional orthologs in *Drosophila* and protein sequences between *Drosophila* and mammals are roughly 40% amidst homologs<sup>3</sup>.

The reproductive cycle of a fly (Image 1) is approximately 10-12 days in length consisting of fertilization, the egg, multiple larva stages, the pupa and finally, the adult fly<sup>3</sup>. The length of each stage of development depends on the temperature in which the fly is exposed to. For a 20°C environment, the length of the egg-larval stage is 8 days and the pupa stage 6.3 days, where as the 25°C exposed flies have an egg-larval period of 5 days and a pupa period of 4.2 days<sup>1</sup>. After fertilization of an egg, which is shortly followed by the formation of a zygote, cleavage of the nuclei, and laying of the egg by the female fly, the next stage in development is the larval stage. The larvae hatch from the egg and partake in 2 molts. The larva is a soft and malleable structure comprising of a non-cellular cuticula and an inner cellular epidermis<sup>1</sup>. It has 12 segments, the head and thoracic segments consisting of 3 separate parts and an abdominal segment consisting of 8 parts. During each molt, the complete cuticle of the larva, along with the mouth, spiracles and cuticular specific structures, are discarded and completely reconstructed<sup>1</sup>. These organisms are hungry and active creatures that can grow to be approximately 4.5 millimeters in length in the third and final larva stage<sup>1</sup>. After the 5-8 days of the larva stage, the growing insect gradually morphs into a pupa. Firstly, the larva undergoes muscular movement which condenses the larval cuticle, forming the puparium case, and discards the larval segmentation<sup>1</sup>. Inside the case, the organism breaks from the epidermis and becomes a prepupa without a head, legs or wings<sup>1</sup>. Approximately 12



**Image 1:** The Reproductive Cycle of *Drosophila*.

hours after the puparium develops, pupation occurs. During pupation, muscular movement allows for the wings, halteres, and legs to present themselves to the surface and the formation of the head, thorax and abdomen structures that are seen in the final adult fly<sup>1</sup>. Lastly, in the metamorphosis process of the insect, specific larval tissues and organs are broken down thus exposing the final adult structures of the *Drosophila*<sup>1</sup>. When a fly enters the final stage of development and escapes from the pupa shell, it is light in color and takes a few hours to fully adapt to the correct color and appearance of a normal adult<sup>1</sup>. All in all, female flies can lay as many as 100 eggs per day in a 20-day time span<sup>4</sup>. This relatively quick developmental cycle with abundance of offspring makes this organism adequate for experimentation. Because of its distinct reproductive cycle, it is relatively simple to perform research on stages of development ranging from embryo to adult thus allowing scientists to undergo experimental genetic crosses and complete behavioral studies.

Female *Drosophila* and male *Drosophila* have distinct features that clearly identify one from the other. Female *Drosophila* are larger in size and have a longer abdomen compared to males. Females also have alternating dark and light stripes on the posterior portion while the segments on a male are joined consisting of one dark band. Females and males also have definite recognizable sex characteristics that set them apart. Male flies have sex combs, or black bristles on the distal side of the foreleg, which are not present in females<sup>1</sup>. External genitalia in females are located at the edge of the

abdomen while male genitalia are located ventral to the edge of the abdomen<sup>1</sup>. During larval development, males have large white testicular tissue located at the posterior third portion of the larva while females have ovarian tissue, which is smaller in size<sup>1</sup>.

Using humans as research subjects for biology creates ethical issues making other organisms such as, mice, zebrafish and especially *Drosophila* a more sophisticated option. Even so, there are restrictions when performing research on vertebrates as subjects, thus making *Drosophila* an ideal animal for research studies<sup>4</sup>. *Drosophila* are optimal organisms for investigation because research can be performed on all stages of development. Because of undifferentiated tissue in the larva, molecular and genetic studies are possible in the pupa extending to the adult stage<sup>3</sup>. The primary organs in the fly, such as the heart, liver, lungs, and brain, undergo the same tasks as do those in mammals. The adult fly brain is particularly impressive with at least 100,000 neurons forming connections and partaking in complex functions such as circadian rhythm, sleep, memory, learning, feeding, grooming, flight navigation, aggression and courtship<sup>3</sup>. To scientists' advantage, there are quite a few drugs that have similar effects in mammals as they do in *Drosophila*<sup>3</sup>. Additionally, many cellular mechanisms including membrane trafficking, membrane cell death, synapse formation, neuronal communication, learning and memory production, motor output, and sensory output are alike in mammals and flies<sup>2</sup>. These neurobiological similarities

make advances and insight in neurodegenerative diseases possible.

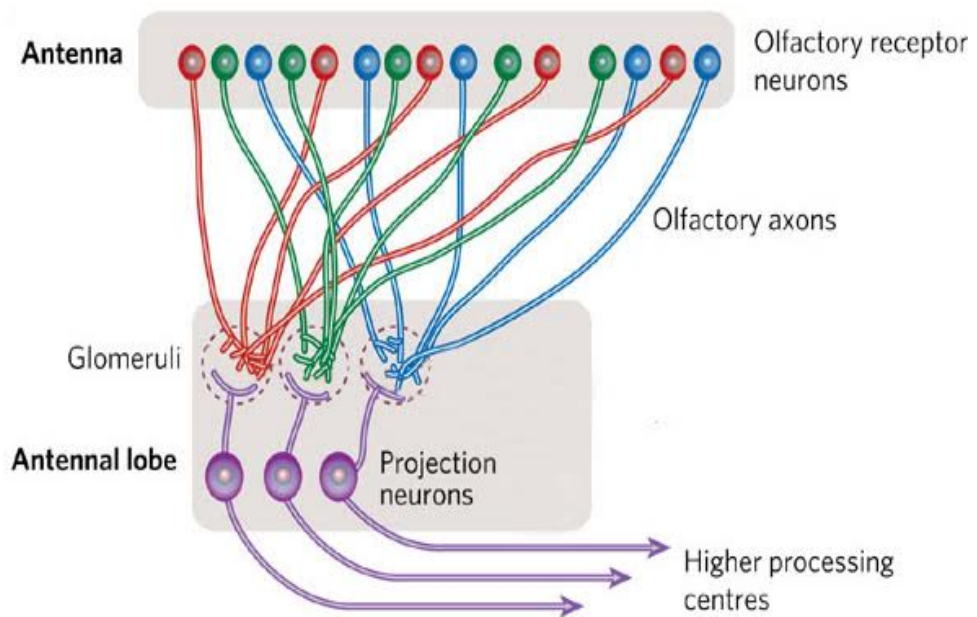
Advances in diseases such as Alzheimer's disease (AD), Parkinson's disease, frontal-temporal dementia, Huntington's disease and motor neuron diseases have all been possible with use of *Drosophila* as an experimental model<sup>2</sup>. Because *Drosophila* have a definite inheritance pattern, a pedigree that encompass at least three generations, and genetic homogeneity, scientists have the ability to study all areas of development and have access to a large number of genetically identical samples, making these organisms valuable subjects for genetic, molecular and neurobiological investigation<sup>2</sup>.

The study of human viruses has also advanced with use of *Drosophila* as a model. In addition to its quick lifecycle, abundance of offspring, and simple maintenance as stated above, viral research in *Drosophila* is ideal because *in vivo* tissue investigations of this insect allow viral genes to be studied in an environment that similarly represents that of a viral infection<sup>5</sup>. In addition, there are fewer genes in *Drosophila* compared to humans, making it easier to study the effects that these viruses have on genes as well as potential targets of the fly allowing for the discovery of drug treatment in the future<sup>5</sup>. These organisms have been used to study such viruses as dengue virus, Epstein Barr virus, hepatitis B virus, human immunodeficiency virus (HIV), human cytomegalovirus, influenza A virus, severe acute respiratory system coronavirus (SARS-CoV), simian vacuolating virus 40, sindbis virus, vaccinia virus, vesicular stomatitis virus, and west Nile virus<sup>5</sup>. Specifically, viral

research with use of *Drosophila* as a model has identified that there is an increased in apoptosis during eye formation with the expression of SARS-CoV 3a protein as well as apoptosis in the cytochrome c mitochondrial pathway<sup>5</sup>. Another example, which is seen in studies involving HIV, has shown that the expression of Nef, a HIV membrane-associated protein, is involved in the deterioration of T-Cell operation and furthers the advancement of AIDS in an individual<sup>5</sup>. Examination of Rev, another HIV protein, in *Drosophila* cells propose that the action of this protein in insects parallels the action in humans and that Tat, yet another HIV protein, may also contribute to the development of HIV disease<sup>5</sup>. Further investigation of these viruses can help discover potential drug therapies and help to better understand the workings and targets of these viruses in organisms.

### **The Olfactory and Visual Systems:**

The olfactory system is fundamental for the existence and life style of numerous creatures. Organisms are exposed to a diversity of odors daily which then must be interpreted in order to respond to reproductive related stimuli, evade predators, find food sources and sufficient shelter in order to survive. An ideal subject that represents the basic layout of the olfactory system is *Drosophila* (Image 2). In fruit flies there are approximately 1,300 olfactory receptor neurons (ORNs), 62 odorant receptors (ORs) and about 49 glomeruli<sup>6</sup>. This is in comparison to approximately 2 million ORNs, 1,000 ORs and about 2,000 glomeruli in a mouse, making the simpler fly anatomy much



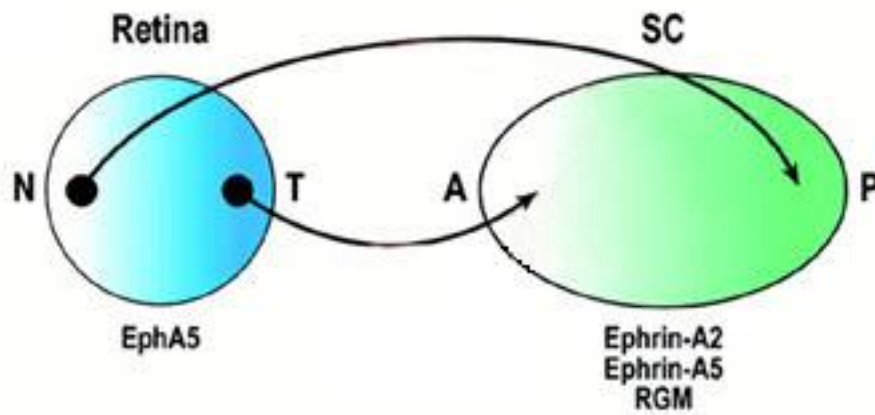
**Image 2:** The Olfactory System in *Drosophila*.



easier for experimentation<sup>6</sup>. In the olfactory system, odorants are exposed to ORs located on ORNs which are contained in sensilla found in the antenna and maxillary pulp<sup>6,7</sup>. The ORNs then synapse onto glomeruli located in the antennal lobe (AL) which then synapse onto projection neurons (PNs) which project their axons into the protocerebrum, and finally terminate into higher order brain centers<sup>6,7</sup>. There are 38 ORN classes and 62 ORs with the majority of ORs being expressed on an individual ORN and only 6 classes of ORNs expressing more than one type of receptor<sup>6</sup>. These ORNs that express the same OR project in the AL into a single glomerulus, with 49 glomeruli in total<sup>6</sup>. This concept of one OR represented on one ORN which projects into a single glomerulus in the AL brings about the one-receptor-one glomeruli theory<sup>6</sup> (Image 2). ORNs are located in four possible sensory sensilla known as basiconic, trichoid, coeloconic and intermediate, which typically house 2-4 ORNs<sup>6</sup>. Each sensilla type is represented on the antenna, but the maxillary pulp only contains the basiconic sensilla<sup>6</sup>. The ORNs in each sensilla extend a solitary axon to glomeruli in specific regions of the AL depending on the sensilla they originate from.<sup>6</sup> ORNs in the trichoid sensilla travel to the lateral anterior portion of the AL, antennal basiconic sensilla to the medial portion, maxillary palp basiconic sensilla to the central-medial portion, and antennal coeloconic sensilla to the posterior portion<sup>6</sup>. This sensilla arrangement represents a topographic organization. Even though ORNs are located next to each other in the same sensillum, and then extend to specific regions in the

AL, this neighboring arrangement is not maintained in the glomeruli<sup>6</sup>. Further investigation of the AL has shown spatial separation depending on odorant type, with glomeruli that react to aromatic odorants located at the ventral-central region and those that react to aliphatic odorants located in the medial region<sup>6</sup>. As with the lack of relationship seen regarding location of ORNs within sensilla versus their location in the glomeruli, clusters of glomeruli that respond to specific odorants are not spatially similar to the arrangement of the sensilla in the antenna<sup>6</sup>. This organization of the AL in the *Drosophila* is also seen in the mammalian olfactory bulb (OB) with specific chemical classes stimulating specific glomeruli<sup>6</sup>.

The development of sensory systems in the brain has been a long studied subject for molecular neurobiologists. Both visual and olfactory systems alike are comprised of neurons which send messages in an orderly manner to the higher order centers of the brain. The most studied and prominent model used to study neuronal projection and development as well as molecular cues is the visual system or retinotectal system. In the retinotectal system the visual world is transferred into a platform the brain can understand by taking images represented on the retina. These images from the retina are then transferred onto the optic tectum/superior colliculus (OT/SC) creating a topographic illustration of the environment<sup>8</sup>. Retinal ganglion cells (RGCs) in the retina exit the eye and travel past the optic nerve, the optic chiasm, and the optic tract finally ending in the midbrain where the



**Image 3:** Topographic map of the visual system in mammals. The development is dominated by the expression of RGM Ephrin-A and its receptor EphA.

OT/SC is located<sup>8</sup>. When transferring images from the retina onto the OT/SC, the temporal-nasal axis of the retina represents the anterior-posterior axis of the OT/SC and the ventral-dorsal axis of the retina represents the dorsal-ventral axis of the OT/SC<sup>8</sup>. The process of development is controlled by the interactions between repellent guidance molecule (RGM) Ephrin-A and its receptor EphA which are expressed in an increasing anterior to posterior fashion in OT/SC<sup>8</sup> (Image 3). Growth cones with a higher quantity of EphA receptors that originate from the temporal retina will terminate at anterior portions of the OT/SC where there are less Ephrin-A ligands<sup>8</sup>. On the other hand, growth cones that stem from the nasal retina, and have a lower density of EphA receptors, will end at more posterior regions in the OT/SC where there are more Ephrin-A ligands<sup>8</sup>. During development, some growth cones of the RGCs project past their appropriate targets in the termination zone (TZ), located in OT/SC, in the process of overshooting<sup>8</sup>. *In vitro* experiments have shown that if EphA is overexpressed or expressed ectopically, the accurate extension of RGCs growth cones is reduced<sup>8</sup>. Like EphA, EphB receptors are represented on RGCs and shape the arrangement of RGCs in the OT/SC<sup>8</sup>. EphB receptors and Ephrin-B ligands work in concert to establish the ventral-dorsal axis of the OT/SC<sup>8</sup>. Ephrin-B ligand is expressed in an increasing ventral-dorsal gradient in the OT/SC with its receptors EphB2 and EphB3 expressed in a high ventral to low dorsal pattern and its receptor EphB1 expressed in a uniform gradient across the RGC layer<sup>8</sup>. The process of

topographic branching follows the initial overshoot of growth cones and involves guidance molecules aiding EphA and Ephrin-A in directing axons to their correct destination in the OT/SC<sup>8</sup>. This refinement of the retinotectal map involving the elimination of overshooting RGC axons, the deletion of branches and ectopic arbors outside of the TZ, brings about the proper arrangement in the OT/SC.

Both the olfactory and visual systems take stimuli from the environment and translate them into a format the brain can understand. In the retinotectal system, a specific location in the retina precisely correlates spatially to its target position in the tectum forming a topographic relationship<sup>8</sup>. In the olfactory system, this spatial relationship is not maintained, and even though one OR is expressed per ORN, which projects to one glomerulus in the OB, these ORNs that are neighbors in the olfactory epithelium are not guaranteed to be neighbors in the OB<sup>8</sup>. The olfactory and retinotectal systems also differ in that RGC axons will overshoot their final destinations in the OT/SC and partake in branching and refinement to develop the final topographic map<sup>8</sup>. On the other hand, in the olfactory system, ORN axons expressing a certain OR will travel from the olfactory epithelium to a destined glomerulus and will not overshoot or participate in revision<sup>8</sup>. In the olfactory system, ORNs project depending on the type of OR that they express and have different affinities for varying guidance ligands dispersed in the OB where in retinotectal development, RGCs map depending

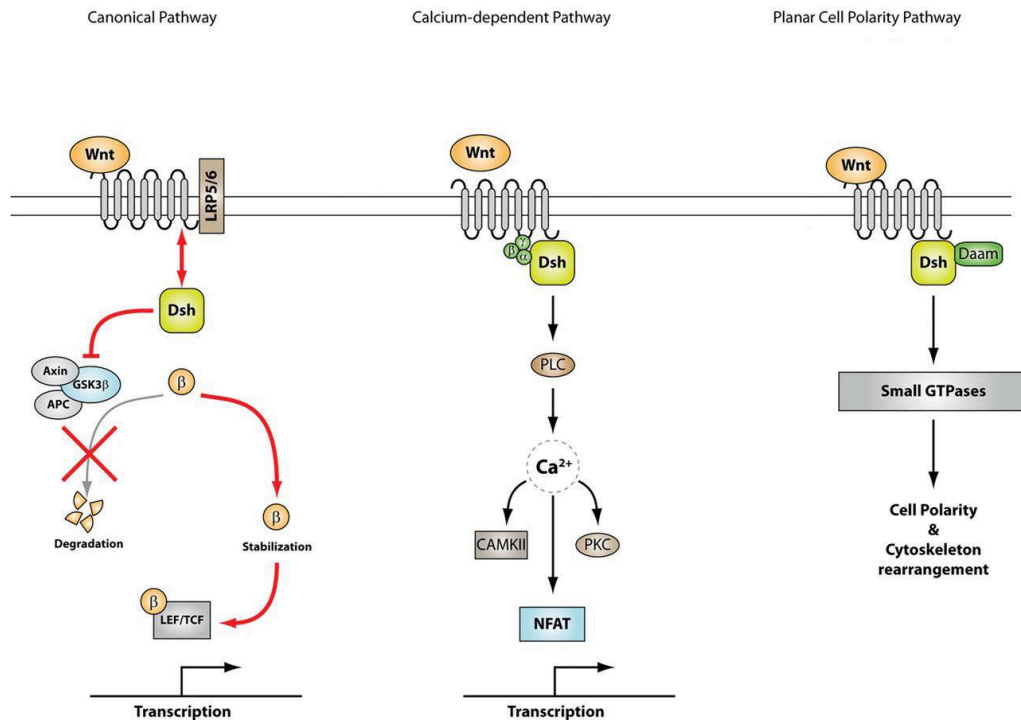
on the expression of the same EphA receptors on axons which effects how they respond to the Ephrin-A ligand gradient.

Despite the differences in the molecular development of the olfactory and visual systems, there are a few similarities. Both these systems are dominated by molecular guidance mechanisms in contrast to the activity-dependent mechanisms which are essential in the development of other axon projections in other systems<sup>8</sup>. Yet another similarity is that in both systems, the driving force are guidance cues which assist development along the anterior-posterior and dorsal-ventral axes, with EphA and Ephrin-A as well as EphB and Ephrin-B involved in the unfolding of the anterior-posterior and dorsal-ventral axes respectively<sup>8</sup>. In the olfactory system, ORs are involved in the establishment of the anterior-posterior and dorsal-ventral axes although much of the molecular basis of this development is still under investigation. All in all, both the olfactory and visual systems use guidance molecules for the development of essential neurobiological structures to help organisms decipher their surroundings.

### **Wnt Proteins:**

There are a considerable number of proteins that aid in the development of an organism. Wnts, a group of glycoproteins, are one such family of molecules which are essential in the development of the central nervous system. Wnt proteins play a huge role in the shaping and appropriate wiring of the nervous system including cell polarity, cell fate, embryonic

patterning, neuronal migration, axon guidance, axon outgrowth, and the arrangement of neural circuits<sup>9</sup>. Wnt proteins function as a ligand by binding to the seven transmembrane protein frizzled (Fz) receptor which activates disheveled (Dvl) down-stream thus initiating transduction<sup>10</sup>. The Wnt pathway may also function through the tyrosine kinase receptor Ryk which interacts with the Fz and Dvl pathway<sup>10</sup>. In total, there are three pathways involved in Wnt signaling which include: the canonical pathway or Wnt/ $\beta$ -catenin pathway, the PCP pathway and the Wnt/calcium ( $\text{Ca}^{2+}$ ) pathway<sup>10</sup> (Image 4). In the canonical pathway, the scaffolding protein Dvl is activated after the Wnt protein binds to its Fz receptor as well as the low density lipoprotein receptor-related protein 5/6 (LRP5/6)<sup>10</sup>. This is followed by the dissociation of the complex formed by axin, adenomatous polyposis coli (APC), glycogen synthase kinase 3 $\beta$  complex (GSK-3 $\beta$ ) and  $\beta$ -catenin which in-turn allows for the accumulation of  $\beta$ -catenin in the cell<sup>9,10</sup>. This build-up results in increased interaction of  $\beta$ -catenin with transcription factor (TCF) and lymphoid enhancing factor to stimulate transcription of genes involved in synaptogenesis and cell fate<sup>9,10</sup>. In the absence of Wnt protein,  $\beta$ -catenin is created, but quickly deteriorated by GSK-3 $\beta$ <sup>9</sup>. In regards to the PCP pathway, Wnt binds to Fz thus activating Dvl which produces an alteration in actin and microtubule activation which results in the activation of Rho-GTPases and c-Jun-N-terminal kinase (JNK) and finally induces cell and tissue polarity as well as dendritogenesis<sup>9,10</sup>. The last Wnt pathway is the  $\text{Ca}^{2+}$  pathway which



**Image 4:** The three pathways involved in Wnt signaling: The canonical pathway or WNT/ $\beta$ -catenin pathway, the WNT/ $Ca^{2+}$  pathway and the PCP pathway.



involves the binding of Wnt ligand to its receptor Fz and the activation of Dvl leading to the increase in intracellular  $\text{Ca}^{2+}$ <sup>9</sup>. The increase in  $\text{Ca}^{2+}$  stimulates protein kinase C (PKC) and  $\text{Ca}^{2+}$ /calmodulin-dependent kinase II (CaMKII) as well as the nuclear translocation of the transcription factor nuclear factor of active T-Cells (NFACT) which activates gene transcription of cell fate and cell movement<sup>9</sup>.

Wnt protein ligands are essential in the development of the anterior-posterior axis of an organism. During development of the spinal cord, axons migrate toward the ventral midline via two chemo-attractants netrin-1 and sonic hedgehog (Shh), as well as chemo-repellent bone morphogenetic protein (BMP)<sup>11</sup>. As soon as axons cross the midline, they no longer respond to these cues and respond to the chemo-repellents Slit and semaphorin instead thus initiating the anterior-posterior growth of axons<sup>11</sup>. In experiments with mouse embryos, it has been discovered that after the axons crossed the midline, Wnt7b, Wnt5a and Wnt4 were expressed in locations where axons started to travel anteriorly<sup>11</sup>. Of the Wnt ligands, Wnt4 was abundant in the floor plate and the ventricular zone representing a decreasing anterior-posterior gradient and Wnt7b was found on the lateral sides of the floor plate where axons that have just crossed the commissure take an anterior direction<sup>11</sup>. Not only were Wnt proteins expressed in these areas of the developing mouse embryos, but Fz genes Fz3, Fz8 and Fz9 were expressed in the spinal cord during the time of development when the axons take an

anterior route<sup>11</sup>. To prove the importance of the Wnt-Fz pathway during the development of the notochord, secreted frizzled related proteins (sFRPs), Wnt-Fz antagonists, were exposed to COS cells and were shown to be significantly impaired<sup>11</sup>. These abnormalities include: an inability of axons to switch to the anterior-posterior direction, axons that stalled, and axons that randomly rotated along the anterior-posterior axis<sup>11</sup>. The deformities in the molecular development of the Wnt-Fz antagonist exposed COS cells compared to the controls shows the importance of the Wnt-Fz pathway in the accurate development of nervous system.

Wnt also plays a repulsive role in the development of the spinal cord. In studies performed by Liu *et al.*, involving the vertebrate corticospinal tract (CST) axons during development, it was shown that these growing spinal cord axons are repelled by ligands Wnt1 and Wnt5a interacting with their tyrosine kinase receptor Ryk. When an anti-Ryk was injected into CST axons, these axons were interrupted just anterior to the injection sites<sup>12</sup>. This shows that the tyrosine kinase transmembrane protein Ryk, acts as a repellent of CST axons<sup>12</sup>. Another experiment in this study showed that secreted frizzle-related sequence protein 2 (sFRP2) blocked Wnt-Fz interactions but did not affect the binding of Wnt-Ryk proving that Fz is not necessary for the repulsive activities of Wnt and Ryk in the CST<sup>12</sup>. On the other hand, Ryk antibodies blocked Wnt-Ryk interactions but did not touch the binding of Wnt onto its receptor Fz<sup>12</sup>. This establishes that Wnt repulsion, specifically

through Wnt1 and Wnt5a via Ryk receptor, is involved in the posterior turning of CST axons in the spinal cord<sup>12</sup>. Liu *et al.* also discovered that Wnt5a, Wnt1, Wnt8a and Wnt9a are expressed in the grey matter surrounding the dorsal funiculus branching from the cervical to thoracic spinal cord with Wnt5a and Wnt1 expressed in greater amounts. These 4 Wnts were expressed in a high to low gradient along the anterior-posterior axis thus guiding CST axons inside the spinal cord<sup>12</sup>.

Axon growth and branching is guided by the involvement of Wnt ligands. In studies performed by Krylova *et al.* on mouse spinal cords, Wnt3 was shown to contribute in the ventral projection of neurotrophin-3 (NT-3) muscle afferents, their branching cessation and their eventual synapse with target motor neurons. Wnt3 was shown to affect the same NT-3 axon in two different ways: by aiding in the growth and branching of the axon as well as inhibiting axon extension<sup>13</sup>. Neurons that were isolated with NT-3 and exposed to Wnt3 showed an increase in growth cone size as compared to those that were not exposed to Wnt3<sup>13</sup>. However, when Wnt3 was exposed to NT-3 axons, there was a 25% reduction in axon length compared to the control<sup>13</sup>. In these experiments, Wnt antagonist sFRP1 was used to verify Wnts involvement in axon remodeling. sFRP1 reversed the results of Wnt3 by reducing the average growth cone size as well as inhibiting the production of secondary and tertiary branching<sup>13</sup>. These experiments showed that Wnt3 delegates the discontinuation of arborization in muscle afferents<sup>13</sup>.

Experiments were also performed to show the effects that Wnt3 had on protein involvement in synapse formation, specifically synapsin I. Dorsal root ganglia (DRG) neurons were cultured and subsequently exposed to Wnt3<sup>13</sup>. In control neurons, synapsin I was confined in small clusters across the axon with a small number of large clusters<sup>13</sup>. When sensory axons were exposed to Wnt3, there were large numbers of synapsin I clusters along the axon implying Wnt3 contributes to presynaptic discrimination<sup>13</sup>.

Not only has research with Wnt ligands unveiled the extent that these proteins have on development, branching and migration of axons, but these ligands are also responsible for the formation of synapses. Wnt7a has been found to participate in the synaptogenesis of cerebellar mossy fiber granule cell neurons (GCs). To visualize the influence that Wnt7a has on synapsin I regarding mossy fibers, Hall *et al.* stained glomerular rosettes of the cerebellum with synapsin I. After staining, the wildtype mice showed rosettes of various sizes, but staining in Wnt7a mutants were smaller overall<sup>14</sup>. This smaller size stain trend was visualized in mutants in earlier postnatal glomerular rosette showing that mutant mice have poor glomerular rosette development compared to non-mutants<sup>14</sup>. This may suggest a decrease in the collection of synapsin I or a delay in the accumulation of synapsin I of neurons that lack Wnt7a<sup>14</sup>. When the Wnt antagonist sFRP1 was exposed to axons, axon remodeling of GCs were interrupted and growth cone size decreased<sup>14</sup>. Further evidence shows that mossy axons that are exposed to

Wnt7a have an increased axonal area of 36% as opposed to axons not exposed to this ligand<sup>14</sup>. Wnt7a also promoted thickness and spreading of the axon shaft with a 30% increase in axonal area and growth cone size and an overall increase in growth cone complexity<sup>14</sup>.

In the olfactory system, Wnt5 plays a crucial role in the proper development and migration of neurons in ALs of *Drosophila*. When Wnt5 is overexpressed, the AL becomes large in size and forms abnormally with ectopic structures<sup>15</sup>. Structures that resembled glomeruli appear at the commissure enlarging the commissure at the midline<sup>15</sup>. In mutant Wnt5 flies, these organisms showed phenotypes such as the absence of ALs connected by commissures and glomeruli that were closer in distance compared to wildtype flies which resulted in lobes that were misshapen as well as shrunken near the dorsomedial aspect of the lobe<sup>15</sup>. These structures represented a “heart shaped” figure instead of the round structure in controls<sup>15</sup>. Another characteristic of Wnt5 mutants is that ORN axons of these mutant flies took unusual paths toward their final destination in the brain and many times would not end in the correct position<sup>15</sup>. When mutants were rescued, the ALs developed a round shape instead of the abnormal “heart-shape,” had more ALs that were connected by a commissure (55% instead of 30% in mutants), and glomeruli were located farther from the dorsal edge of the ALs as seen in controls<sup>15</sup>. Wnt5 is also necessary for the proper development of the AL and PNs. During development, PN dendrites

experience rotation in the AL before they resolve in their appropriate locations<sup>16</sup>. Many neurons in the AL express elevated levels of Wnt5 showing that Wnt5 is essential for olfactory organization<sup>16</sup>. Wnt5 protein is expressed in AL neuropils from a dorsolateral-high to a ventromedial-low arrangement with its known receptor Drl/Ryk also expressed in a dorsolateral to ventromedial pattern in the PNs<sup>16</sup>. When Wnt5 is overexpressed, there is a malformation of dendritic arrangement with a loss of Wnt5 resulting in dendrites inability to migrate ventrally<sup>16</sup>. In addition, loss of Drl results in atypical ventromedial destination of dendrites with an overexpression of this protein producing dorsolateral termination of dendrites<sup>16</sup>. Thus, Wnt5 guides PN dendrites to their correct position in the AL by antagonistic measures and Drl inhibits Wnt5 communication allowing these dendrites to end at their destined glomerulus<sup>16</sup>. Without correct Wnt5 expression during development, abnormalities in the olfactory system will inevitably arise.

Mutations and abnormalities in Wnt signaling and Wnt signaling proteins have resulted in many disorders and diseases. One such disorder is AD, a debilitating neurodegenerative disorder marked by declining cognitive activity and defective synapses. AD is characterized by senile plaques formed by extracellular deposits of amyloid- $\beta$  ( $A\beta$ ) peptides and neurofibrillary tangles (NFTs) which are formed by intracellular aggregates of hyper-phosphorylated tau protein<sup>10</sup>. Alteration in Wnt signaling has been shown to play a significant role in AD. In AD individuals,  $\beta$ -catenin levels are diminished and Dkk1, a Wnt

antagonist, is inflated in postmortem brains of humans infected with AD and in AD brains of transgenic mice<sup>10</sup>. A $\beta$  binding to the extracellular cysteine-rich domain (CRD) Fz5 receptor near or at the Wnt binding location prevents the canonical Wnt pathway<sup>10</sup>. It has also been shown that when cultured rat hippocampus neurons were presented with A $\beta$ , destabilization of  $\beta$ -catenin levels, increase in GSK-3 $\beta$  activity and reduced expression of Wnt target genes occur, all of which are involved in the overall suppression of the Wnt signaling pathway<sup>10</sup>. A $\beta$  exposure can also result in the activated expression of Dkk1 which in-turn increases Dkk1 thus allowing it to bind to LRP5/6 inhibiting its ability to interact with Wnt proteins<sup>10</sup>. This results in the inability of Wnt to inhibit GSK-3 $\beta$  promoting tau hyper-phosphorylation and NFT formation ending in the apoptosis and neurotoxicity of A $\beta$  peptides to dominate and infect cells<sup>10</sup>. It has also been shown that A $\beta$  increased the protein clusterin which encourages the expression of Dkk1<sup>10</sup>. The C-terminal of Dkk1 limits the binding of Wnt to the LRP5/6 and thus actuates the gene transcription involved in features characterized by AD<sup>10</sup>.

It has been suggested that schizophrenia, yet another debilitating neurological disorder, may be linked to over expression of Wnt1 and may result in the transformed cell adhesion, synaptic reorganization and altered plasticity in individuals with this disorder<sup>17</sup>. Specifically, it has been shown that single-nucleotide polymorphisms (SNPs) in Fz3 are associated with the

likelihood of developing schizophrenia<sup>17</sup>. It has also been suggested that GSK-3 $\beta$  activity differs in those with schizophrenia<sup>17</sup>.

Abnormalities in the Wnt pathway not only contribute to neurological deficits, but are also linked to other systems of an organism. For example, deformities in the Wnt pathways are linked to cardiovascular disease. It has been found that after a heart attack was induced in Dvl1/Dvl1-null mice, those in the experimental group were approximately two-thirds more likely to develop a ruptured infarct compared to the control group<sup>17</sup>. In addition, when sFRP1 was expressed in high quantities in mice with induced heart attacks, infarct size was decreased and cardiac operation was improved<sup>17</sup>. Mutation in the Wnt receptor Fz4 and co-receptor LRP5 are responsible for many incidents of familial exudative vitreoretinopathy (FEVR), a retinal vascular disorder. In this disorder, the peripheral capillaries do not mature thus ending in leakage, bleeding, scarring, compensatory neovascularization and eventually blindness<sup>17</sup>. Abnormalities in bone density have also been related to the Wnt pathway. There is a link between gain-of-function in the extracellular portion of LRP5, a Wnt co-receptor, resulting in increased bone density of mice and humans<sup>17</sup>. Just as mutations in LRP5 result in increased bone density, loss-of-function mutations of LRP5 in humans as well as mice end in decreased bone mass which can be seen in those with osteoporosis-pseudoglioma syndrome (OPPG)<sup>17</sup>. Wnt5a and Wnt7b deficient mice indicate the importance of these ligands in the proper development of the lungs. When



$\beta$ -catenin is knocked out in mice, there is a deformation of peripheral airways and gas exchange in the lungs resembling the crucial role that the  $\beta$ -catenin pathway plays in the migration as well discrimination of lung cells<sup>17</sup>.

Specifically, mice that have insufficient levels of Wnt5a and Wnt7b exhibit idiopathic pulmonary fibrosis<sup>17</sup>. Wnt/ $\beta$ -catenin pathway is fundamental in the proper development of skin and the fate of stem cells aiding in their determination to restore themselves, as well as their ability to differentiate<sup>17</sup>.

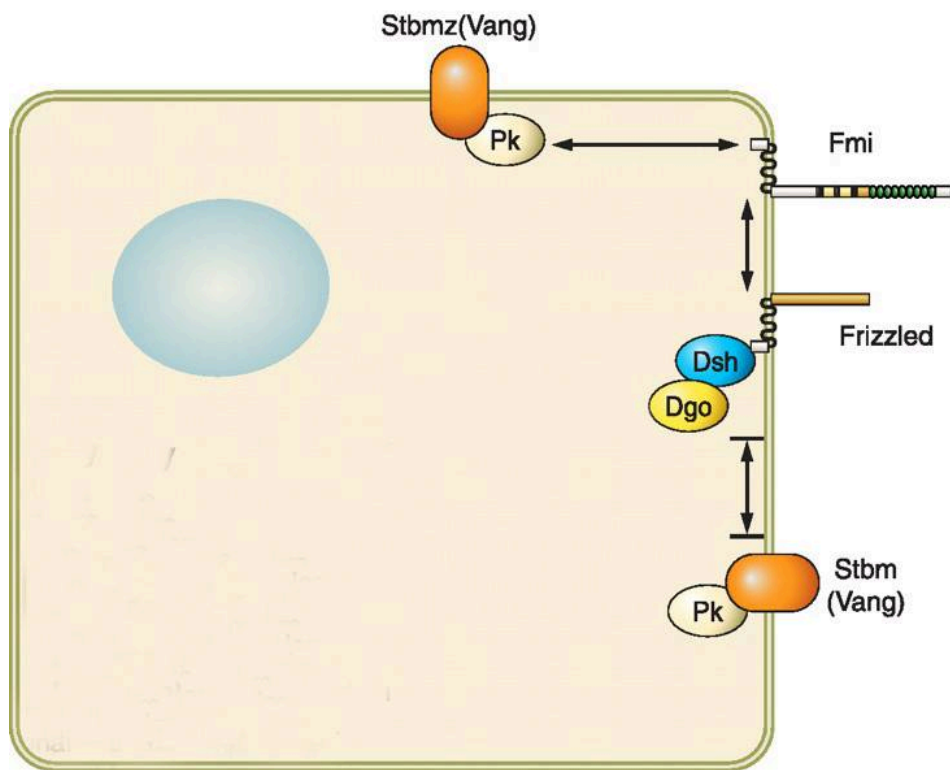
There is an association between mutations in the  $\beta$ -catenin pathway and skin tumors known as pilomatricomas<sup>17</sup>. During normal fibroblast recruitment after a skin wound, these cells travel to the skin and form a collagen complex. It is suggested that Wnt/ $\beta$ -catenin pathway abnormalities are identified with excessive fibromatosis resulting in the uncontrolled process of wound healing<sup>17</sup>.

### **Planar Cell Polarity Pathway:**

Planar polarity can be described as the persistent orientation of cells or structures in a tissue<sup>18</sup>. Even though polarity is largely studied at the cellular level, specifically pertaining to PCP, polarity can also refer to subcellular levels, such as the organization of many cilia on a cell or in tissues as a whole, such as fly wing hairs or mouse limb hairs<sup>19</sup>. Whether at the cellular level or a broader tissue level, planar polarity consists of cells interacting with each other in coordination with neighboring cells and long-range arrangements to orient themselves with axes of the tissue<sup>19</sup>. There are two

classifications which regulate the orientation of nearby cells: the PCP pathway and the Fat/Dachsous (Ft/Ds) pathway. The PCP pathway in *Drosophila* consists of six core proteins that govern the cell-cell network which include: the seven pass transmembrane protein Fz, cytosolic proteins Dvl, Diego (Dgo) and Prickle (Pk), the four-pass transmembrane protein Strabismus (Stbm) (also known as Van Gogh {Vang}), and the seven-pass transmembrane cadherin Flamingo (Fmi) (also known as Starry Night {Stan})<sup>19</sup> (Image 5). These proteins are located asymmetrically and within cells. They are localized asymmetrically in the cell in that Fmi is positioned distally and proximally, Vang and Pk concentrated distally, and proteins Fz, Dgo and Dvl placed proximally in the cell<sup>19</sup>. By establishing an asymmetrical pattern, these PCP proteins are allowed to adequately interact with each other and create proper positioning in a cell<sup>19,20</sup>. The asymmetry amongst the proteins is thought to be a consequence of intracellular communication between proximal and distal elements and heterophilic interactions<sup>19</sup>. These PCP proteins are encoded by PCP genes which are conserved in both invertebrates as well as mammals. These genes have orthologues in vertebrates, the most common of which are FZD3, FZD6, CELSR1, CELSR2, CELSR3, VANGL1, VANGL2, DVL1, DVL2, DVL3, PRICKLE1, PRICKLE2, PRICKLE3, PRICKLE4 and ANKRD6<sup>18</sup>.

In the Ft/Ds system, Ft and Ds function by encoding cadherins that bind to one another at the exterior of the cell which is controlled by the



**Image 5:** PCP proteins in *Drosophila*.

phosphorylation activity of Golgi protein four-jointed (Fj)<sup>19</sup>. Phosphorylation of Ft by Fj increases its binding attraction for Ds while the phosphorylation of Ds decreases its binding attraction toward Ft<sup>19</sup>. Cells without Ft, Ds or Fj display defects in polarity and which is notably seen in *Drosophila* wing cells.

Effectors, elements that work downstream in the Ft/Ds pathway, carry out the processes that result in cell polarity<sup>19</sup>. These factors are precise in regards to cell and tissue type and therefore represent the varied assortment of potential effects in an organism<sup>19</sup>. Such planar polarity effectors in the *Drosophila* wing are Fuzzy (Fy), Inturned (In) and Fritz (Frtz) all of which affect the positional outcome of trichome and bristles on the wing<sup>19</sup>. The GTPase RhoA and its effector *Drosophila* Rho-kinase (Drok) are other proteins involved in the Ft/Ds pathway, specifically involved in actin development<sup>19</sup>. RhoA is notably important in that loss of this protein results in the prevention of cell division in the *Drosophila* wing<sup>19</sup>.

In the PCP pathway, the polarization of hair in cells are dominated by bridges between PCP proteins, specifically transmembrane protein Stan acting with other transmembrane proteins such as Fz and Vang<sup>21</sup>. In *Drosophila*, epithelial cells are polarized by a gradient of Fz and Stan proteins thus producing bridges between these cells<sup>21</sup>. By doing so, these neighboring cells have the ability to read as well as compare levels of Fz between one another allowing cells to point in the same direction<sup>21</sup>. The proper polarization in the Stan system occurs when Stan in one cell makes a bridge with Stan

interacting with Fz in a neighboring cell assisted by the protein Vang<sup>21</sup>.

Although Stan has the capacity to produce bridges between cells without Vang, Vang aids in the bridge making between Stan and Fz and helps Stan accept approaching Stan-Fz signals thus increasing the effectiveness of these protein bridges<sup>21</sup>.

In the wing of *Drosophila*, each cell contains a hair that distends from the distal tier of the cell which will present a deformity in cell polarity when there is a mutation in PCP genes, showing that these genes are fundamental in the construction of epithelial hairs<sup>18</sup>. The PCP pathway is essential for the accurate development of cilia in most vertebrate cells and is necessary for the synchronized movement of cilia lining the airway, reproductive system, and cerebral ventricles<sup>18</sup>. In studies involving mice with a mutation in *Celsr2* and *Celsr3*, cilia are randomly distributed and are unable to produce the natural flow of cerebral spinal fluid<sup>18</sup>. Flaws in cilia development increase the likelihood of developing hydrocephalus and individuals with cilia related pathologies, such as Kartagener and Bardet-Biedl syndromes, are more likely to have hydrocephalus<sup>18</sup>. In a mouse lacking *Vangl1* and *Vangl2*, evidence has shown that there is an abnormal orientation of tracheal cells<sup>18</sup>. Mutations in PCP genes have also been connected to defects in the cilia of the nephron and collecting duct resulting in cystic renal disease<sup>22</sup>.

The PCP pathway is also necessary for neural tube closure, as development of the neural plate into the neural tube during neurulation

involves adjustments and migration of cells. Craniorachischisis, a disease of the neural tube, results from the inactivation of Vangl2, Dvk1, Dvk2, Celsr1, Fzd3 and Fzd6 in mice as well as VANGL2 and CELSR1 in humans<sup>18</sup>. This severe neural tube closure defect is linked to alterations in VANGL1, VANGL2, FZD6, PRICKLE1 and CELSR1 genes in humans<sup>18</sup>.

PCP has also been found to be associated with the migration of neurons as well as dendritic and axon guidance. The journey of facial branchiomotor (FBM) neurons depends on PCP proteins. In the brainstem of mice, FBM neurons begin in the medial rhombomere 4 (r4) followed by the protrusion of their growth cones in the direction of the facial muscles, which then migrate caudally and finally travel laterally and dorsally in rhombomere 6 (r6) forming the subpial facial nucleus<sup>18</sup>. This process is involved with the polarity pathway, and was first detected from research in zebrafish indicating that Vangl2, Fzd3a, Prickle1a and Prickle1b are responsible for the movement of FBM neurons in both cell autonomous and non-cell autonomous aspects<sup>18</sup>. As with mutant zebrafish, the movement of FBM neurons in knockout Celsr2 mice were abnormal with neurons migrating dorsolaterally in r4 and rhombomere 5 (r5) instead of in r6<sup>18</sup>. In transplantation research, neurons with mutation in Vangl2, Fzd3 and Celsr2 did not migrate appropriately compared to non-mutants<sup>18</sup>. In regards to dendritic migration, as seen in brain slices of mammals, the reduction of Celsr2 produces shorter dendrites in cortical pyramid neurons, and a decrease in arborization in

Purkinje cells, whereas the silencing of *Celsr3* results in the overabundance of dendrites<sup>18</sup>. In *Caenorhabditis elegans*, the PCP gene *Fmi1* dominates migration of axons in the ventral nerve cord and works with the Wnt pathway to determine the expansion of anterior-posterior axons of the ventral D-type GABAergic neurons<sup>18</sup>.

As with craniorachischisis, there are many other debilitating and deadly outcomes of dysfunction in the PCP genes. Other disorders involved in mutations of PCP genes are spina bifida, palate cleft defects, heterotaxia and cystic kidney disease<sup>20</sup>. Abnormal levels of PCP proteins, alterations and inappropriate location of these proteins have also been linked to epithelial tumors as well as non-epithelial neoplasia such as Chronic Lymphocytic Leukemia and Acute Myeloid Leukemia<sup>20</sup>.

#### **Question of Interest:**

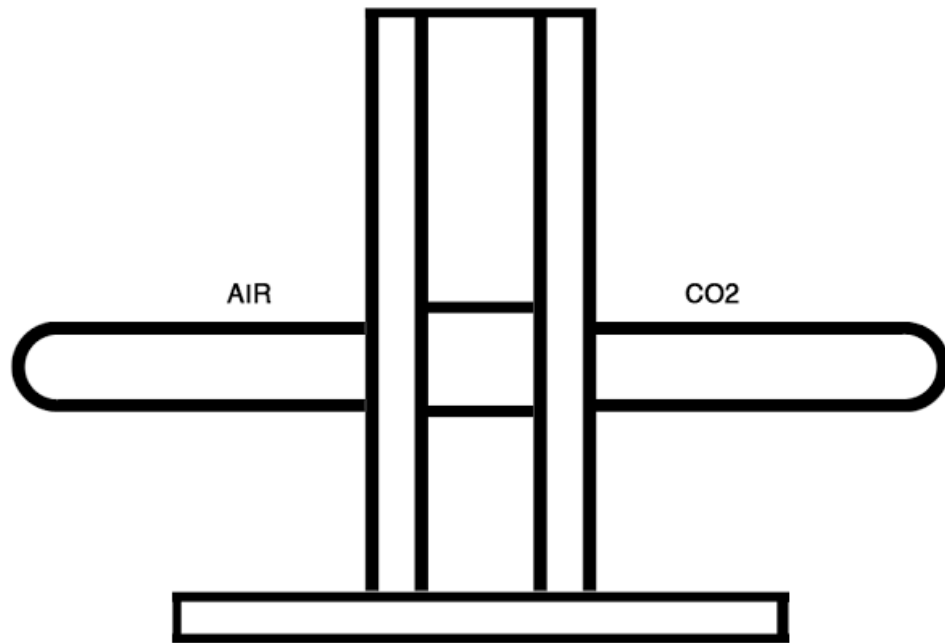
As evident from previous research, Wnt protein is fundamental in the development of the olfactory system. From recent investigation in Dr. Huey Hing's lab, it has been shown that Vang protein also plays a role in the olfactory system. *Drosophila* with *Vang6* mutant gene show similar phenotypic abnormalities in the olfactory system as *Wnt5* mutant *Drosophila*. Along with this atypical molecular and anatomical component, there is also a behavioral irregularity seen in *Drosophila*. Previous research from a collaborating lab at the University of California at Riverside (UCR) has shown that *Wnt5* mutants had no aversion to CO<sub>2</sub>, a repellent odor, and chose CO<sub>2</sub>

and air at equal rates. These flies have a defective response to the CO<sub>2</sub> and are unable to detect a difference between the repulsive CO<sub>2</sub> versus the favorable air as were their wildtype counterparts. Wildtype flies have no abnormality in their olfactory system and therefore are able to detect and will choose air over CO<sub>2</sub>. This brings about the question that if Wnt5 mutant flies and Vang6 mutant flies show similar molecular and anatomical characteristics there is a possibility that their behaviors may also be similar. **It is my goal to investigate if flies with a mutation in the Vang6 gene show a similar disposition as do those with a Wnt5 mutation. Using similar tools and methods, I hope to show the behavioral characteristic represented by Vang6 mutant flies and verify the normal behavior of wildtype flies. I will investigate whether Vang6 mutant *Drosophila*, the experimental group, are able to discriminate between air and CO<sub>2</sub>, and verify that wildtype WT1118, are able to show a distinct behavioral tendency to prefer air.**



## **Chapter 2: Methods**

Two strains of *Drosophila* were used during the behavioral assay: Vang6 mutant and wildtype WT1118. Both strains were grown in stocks in order to obtain a sufficient number of subjects to perform trials. Once *Drosophila* fully matured, they could be separated in order to execute trials. *Drosophila* were sorted using a plastic makeshift tube apparatus, after briefly being knocked out with CO<sub>2</sub>, and were individually separated into female and male groups. 50 *Drosophila* were selected, 25 female and 25 male, and were placed into behavioral vials with only one-half of a Kim-wipe and 3mls distilled water for a drinking source. Behavioral vials had 50 flies each for both Vang6 and WT1118 strains. Subjects were then starved for 24 hours and kept in a 25° C incubator. After 24 hours, approximately 3-4 hours before sunset when *Drosophila* are most active, the animals were ready for experimentation. All light sources were decreased, including light switches turned off and window blinds closed, in order to decrease this extraneous variable. Fifteen ml culture tubes were labeled individually with air or CO<sub>2</sub>. The tubes were covered around the rim with Parafilm in order to keep a tight seal while tubes were in the T-maze apparatus (Image 6). *Drosophila* were transferred from behavioral vials to a separate 15ml culture tube and the tube was quickly placed into the entry hole of the T-maze. Flies were transferred into the first chamber and the elevator was slid halfway in order to keep subjects contained in the apparatus. The air tube was also placed into position and the



**Image 6:** T-Maze Model. Flies are placed in the middle chamber and provided with an Air or CO<sub>2</sub> tube option.

CO<sub>2</sub> tube was covered with a foam cap. The maze was set standing vertically under a box to decrease light contact. During this time, CO<sub>2</sub> for the test component tube was collected. CO<sub>2</sub> was collected using a 1ml syringe. A small piece of plastic tubing was placed on the end of the syringe to exclusively collect CO<sub>2</sub> into the syringe. CO<sub>2</sub> would be collected from the CO<sub>2</sub> dispenser after 30 seconds to decrease the amount of air being collected. The syringe was filled slowly with 1ml of CO<sub>2</sub>, flipped 180 degrees, and the syringe was emptied 0.5mls. This was performed in order to once again reduce air in the syringe. The syringe was then flipped 180 degrees once more with the end of the syringe facing down and placed into the CO<sub>2</sub> tube. 0.05mls was placed into the tube resulting in a total of 0.34% of the tube containing CO<sub>2</sub>. The foam cap was moved and immediately placed into the T-maze, followed by immediate lowering of the elevator into position so the *Drosophila* chamber was exposed to both air and CO<sub>2</sub> tubes. Both the air and CO<sub>2</sub> tubes were placed at a horizontal level. At this time the box was placed over the T-maze for 1 minute. After 1 minute, the box was lifted and the T-maze elevator was raised to the half way position. *Drosophila* from both air and CO<sub>2</sub> culture tubes were placed into separate vials using the funnel method. This was also done for the flies still inside the T-maze that did not make a choice, the undecided group. The 3 samples, air, CO<sub>2</sub> and undecided, were then knocked out with CO<sub>2</sub> and counted. Male and female subjects were recorded for all three samples. Flies were then sacrificed, thus ending the

procedure. This method was used for both Vang6 mutant flies and WT1118 flies. Trials were performed with the air tube alternating on the right or left side as to eliminate the direction of the tube as an extraneous factor. A two-tailed t-test was performed using the data.

### **Chapter 3: Discussion & Results**

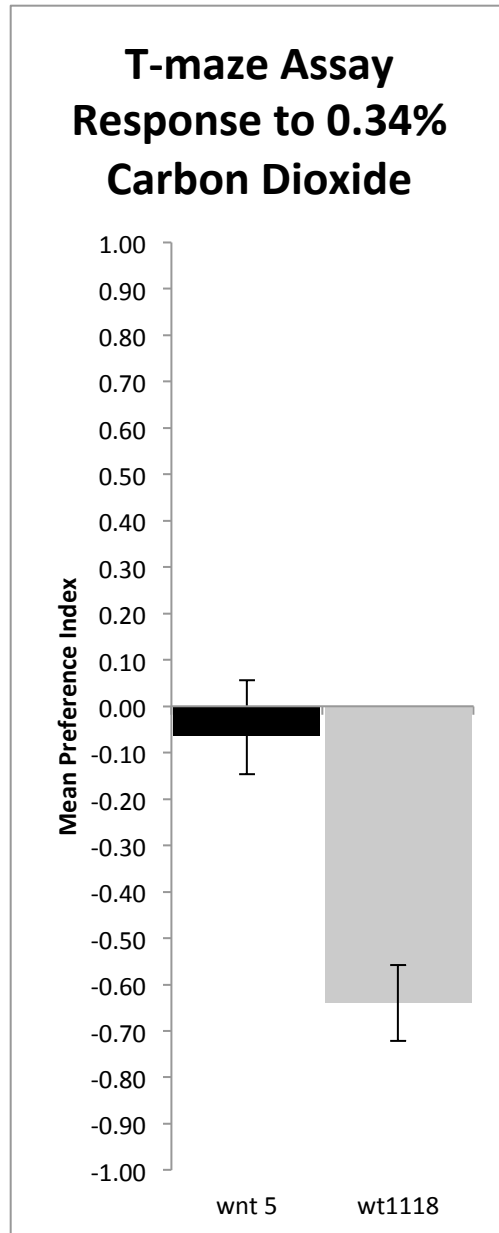
After performing a two-tailed t-test, and comparing these values to the p-value of 0.05, it is quite clear that there was no significant difference between WT1118 flies and mutant Vang6 flies and the tube they selected. The t-test value is 0.092 in females and 0.147 in males meaning that Vang6 flies were just as likely to pick the air or CO<sub>2</sub> tube as the WT1118 flies. The standard error of measurement (SEM) value in WT1118 males is 0.08 and 0.14 in females and the SEM value for Vang6 mutant males is 0.136 and 0.129 in females. Even though wildtype female flies were more likely to choose air over CO<sub>2</sub>, with females 48% likely to choose air and males 35% likely, the values are still not significant (Table 1 and Table 2). This is not what was expected after seeing the anatomical and molecular similarities that Wnt5 mutants had with Vang6 mutants in Dr. Hing's lab. The data from UCR clearly showed a significant tendency of WT1118 flies choosing air over CO<sub>2</sub> with a two-tailed t-test value of 0.0008 (Figure 1). The wildtype flies do not have abnormal olfactory development and are able to correctly detect the control air odor over the repulsive odor of CO<sub>2</sub>. Wnt5 ligand contributes a great deal to the development of the olfactory system. This is quite evident in the UCR data because the Wnt5 mutant flies equally did not choose air or CO<sub>2</sub>. It would only make sense that due to the similar abnormalities seen between Vang6 mutants and Wnt5 mutants and the important role these

Females	genotype	#	test compound	# control	# test compound	Not Part	Control Compound L/R	PI
	wt1118	1	0.05 CO2	22	1	0	L	-0.91
	wt1118	2	0.05 CO2	16	3	1	R	-0.68
	wt1118	3	0.05 CO2	17	6	0	L	-0.48
	wt1118	4	0.05 CO2	8	8	1	L	0.00
	wt1118	5	0.05 CO2	8	8	5	R	0.00
	wt1118	6	0.05 CO2	19	0	5	L	-1.00
	wt1118	7	0.05 CO2	23	2	0	R	-0.84
	wt1118	8	0.05 CO2	9	9	6	L	0.00
	wt1118	9	0.05 CO2	14	6	2	R	-0.40
	vang6	10	0.05 CO2	6	8	9	R	0.14
	vang6	11	0.05 CO2	7	4	13	L	-0.27
	vang6	12	0.05 CO2	3	13	5	R	0.63
	vang6	13	0.05 CO2	10	7	7	L	-0.18
	vang6	14	0.05 CO2	9	4	11	R	-0.38
	vang6	15	0.05 CO2	12	7	5	L	-0.26
	vang6	16	0.05 CO2	13	4	6	R	-0.53
	vang6	17	0.05 CO2	7	4	11	R	-0.27

**Table 1:** Data from behavioral assay: Female *Drosophila*. 25 flies per trial. Preference index (PI) calculated as follows: (Number of flies in test component - number of flies in control component)/(Number of flies in test component + number of flies in control component). Average value of PI for WT1118 is -0.48. Average value of PI for Vang6 mutants is -0.14.

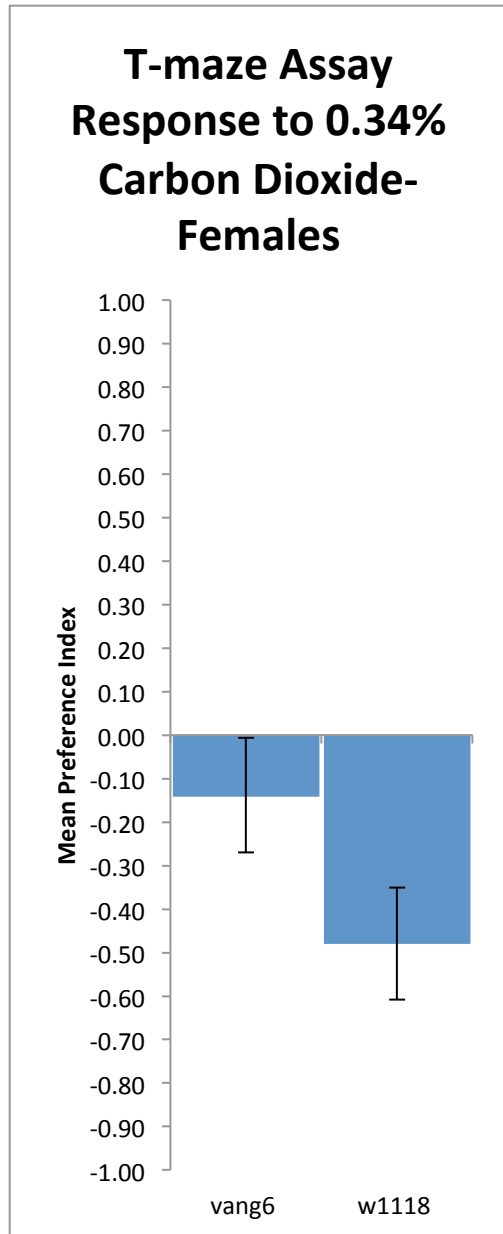
Males	genotype	#	test compound	# control	# test compound	Not Part	Control Compound L/R	PI
	wt1118	1	0.05 CO2	19	3	2	L	-0.73
	wt1118	2	0.05 CO2	15	8	4	R	-0.30
	wt1118	3	0.05 CO2	12	6	4	L	-0.33
	wt1118	4	0.05 CO2	7	8	4	L	0.07
	wt1118	5	0.05 CO2	12	7	5	R	-0.26
	wt1118	6	0.05 CO2	18	5	3	L	-0.57
	wt1118	7	0.05 CO2	12	6	3	R	-0.33
	wt1118	8	0.05 CO2	12	4	5	L	-0.50
	wt1118	9	0.05 CO2	8	5	4	R	-0.23
	vang6	10	0.05 CO2	4	10	8	R	0.43
	vang6	11	0.05 CO2	9	8	7	L	-0.06
	vang6	12	0.05 CO2	10	6	5	R	-0.25
	vang6	13	0.05 CO2	6	1	11	L	-0.71
	vang6	14	0.05 CO2	9	8	1	R	-0.06
	vang6	15	0.05 CO2	7	9	4	L	0.13
	vang6	16	0.05 CO2	12	3	8	R	-0.60
	vang6	17	0.05 CO2	3	4	14	R	0.14

**Table 2:** Data from behavioral assay: Male *Drosophila*. 25 flies per trial. PI calculated as follows: (Number of flies in test component - number of flies in control component)/(Number of flies in test component + number of flies in control component). Average value of PI for WT1118 is -0.35. Average value of PI for Vang6 mutants is -0.12.

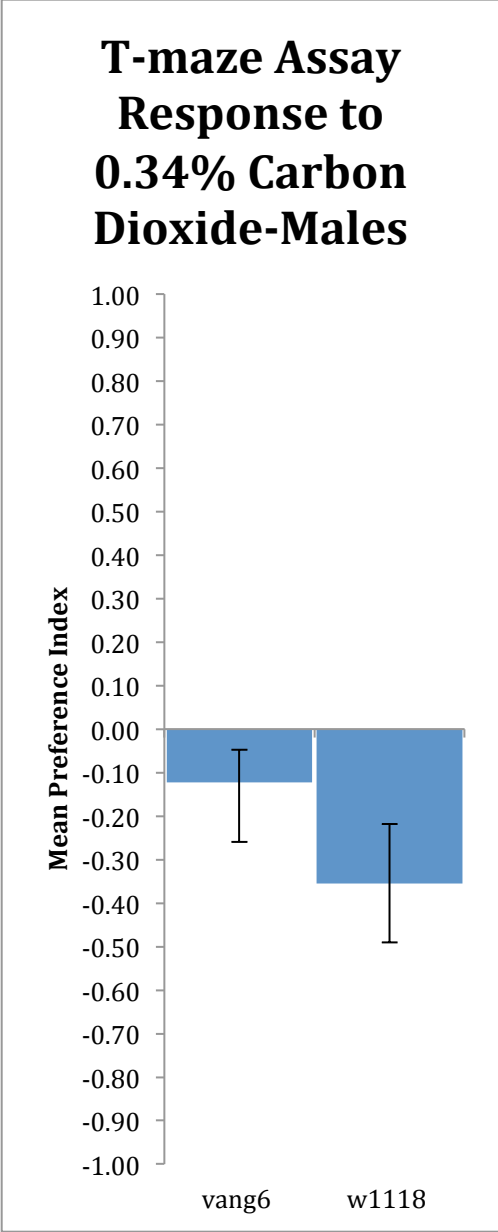


**Figure 1:** Data from UCR using Wnt5 mutant flies and WT1118 flies. A two-tailed t-test value of 0.0008 was calculated. Error bars=SEM. SEM for WT1118 flies is 0.08 and 0.12 in Wnt5 mutant flies.





**Figure 2:** Vang6 mutant and WT1118 fly mean PI for females flies. A two-tailed t-test value of 0.092 was calculated. Error bars = SEM. SEM for WT1118 is 0.14 and 0.129 in Vang6 mutant flies.



**Figure 3:** Vang6 mutant and WT1118 mean PI for male flies. A two-tailed t-test value of 0.147 was calculated. Error bars=SEM. SEM for WT1118 flies is 0.08 and 0.136 in Vang6 mutants.

proteins play in the olfactory system, that the Vang6 mutant flies would also show a significant tendency to have no preference. This is not the case in my data. Even though there was not a significant preference of the control component over the test component, the wildtype flies did select the air more often than the CO<sub>2</sub> which can be seen in both Figure 2 and Figure 3.

Despite the phenotypical similarities between Vang6 and Wnt5 mutants, Vang6 may not duplicate Wnt5 in behavioral aspects. Instead, the Vang6 mutant flies may still be able to detect CO<sub>2</sub> regardless of their anatomical malfunctions. This could be the case because mutant flies did choose CO<sub>2</sub> 12% of the time in males and 14% in females. I do not think this to be so considering the evidence also shows that WT1118 flies did not significantly choose the air over the CO<sub>2</sub> as they should. This could suggest that instead, the apparatus may not be ideal for testing the behavior aspects of flies with use of CO<sub>2</sub>. It appears more likely that there is an issue with the apparatus and the method of the experiment. The method of obtaining CO<sub>2</sub> could have resulted in an inaccurate amount, not the proper value of 0.34%. There could have been more air in the test component tube, despite the precautions and efforts to prevent air from leaking into the CO<sub>2</sub> tube. This could result in the flies choosing air and CO<sub>2</sub> equally regardless of being a mutant or wildtype. It is also probable that CO<sub>2</sub> is not an ideal odor to use with the T-maze apparatus. Wildtype *Drosophila* did not significantly choose air over CO<sub>2</sub> as would be expected. Flies respond to many odors and in this

experiment, only one odor was used which greatly limits the investigation and the phenotypic outcome. For further experimentation, a wider variety of odors could be used with the possibility of using attractive odors as well as repulsive odors. Trials using the neutral odor of air versus an attractive odor or attractive versus a repulsive odor are all viable options. With further research, the behavioral characteristics of Vang6 mutant flies will be thoroughly known. Within the last several years, there has been significant breakthrough in understanding the olfactory system. With the use of *Drosophila*, the molecular and anatomical features of this system have been uncovered. Additional exploration will help further the understanding of Wnt5 and Vang6, their relationship, and the importance they both have in the development of the olfactory system.

## References

- <sup>1</sup>Parvathi, Deepa V., Akshaya S. Amritha, and Solomon FD. Paul. "WONDER ANIMAL MODEL FOR GENETIC STUDIES - *Drosophila Melanogaster* –ITS LIFE CYCLE AND BREEDING METHODS – A REVIEW." *Sri Ramachandra Journal of Medicine* 2.2 (2009): 33-38. Web. 8 Sept. 2015.
- <sup>2</sup>Hirth, Frank. "Drosophila Melanogaster in the Study of Human Neurodegeneration." *CNS & Neurological Disorders - Drug Targets* CNSNDDT 9.4 (2010): 504-23. Web. 8 Sept. 2015.
- <sup>3</sup>Pandey, U. B., and C. D. Nichols. "Human Disease Models in *Drosophila Melanogaster* and the Role of the Fly in Therapeutic Drug Discovery." *Pharmacological Reviews* 63.2 (2011): 411-36. Web. 8 Sept. 2015.
- <sup>4</sup>Jennings, Barbara H. "Drosophila – a Versatile Model in Biology & Medicine." *Materials Today* 14.5 (2011): 190-95. *ScienceDirect*. Web. 29 Dec. 2015.
- <sup>5</sup>Hughes, Tamara T., Amanda L. Allen, Joseph E. Bardin, Megan N. Christian, Kansei Daimon, Kelsey D. Dozier, Caom L. Hansen, Lisa M. Holcomb, and Joseph Ahlander. "Drosophila as a Genetic Model for Studying Pathogenic Human Viruses." *Virology* 423.1 (2012): 1-5. *ScienceDirect*. Web. 30 Nov. 2015.
- <sup>6</sup>Couto, Africa, Mattias Alenius, and Barry J. Dickson. "Molecular, Anatomical, and Functional Organization of the *Drosophila* Olfactory System."

*Current Biology* 15.17 (2005): 1535-547. *ScienceDirect*. Web. 28 Aug. 2015.

<sup>7</sup>Bruyne, Marien De, Kara Foster, and John R. Carlson. "Odor Coding in the *Drosophila* Antenna." *Neuron* 30.2 (2001): 537-52. *ScienceDirect*. Web. 21 Jan. 2016.

<sup>8</sup>O'Leary, Dennis, Paul Yates, and Todd McLaughlin. "Molecular Development of Sensory Maps Representing Sights and Smells in the Brain." *Cell* 96 (1999): 255-69. Print.

<sup>9</sup>Rosso, S., & Inestrosa, N. (2013). WNT signaling in neuronal maturation and synaptogenesis. *Frontiers in Cellular Neuroscience Front. Cell. Neurosci.*, 7(103), 1-11.

<sup>10</sup>Ciani, L., & Salinas, P. (2005). WNTS in the vertebrate nervous system: From patterning to neuronal connectivity. *Nature Reviews Neuroscience Nat Rev Neurosci*, 6, 351-362.

<sup>11</sup>Lyuksyutova, Anna I., et al. "Anterior-posterior guidance of commissural axons by Wnt-frizzled signaling." *Science* 302.5652 (2003): 1984-8. *Academic OneFile*. Web. 10 Aug. 2015.

<sup>12</sup>Liu, Yaobo, Jun Shi, Chin-Chun Lu, Zheng-Bei Wang, Anna I Lyuksyutova, Xuejun Song, and Yimin Zou. "Ryk-mediated Wnt Repulsion Regulates Posterior-directed Growth of Corticospinal Tract." *Nature Neuroscience Nat Neurosci* 8.9 (2005): 147. Web. 10 Aug. 2015.

- <sup>13</sup>Krylova, Olga, Judit Herreros, Karen E Cleverley, Elisabeth Ehler, Juan Pablo Henriquez, Simon M Hughes, and Patricia C Salinas. "WNT-3, Expressed by Motoneurons, Regulates Terminal Arborization of Neurotrophin-3-Responsive Spinal Sensory Neurons." *Neuron* 35.6 (2002): 1043-056. *Science Direct*. Web. 10 Aug. 2015.
- <sup>14</sup>Hall, Anita C, Fiona R Lucas, and Patricia C Salinas. "Axonal Remodeling and Synaptic Differentiation in the Cerebellum Is Regulated by WNT-7a Signaling." *Cell* 100.5 (2000): 525-35. *Science Direct*. Web. 11 Aug. 2015.
- <sup>15</sup>Yao, Ying, Yuping Wu, Chong Yin, Rie Ozawa, Toshiro Aigaki, Rene R Wouda, Jasprina N Noordermeer, Lee G Fradkin, and Huey Hing. "Antagonistic Roles of Wnt5 and the Drl Receptor in Patterning the Drosophila Antennal Lobe." *Nature Neuroscience Nat Neurosci* (2007): 1423-432. Advance Online. Web. 9 Sept. 2015.
- <sup>16</sup>Wu, Yuping, Jay-Christian Helt, Emily Wexler, Iveta M. Petrova, Jasprina N. Noordermeer, Lee G. Fradkin, and Huey Hing. "Wnt5 and Drl/Ryk Gradients Pattern the Drosophila Olfactory Dendritic Map." *Journal of Neuroscience* 34.45 (2014): 14961-4972. Print.
- <sup>17</sup>Moon, R., Kohn, A., Ferrari, G., & Kaykas, A. (2004). WNT and  $\beta$ -catenin signalling: Diseases and therapies. *Nat Rev Genet Nature Reviews Genetics*, 5, 689-699.

- <sup>18</sup>Tissir, F., & Goffinet, A. (2013). Shaping the nervous system: Role of the core planar cell polarity genes. *Nature Reviews Neuroscience Nat Rev Neurosci*, 14, 525-535.
- <sup>19</sup>Goodrich, L., & Strutt, D. (2011). Principles of planar polarity in animal development. *Development*, 138(11), 1877-1892.
- <sup>20</sup>Sebbagh, Michael, and Jean-Paul Borg. "Insight into Planar Cell Polarity." *Experimental Cell Research* 328.2 (2014): 284-95. *Science Direct*. Web. 13 Aug. 2015.
- <sup>21</sup>Struhl, G., Casal, J., & Lawrence, P. (2012). Dissecting the molecular bridges that mediate the function of Frizzled in planar cell polarity. *Development*, 139, 3665-3674.
- <sup>22</sup>Goggolidou, Paraskevi. "Wnt and Planar Cell Polarity Signaling in Cystic Renal Disease." *Organogenesis* 10.1 (2013): 86-95. *NCBI*. Web. 13 Aug. 2015.