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Multisensory integration in weakly electric fish

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ABSTRACT

MULTISENSORY INTEGRATION IN WEAKLY ELECTRIC FISH

by
Andrea Roeser

Animals integrate information from across sensory systems, such as vision and hearing, to improve perception. To understand how neural circuits in the central nervous system integrate information from different senses, the responses of midbrain neurons to two categories of electrosensory stimuli in *Eigenmannia virescens* were studied. The first category of stimulus is electrical signals with frequencies below 50 Hz that are encoded in the activity of ampullary receptors. The second category is amplitude modulations of the electric organ discharge, which are encoded by p-type tuberous receptors. Six multisensory neurons were found that responded to both categories of stimuli. However, when the stimuli were presented simultaneously, the responses to one of the two categories were suppressed. Further, in six neurons that responded to one modality, responses were significantly reduced when the two categories of stimuli were presented simultaneously. These data suggest that multisensory information does not enhance neural responses.

MULTISENSORY INTEGRATION IN WEAKLY ELECTRIC FISH

by
Andrea Roeser

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Mom and Dad,
I will be forever grateful for your love, support, and endless home-cooked meals.
I love you.

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TABLE OF CONTENTS

TABLE OF CONTENTS
(Continued)

Chapter **Page**

TABLE OF CONTENTS
(Continued)

Chapter **Page**

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES
(Continued)

Figure **Page**

CHAPTER 1

INTRODUCTION

1.1 Multisensory Integration

The McGurk effect is a perceptual phenomenon that demonstrates how visual cues can have a profound effect on the perception of auditory information. When a subject is shown a video of a person pronouncing the word 'ga' while the sound 'ba' is played, subjects perceive the sound as 'da' even though their ears heard 'ba'. When subjects close their eyes, they immediately perceive the sound as 'ba' (Tiippana 2014; Ernst & Bulthoff 2004). The McGurk effect shows how visual and auditory information can interact to change perception, and it is an example of a broader phenomenon that is known as "multisensory integration" (Holmes & Spence 2005; Tiippana 2014).

Multisensory integration is the process by which information from different sensory modalities are combined to generate a single percept of the external world (Ernst & Bulthoff 2004; Zahar et al. 2009). Animals routinely rely on multisensory perceptions for survival – including for predator avoidance and prey capture (Bürck et al. 2010). But why do animals use more than one modality to understand the outside world given that, as shown by the McGurk effect, multisensory integration can lead to false perceptions?

1.2 Variability in Sensory Perception

Perceptual errors occur when an animal mis-categorizes or misinterprets sensory information. The McGurk effect perhaps demonstrates a form of perceptual error – where a sound is perceived incorrectly when presented in combination with certain visual cues.

However, the majority of perceptual errors do not result from multisensory integration, but rather from two sources of uncertainties in sensory perception from within single sense (Fetsch et al. 2013).

The first source is variability in the energy fluxes, such as sound radiating or light waves, which the animal receives from an object in its environment. The second source comes from uncertainties generated by the animal's own perceptual systems due to the organization and activity in neural circuits. These two sources of variability are sufficient to routinely generate perceptual errors. Indeed, perhaps the best way to define perception is in relation to variability: perception is an estimate of the state of the external world (Fetsch et al. 2013; Green et al. 2010).

It is interesting that organisms generate perceptions of the outside world in the face of variability. This thesis focuses on one of the two categories of variability– variability in the nervous system. The approach is to use multisensory integration as a tool for understanding how perceptions are assembled from sensory information. This experiment measured the responses of midbrain neurons to two independent forms of electrosensory stimuli, ampullary and tuberous, when presented alone and presented simultaneously.

1.3 Vertebrate Sensing Systems

Animals, including vertebrates, use specialized sensory cells that transduce energy fluxes in the environment into temporal patterns of graded potentials or action potentials. Each sensory cell encodes information from within a limited spatial, spectral, or computational region of stimulus space, which is known as a "receptive field." Receptive fields are the

part of the environment or body that a receptor 'sees' and encodes. For example, somatosensory neurons known as Pacinian corpuscles can have larger receptive fields in the skin that measure high-frequency vibration, whereas Meissner's corpuscles can have smaller receptive fields that encode lower-frequency stimuli (Figure 1.1; Johnson 2001). Most types of sensory cells respond by producing gradient potentials when its receptive field is stimulated, whether it is excited by light waves, temperature fluctuations, vibrations due to touch or sound, or even changes in pressure.

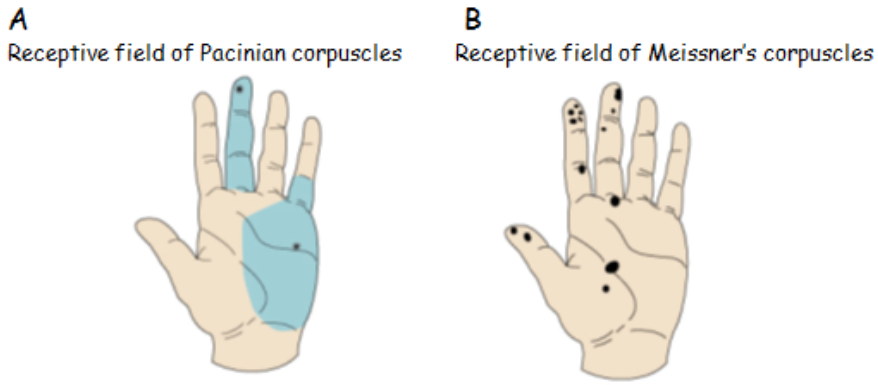

Figure 1.1 Receptive field sizes of sensory receptors found in the skin. (A) Pacinian corpuscles (black dots) are found deep in the skin and have large receptive fields (blue areas). (B) Meissner's corpuscles are found near the surface of the skin and have small receptive fields (black areas).

Source: https://dundeemedstudentnotes.wordpress.com/2012/04/12/sensory-innervation/

The spatial relations of receptive fields are maintained in the brain, forming what are known generically as somatotopic maps. These somatotopic maps represent the surface of the animal in relation to the distribution of sensory receptors. For example, there are more somatosensory receptors on the face than on the shin, so the number of neurons and size of the region in the brain dedicated to the face is larger than the area for the shin. Information from each sensor is independent as it enters the brain, either through

a dorsal root ganglion or cranial sensory ganglion, and remains separate until it reaches multisensory areas such as the midbrain and the telencephalon (Buonomano & Merzenich 1998).

Specific receptor types are often concentrated in specialized organs, such as eyes for photoreceptors and cochlea for hair cells. Cells that respond to a particular category of energy fluxes are known as a "sensory modality." In humans, we typically refer to our five senses as unique modalities – vision, hearing, touch, smell, and taste.

This broader definition of a sensory modality found in humans, however, masks a deeper complexity. Consider that the sense of touch comprises a group of different receptor types that encode different categories of energy fluxes. For example, Pacinian corpuscles encode high frequency vibratory stimuli, whereas free nerve endings encode painful stimuli such as heat. Indeed, sensory systems mediated by receptors that are similar to Pacinian corpuscles, such as Meissner's organs, Merkel's disks, and Ruffini end organs, and sensory systems that are mediated by free nerve endings, which include nociceptors, thermoreceptors, and chemoreceptors, are usually separated into two distinct modalities. The differences include the receptor structure and unique, parallel neural pathways in the brain.

Multisensory integration can occur between the various touch receptors and types of free nerve endings. However, the same type of integration can occur between Pacinian corpuscles and Merkel's disks, for example. Because these two different touch receptors encode slightly different features of the same category of energy fluxes, the information from each has, to some extent, the same similarities and differences that we would observe in codes originating from distinct modalities. For example, the variation seen in

each receptor type will generally be independent, except when there is a stimulus that activates both types of receptors. The activation of both types of touch receptors increases the likelihood that the signals are due to a real event rather than noise as in multisensory integration.

This is also true for other seemingly simpler sensory modalities. Consider that photoreceptors are tuned to particular frequencies of light, and that the visual representation in the brain draws from across photoreceptors. Each group of photoreceptors that are tuned to a particular range of frequencies comprises a unique modality, insofar as they can be stimulated independently of other photoreceptors. The generation of the unified representation of the visual world requires integration across groups of photoreceptors tuned to different frequency ranges.

In the work below, information encoded by two types of electroreceptors were studied: ampullary and p-type tuberous. Ampullary receptors encode exogenous electric fields whereas tuberous receptors are tuned to respond only to modulations of the autogenous electric field. These two types of receptors respond to completely independent electrical stimuli. Further, the pathways for information from these types of receptors are segregated in the brain up to the level of the midbrain. These two systems are generally considered to be separate modalities. But even if one were to lump these into a single modality, electrosensation, the computational challenges for the integration of information between them are nevertheless identical to those used in multisensory integration.

1.4 Variability in Vertebrate Sensory Systems

Neural activity is variable. Neurons can have spontaneous activity that is unrelated to stimulus condition, and neurons respond differently to the same stimulus. This variability introduces uncertainty into the relations between a stimulus and the response to that stimulus. Consider if you were designing a sensor. You might want it to respond to each stimulus with a unique, easily discernable response. In this way, the code generated by your sensor would uniquely identify each stimulus in the environment. Now consider a sensor that responds with some variability. If the variability in the response is large enough, two different stimuli might give rise to the same response. Therefore, if you were decoding a particular output from this sensor, it could represent two possible stimuli (Figure 1.2; Avila-Akerberg & Chacron 2011).

If responses of neurons to different stimuli overlap, then recreating the original stimulus becomes difficult because there could be many stimuli that elicited that response. The degree of overlap in responses to different stimuli affect the ability of the animal to accurately discriminate between stimuli (Figure 1.2; Avila-Akerberg & Chacron 2011; Sadeghi et al. 2007). A solution to this problem is averaging. An animal could average the responses to a particular stimulus that tends to reduce the effects of random variability. This idea is costly, however, as averaging may take time (van Beers et al. 2002).

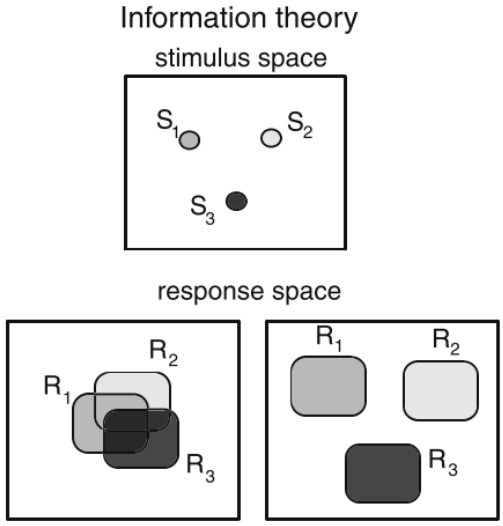

Figure 1.2 Information Theory showing how a stimulus can elicit different responses. In one case, the neurons have high variability and therefore it is difficult to tell how many stimuli there were. The neurons in the other case had low variability, allowing the response to clearly show that there were three stimuli.

Source: Avila-Akerberg & Chacron 2011.

1.4.1 Sources of Variability in Neurons

Neurons are variable ('noisy') due to intrinsic properties like synaptic variability, membrane properties, spontaneous firing, and other cellular and network processes. The noise created from neural sources can lead to errors in accuracy and precision. The more noise there is, the less precise the neuron can be in encoding smaller details of the signal. For example, energy fluxes received by a sensory neuron might have low amplitude, as when trying to see in a dimly-lit room, and thus the changes in activity related to the stimulus may be on a magnitude that is similar to the level of variability in firing. Similarly, increases in noise may also reduce the accuracy of a neural representation. Differences in firing between two stimuli, such as the face of your grandmother and that of your grandfather, may not be easily discerned if the level of variability in spontaneous firing is too high (van Beers et al. 2002; Faisal et al. 2008).

Each sensory modality, however, is not encoded by a single neuron, but rather many neurons. This allows information encoded by different neurons to be combined within a given modality and noise to be reduced (van Beers et al. 2002; Zahar et al. 2009; Faisal et al. 2008). This seems paradoxical, however, as one can imagine that with the addition of each neuron, each with its own spontaneous activity and variability, the total variability of the system will increase. Further, one would think that summing responses from multiple neurons would increase variability and make it more difficult to decode information.

How then is noise reduced if responses from neurons are combined? If the variability or noise in each neuron is random and/or specific to that neuron, then when the information of many neurons is averaged, only activity that is correlated between the neurons will remain. If a stimulus leads to correlations in activity across sensory neurons, then stimulus-related activity will remain and variability will be filtered (van Beers et al. 2002).

1.5 Multimodal Integration

A goal of multisensory integration is to reduce the influence of variability by focusing on correlated information between the independent sensory streams. This relies on the assumption that activity is random in each sensory modality. Truly random activity will be uncorrelated. In contrast, if we assume that salient stimuli simultaneously activate multiple modalities, we would expect that stimulus-related activity might be correlated in time across modalities. In other words, the idea is that correlations of spiking activity between two streams of information occur at a low rate in the absence of a stimulus

whereas stimulus-related correlations would occur at a high rate. Therefore, by passing correlated signals and rejecting uncorrelated signals, multisensory integration can increase the likelihood of correct identification of salient signals in the environment (Tiippana 2014; Eimer 2004).

1.6 Strategies for Multisensory Integration

A challenge in the implementation of multimodal integration is that each modality uses independent signals that can have dramatically different spatiotemporal properties. Consider the integration of visual and auditory signals from a drummer: arms moving in a rhythmic pattern hitting the drum to cause a distinct sound. First, the visual signal will travel at the speed of light and the acoustic signal at the significantly slower speed of sound. Given that sound travels at roughly 1 meter in 3 milliseconds, even distances of just a few meters can lead to biologically relevant disparities between the arrival of visual and acoustic cues. Second, 3D visual information is projected onto the 2D surface of the retina, providing a spatial representation of the visual world, whereas acoustic information is encoded as a single stream of information. In most animals, spatial information for salient stimuli can be computed from differences between the acoustic signals at the ears (Zahar et al. 2009).

How does the brain use the idea of cross-modal integration to generate a single perceptual object? There are several theoretical strategies to solve this problem, and evidence for several have been described in animal systems. The first is a 'winner-take-all' competition where the modality that is more reliable wins (absolute dominance). Second is where the modalities are weighed equally (simple averaging). Third,

information from the modalities can be mixed and contribute in varying strengths, allowing more reliable modalities to have a greater input (maximum-likelihood estimation [MLE] theory or Bayesian theory; Battaglia et al. 2003). These three approaches are described below.

1.6.1 Absolute Dominance

The computationally simplest idea for resolving differences between information from different modalities is not to integrate at all. In this strategy, the most reliable modality will win and the information from all other senses will be discarded. In this way, variability due to noise will not be reduced or eliminated (Deneve & Pouget 2004; Battaglia et al. 2003).

This form of integration might be the best option in situations where only one modality can be trusted. An example is if a person is trying to listen to music coming from a speaker. Any visual information coming into the brain will not help understand the music, so the absolute dominance approach could be useful in just using any auditory information coming in to listen to the music played. In fact, people tend to close their eyes when listening intensely to something, possibly reducing noisy information coming from unrelated visual information (van Beers et al. 2002; Deneve & Pouget 2004; Battaglia et al. 2003).

1.6.2 Simple Averaging

Simple averaging is an approach in which information from different modalities are combined, but are not independently 'weighed.' This solution takes in information from the modalities, gives them the same weight or value, and computes the arithmetic mean. It is a computationally simple solution and works well if the sensory information encoded

by each modality can be trusted equally. Image a person is attempting to cross a busy city street: the auditory and visual stimuli should be weighed equally to ensure every car is seen and heard. Noise is reduced because more than one modality is being used, however, if one of the modalities is not very reliable, then the incorrect conclusion might be made (van Beers et al. 2002).

1.6.3 Maximum Likelihood Estimate (MLE) Theory

The MLE theory, also known as the Kalman filter, evaluates sensory information in relation to its variance. Lower variance indicates that a signal, be it a signal in the environment or a representation of a signal in the brain, may be more reliable. For MLE, modalities found to have less variability/be more reliable are given a greater weight for generating an estimate of a signal. MLE theory evaluates the variability of signals within a window of time and space, and assumes that the variance is normally distributed. MLE does not include information from previous estimates of variance, and further does not include prior assumptions about the structure of signals. In this way, MLE is a 'bottom-up' approach because it relies solely on incoming sensory information. A MLE system could generate an absolute dominance system, by evaluating one modality as a zero and the other modality a one. Also, an MLE system could be reduced to simple averaging giving all the modalities equal weights. In an MLE system, the weighting would be tuned as variance from signals changed over time, causing it to be different than absolute dominance and simple averaging because there are no predetermined assumptions for the weights of each modality (Deneve & Pouget 2004; Knill & Pouget 2004; Battaglia et al. 2003; Faisal et al. 2008).

MLE provides the brain with greater flexibility, but requires far more computing power than a fixed absolute dominance or simple averaging system. This additional computational demand arises because the variance in each modality must be measured, then the modalities must be weighed, and lastly, the modalities must be optimally combined (Deneve & Pouget 2004; Battaglia et al. 2003; Knill & Pouget 2004; Ernst & Bulthoff 2004; Angelaki 2009).

1.6.4 Bayesian Theory

Bayesian interference is similar to MLE theory because both use the variance of each modality to determine the weight of the modality, but Bayesian systems also track time and space for this weighting process. Bayesian systems use prior knowledge that is updated with current estimates of variance to generate optimal weights. The more data collected, the more likely an appropriate distribution of weights will be achieved because there is more information to compare. This model is a 'top-down' process because contextual information and current sensory information are used, unlike MLE that only uses current sensory information. This model is the most costly in terms of computational demand because modalities must be weighed, like in MLE, but Bayesian theory also depends on memory: information from the past is used to determine present outcomes (Ernst & Bulthoff 2004; Deneve & Pouget 2004; Knill & Pouget 2004; Battaglia et al. 2003; Angelaki 2009).

1.7 Posture Control in Humans Utilizes Multimodal Integration

Humans control their posture using information from multiple sensory systems, including vision, proprioceptive, and vestibular modalities. How information from these modalities

is combined to maintain our upright posture has been a focus of intensive study (Oie et al. 2002; Barela et al. 2014). Bipedalism, upright walking on two legs, requires control systems to ensure stability and posture. The human body has a heavy torso, making it top-heavy and prone to falling. Most devices created by humans, such as cars, are designed more intelligently with most of their weight close to the ground or distributed over a wide base, contributing stability. How is it that humans are able to move about without constantly falling and right themselves if they are about to fall?

When standing upright, humans are constantly making small movements called postural sway, providing the brain with sensory feedback. These small movements are created to generate torque to stabilize the body, and come from integrating visual, vestibular, and proprioceptive information. Information from all of these modalities are not always available or reliable, such as when a person's eyes are closed or they are looking at a moving object when they themselves are motionless. To compensate for variability, the postural control system must use a type of Kalman filter to adjust to different situations (Oie et al. 2002; Zhang et al. 2007; Barela et al. 2014).

Postural sway and balance control have been studied by placing human subjects in environments with conflicting sensory cues. In a study done by Oie et al. 2002, subjects were asked to stand on a platform while facing a screen. Subjects were also asked to place one finger on a movable surface (Figure 1.3). The experimenter then made small-amplitude oscillatory movements in a projected visual scene in front of the subject, and via the movements of the surface where the finger was placed. Subjects will make small 'sway' movements – changes in posture – in response to either category of stimuli. As the stimuli could be independently and simultaneously controlled, the investigators could

measure the sway responses to conflicting multisensory cues. For example, the visual stimulus could be presented at 0.20 Hz, while the somatosensory stimulus on the touch surface was at 0.28 Hz (Oie et al. 2002).

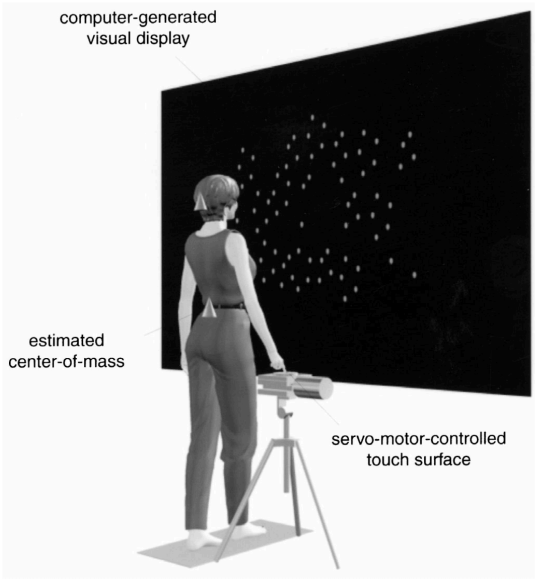

Figure 1.3 Experimental set-up. The subject is standing, facing the visual display while placing a finger on the touch surface. Stimuli are shown on the visual display and given through the touch surface.

Source: Oie et al. 2002.

The results showed that posture was controlled through re-weighting of sensory information. The weights were based on the variance and motion amplitude of the stimuli. Gain was found to also depend on the motion amplitude of the stimulus for both visual and touch surface stimuli— as the amplitude of the stimulus decreased, so did the gain indicating that particular modality was less reliable. Gain was measured by finding the ratio between the amplitude of the response and the amplitude of the stimulus; it measures the control stimulus motion has on induced postural sway. Having a gain equal to 1 means that the stimulus and postural response amplitudes are the same (Oie et al. 2002).

In the case of posture control, vision is a less reliable modality because it mainly encodes movement in the environment, not body movement. Visual information can encode environmental movement, such as watching a person skateboarding past you, or self-motion, such as looking down at your feet as you go down a flight of stairs, or a combination of both. Therefore, the nervous system must learn to weigh visual input. In the experiment described above, when a subject is shown a small amount of moving dots, the visual stimulus has little affect on postural sway. This indicates that the body might interpret this information as motion in the environment and may weigh this information as less important to posture control. If the number of moving dots is greatly increased, then postural sway begins to follow the moving stimulus, showing the nervous system then assumes the stimulus is encoding self-motion (Oie et al. 2002).

1.8 Multisensory Integration in Non-Human Animals

Multisensory integration has been studied in owls, cats, flies, lobsters, weakly electric fish, and many other species. A goal of these studies is to reveal how sensory information from different modalities is integrated to generate a percept of signals in the environment for behavioral control. The next sections describe how various animals use multisensory integration to locate moving stimuli or prey. Owls integrate visual and auditory information to capture moving prey at night; neurons in the superior colliculus of the cat are able to combine information from multiple modalities to locate prey; lobsters use either visual or proprioceptive information to control their eye movements; and weakly electric fish weakly electric fish use two different types of electroreceptors to gain

information about their environment (Bürck et al. 2010; Abbott et al. 2016; Eimer 2004; Battaglia et al. 2003; Zahar et al. 2009; Fortune 2006).

1.8.1 Owls

Barn owls are crepuscular animals that typically feed on small mammals. Owls can use both visual and auditory cues to localize prey moving on the ground. When hunting, the auditory and visual information are noisy due to external auditory noise in the environment and noise caused by low light levels. Nonetheless, owls are able to align auditory and visual receptive fields found in their optic tectum (the avian superior colliculus) with extreme accuracy. Studies on barn owls have demonstrated how vision and hearing can be linked through multisensory integration (Eimer 2004; Battaglia et al. 2003; Zahar et al. 2009).

If the vision of a young barn owl is skewed through using displacing prism spectacles, or if its hearing is impaired by an earplug placed in one ear, the owl will reweigh these modalities. The young owls rely more heavily on vision and weigh its input more highly than auditory information, even if the vision and not the auditory information is skewed. When the juvenile owl is given the displacing prisms, its brain alters the spatial auditory map to match the skewed vision (Eimer 2004; Battaglia et al. 2003; Zahar et al. 2009).

Adult barn owls that are given the prisms are able to adjust their spatial auditory maps only if they were exposed to them as juveniles, illustrating that prior skills leave a ‘skeleton’ even if they are not used for a long period of time. The owls, therefore, weigh visual stimuli higher than auditory stimuli and are able to use multisensory integration to

alter how they view their surroundings based on this reweighing (Eimer 2004; Battaglia et al. 2003; Zahar et al. 2009).

1.8.2 Cats

The deep layers of the superior colliculus (SC) in cats, like the optic tectum in owls, is a site of multisensory integration where sets of neurons respond to visual, auditory, and somatosensory stimuli. These multimodal SC neurons use multisensory enhancement when encoding most stimuli, which is thought to be a trademark of multimodal integration; multisensory enhancement is when a neuron's response to two or more stimuli is greater than its response to the best single modality— but only when the stimuli are correlated in time and space. Multisensory enhancement has been found to dramatically increase the superior colliculus' ability to control orientation, such as a saccadic eye movement, in alert cats (Perrault et al. 2005; Wallace et al. 1998; Anastasio et al. 2000).

Anastasio and colleagues (2000) have used the Bayesian interference theory to describe how the cat SC neurons interpret unimodal and bimodal stimuli. The result given through multisensory enhancement can be explained if neurons use the visual and auditory input they receive to make probabilities representing whether or not a target is in their receptive field. Therefore, if two weak stimuli are coherent, multisensory enhancement will cause the neuron to respond with greater strength than if each modality was presented separately. If a cat hears a bird and sees a faint rustling in a bush near where the sound is coming from, multisensory enhancement causes its eyes to orient to the place it saw and heard the rustling (Figure 1.4; Perrault et al. 2005; Wallace et al. 1998; Anastasio et al. 2000).

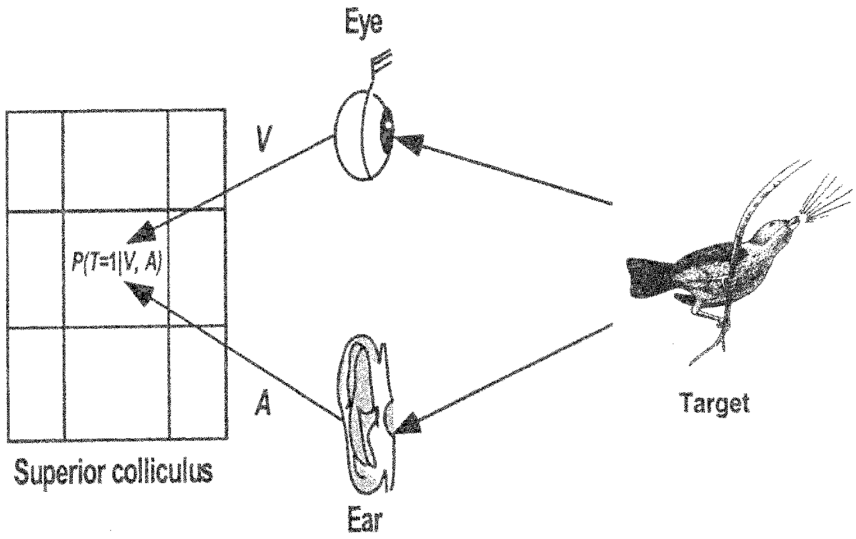

Figure 1.4 Diagram illustrating how the SC of a cat is able to take in two modalities (vision (V) and hearing (A)) and integrate them to generate the correct position of a target.

Source: Anastasio et al. 2000.

1.8.3 Lobsters

Multisensory integration has also been studied in the spiny lobster, *Palinurus vulgaris*. It has been shown that the ability of tethered lobsters to track objects depends on visual and proprioception information from the legs. A lobster is placed on an oscillating platform with an oscillating visual stimulus mounted over its head. The visual stimulus was created by putting dark stripes on a piece of clear Plexiglas that was in the shape of a half-cylinder (Neil et al. 1983).

When a lobster was presented with proprioceptive inputs that are the same frequency, but are out of phase, the eyes move with the visual stimulus for low frequencies, and with the platform for high frequencies. Interestingly, when the platform and visual stimulus frequencies were different, the lobster's eyes still followed the visual stimulus for low frequencies, but was unable to follow either the visual or platform for high frequency stimuli. These results show that eye movements of lobsters are not

determined by simply averaging modalities— there is a difference between the weights of the information from the optokinetic and proprioceptive systems. Lobsters control eye movements by using information from their visual and proprioceptive systems; depending on the stimulus frequency, lobsters use information from one or neither of the modalities to track the stimulus (Neil et al. 1983).

1.9 Weakly Electric Fish as a Model System

Weakly electric fish generate an electric field on the order of volts around their body that they use to sense nearby objects and in communication. The electric field is normally kept at a constant frequency. *Eigenmannia*, a type of weakly electric fish, have two types of receptors embedded in their skin, ampullary and tuberous, that encode two different electrosensory modalities. Ampullary receptors are phylogenetically ancient and are found across aquatic species. These receptors respond to exogenous electric fields in the environment in a range of frequencies typically below 100 Hz (Fortune & Rose 1997; Stöckl et al. 2014).

Tuberous receptors encode information contained in the fish's own electric organ discharge (EOD). Tuberous receptors are composed of two subtypes – P-type and T-type. T-type tuberous receptors fire in line with the fish's EOD frequency, meaning if the fish has a 250 Hz EOD, the receptors will also fire at 250 Hz. P-type tuberous receptors are amplitude modulated and encode signals resulting from perturbations in the fish's own electric field. Changes in the electric field can be caused by objects close to the animal, like small prey or plants, or by the EOD of another fish; these changes alter the amplitude

of the EOD on the fish's skin, which stimulates the P-type tuberous receptors (Krahe & Maler 2014).

Ampullary and p-type tuberous receptors are found along the whole body of the fish, but are highly concentrated around the head. Because these two receptor types are found near each other (Figure 1.7), and are distributed all over the fish, their responses are spatially and temporally congruent. Information from ampullary and P-type tuberous receptors is transmitted to the brain via VIIIth nerve afferents, where they terminate in a structure called the electrosensory lateral line lobe (ELL). The ELL then sends afferents to the midbrain torus semicircularis (Ts).

There are neurons in the midbrain of the fish that have been found to respond to both ampullary and tuberous stimuli (Rose & Cali 1992). By presenting a fish with multimodal stimuli, multisensory integration can be studied in anaesthetized animals without the need of visual or mechanosensory inputs. By working with an anaesthetized animal, environmental cues are removed from the system, which enables us to control all relevant sensory stimuli. In addition, *Eigenmannia* is a well-studied animal; the neural circuits in the brain have been studied and identified in previous anatomical and neurological studies. This knowledge allows us to easily identify the area of the brain where multimodal integration occurs (Figure 1.10; Heiligenberg et al. 1981; Rose & Heiligenberg 1985; Rose & Call 1992).

1.10 Electrosensory Systems

Electroreceptors are found in many animals including different species of fish, rays, sharks, and even the Platypus. Receptors are embedded in the skin of the animal and

respond to changes in electrical fields found in the water. These changes can be produced by the geomagnetic field of the earth, living organisms in the environment, lightening, among other sources. Animals use information from electric fields for navigation, prey capture, predator avoidance, and in social communication (Fortune 2006).

Most species with electrosensory systems, like sharks and rays, have ampullary receptors that detect exogenous, low frequency signals. Ampullary receptors are found along the entire body, but are more numerous in the head. The low frequency signals are not limited to muscle activity in other animals and the swimming movement of nearby prey (Rose 2004).

Interestingly, electrogenic fish are able to generate their own electric fields and have specialized tuberous receptors that encode changes in that field. The information from electroreceptors are used in communication, prey capture, predator avoidance, and in navigation through their environment (Rose 2004; Fortune 2006; Krahe & Maler 2014).

1.11 Strongly and Weakly Electric Fish

Electrogenic fish are found in South America (Gymnotiformes) and Africa (Mormyriformes), in other orders (Figure 1.5). Weakly electric fishes emit an electric field via a specialized electric organ that is used as an additional sensory modality (Heiligenberg 1981). There are also strongly electric fish, such as electric eels, catfish, and the skate Torpedo, that generate electric fields with sufficient current to stun prey—at voltages ranging from 10 to 600 Volts. Weakly electric fish on the other hand, like

Eigenmannia and *Apteronotus*, generate low amperage currents with voltages that are typically below about one Volt (Rose 2004).

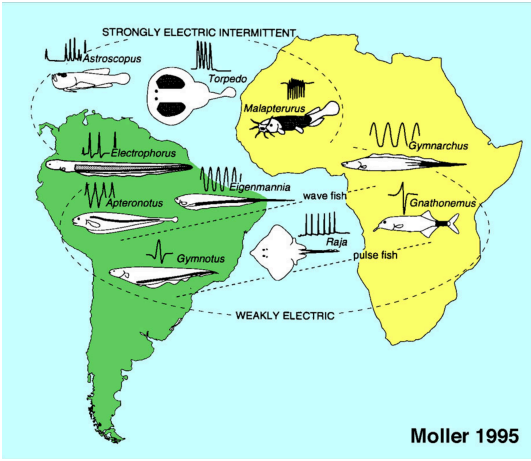

Figure 1.5 Map showing the geographical distribution of electric fishes. The waveform of the electric organ discharge (EOD) is shown next to each fish; it varies between species.

Source: Moller 1995.

Weakly electric fishes can be divided into two categories in relation to the electric signal that they produce. Some species generate short pulses, typically below a few milliseconds in duration and often less than 1 millisecond, which are separated by longer interpulse intervals. These species are known as "pulse-type" weakly electric fishes. Other species produce electric pulses with durations that are roughly equivalent to the duration of the interpulse interval. Further, the waveform of pulse and subsequent interval appear pseudosinusoidal: these species are known as "wave-type" weakly electric fishes. Wave-type weakly electric fishes often maintain a nearly constant production of electrical pulses, resulting in a constant-frequency electrical signal (Hitschfeld et al. 2009; Emde et al. 1999).

1.11.1 The Wave-type Weakly Electric Fish *Eigenmannia virescens*

This study will focus on *Eigenmannia virescens*, a Gymnotiform species that is found throughout the Amazon basin. These fish live in white- and black-water rivers, streams, and lakes. They are social, forming shoals of individuals, and are most typically found in small groups of three to five fish (Figure 1.6 a; Tan et al. 2005).

When in these groups, *Eigenmannia* communicate with each other and navigate through the environment using their electric fields. The electric field is generated by an electric organ (EO) found along either side of the fish. The EO creates what is called the electric organ discharge (EOD) that can be detected up to one meter away from the fish's body (Stamper et al. 2013). In *Eigenmannia* the field is pseudosinuoidal with fundamental frequencies between 200 and 700 Hz (Tan et al. 2005; Ramcharitar et al. 2005).

Normally, individual *Eigenmannia* maintain their EOD at a constant frequency (Figure 1.6 a). Nevertheless, *Eigenmannia* are able to raise or lower the EOD frequency to generate communication signals or avoid detrimental electrosensory conditions that can arise due specific social interactions (Figure 1.6 b; Rose & Heiligenberg 1985; Rose 2004; Fortune 2006).

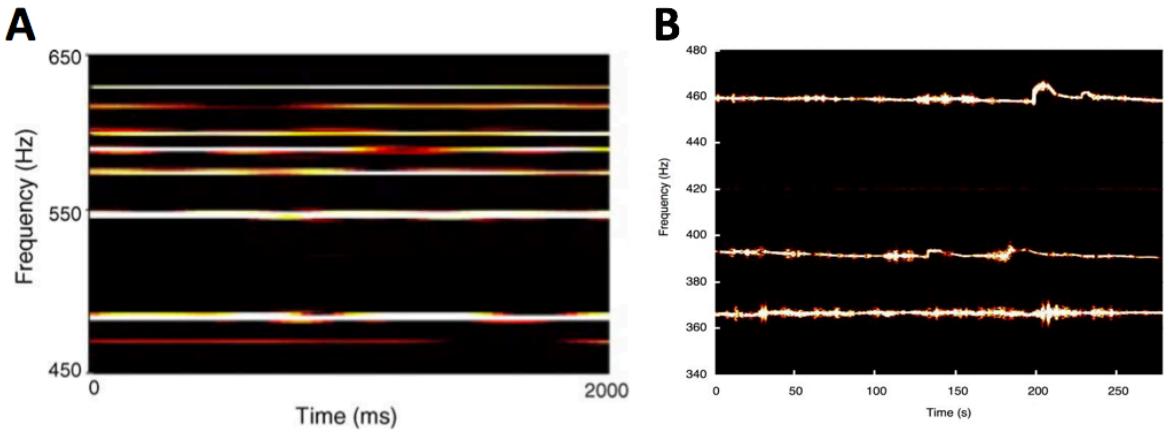

Figure 1.6 Sonograms of the EOD from *Eigenmannia virescens* (A) Eight wild *Eigenmannia* recorded in the Amazon basin; each horizontal line is the EOD of one individual. (B) Captured *Eigenmannia* recorded in a large tank; the increases in frequency are the fish.

Source: Tan et al. 2005.

1.11.2 Tuberous and Ampullary Electrosensory Receptors

Conspecifics or objects in the environment can perturb a fish's EOD causing changes in timing and amplitude of the electric signal along the surface of the skin. The changes in the waveform stimulate the tuberous receptors in the skin of the fish, and any exogenous signals stimulate the ampullary receptors. Both types of receptors are located in jelly-filled pits in the skin and are densest on the head, but are found along the entire body (Figure 1.7; Fortune 2006; Rose 2004).

Tuberous and ampullary receptors encode two separate modalities through utilizing signal transduction. The ampullary receptors respond to exogenous signals found in the environment that are not created by the fish, but rather from other animals or objects—a passive sense. Tuberous receptors are only able to encode signals that create amplitude modulations in the self-generated electric field of the fish— an active sense.

Another difference between ampullary and tuberous receptors is that ampullary receptors are ancient and phylogenetically widespread— many animals have them including sharks and platypus. Tuberous receptors, however, were created in a duplication event where the ampullary circuitry was doubled, and the fish also acquired the ability to create and emit their own electric field. Both types of electroreceptors respond to electric signals found within the water.

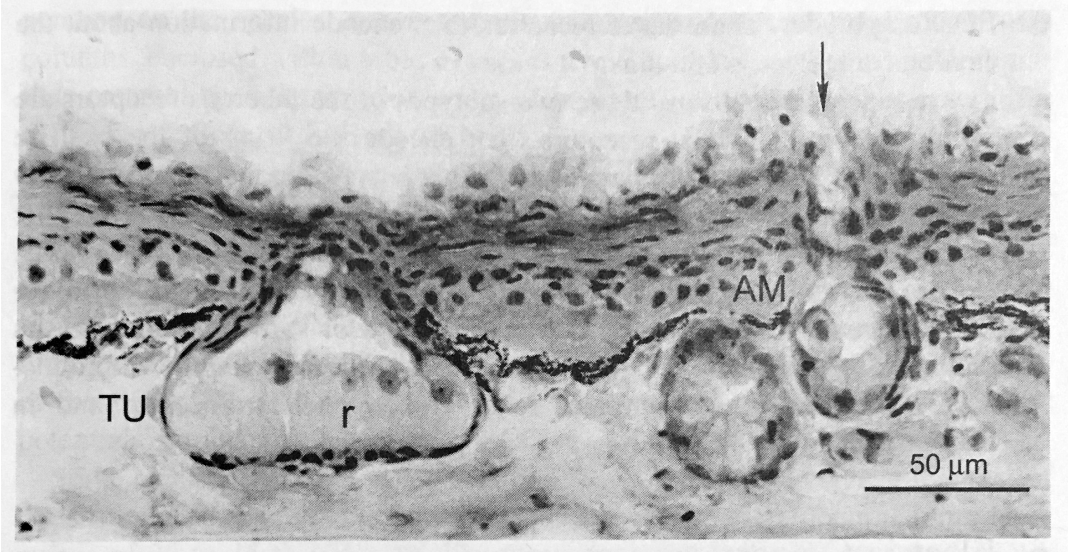

Figure 1.7 Photomicrograph of tuberous and ampullary receptor organs in the skin of *Eigenmannia*. The tuberous receptor (TU) on the left is made of individual receptor cells (r). The ampullary receptor (AM) on the right has a long canal (arrow).

Source: Bullock et al. 2005.

1.11.3 Global and Local Stimuli

Depending on what object is creating the perturbation, two types of salient electrosensory stimuli can be created: global and local (Figure 1.8). Global stimuli stimulate tuberous electroreceptors and local stimuli are able to stimulate both ampullary and tuberous electoreceptors.

Global stimuli activate a large number of receptors across the surface of the animal simultaneously. If a fish encounters communication signals from a conspecific, high-frequency amplitude modulations (AMs), or 'beats', are made that are spatially diffused across the skin (Figure 1.8C— global). Beats are created when electric fields of two or more fish interact— the fields sum and their frequency difference is the beat rate. *Eigenmannia* and other Gymnotiforms are able to chirp as a form of communication, creating beats. A chirp is a shift in the frequency in the fish's electric field that can last from a few milliseconds up to tens of seconds (Figure 1.6B — refer to the 460 Hz fish). By emitting a chirp, a fish creates global stimuli that are short amplitude modulations (20 Hz or greater; Chacron et al. 2003; Rose 2004; Tan et al. 2005; Fortune 2006).

Studies on *Eigenmannia* in the wild have shown that they tend to live in shoals, which might help avoid predation. The global stimuli created from being near other conspecifics might also enhance the fish's sensory perception. Continuous high-frequency interference patterns have been shown to create short-term synaptic depression in neurons found in the midbrain of the fish. This type of depression can enhance a fish's ability to process moving electrosensory images, like prey objects. Therefore, it might be beneficial for weakly electric fish to live in aggregates (Tan et al. 2005).

Local stimuli only activate a small, localized portion of receptors. When a fish is near a small prey item, the AMs created are low in frequency and localized to a small portion of the skin (Figure 1.8C— local). Other objects found in the water such as leaves and roots can also create localized amplitude modulations. Through watching feeding behavior, models have shown that the most salient information during prey capture is

created by beat rates of 10 Hz or below. These local stimuli are created by motion of the fish (Fortune 2006; Rose 2004).

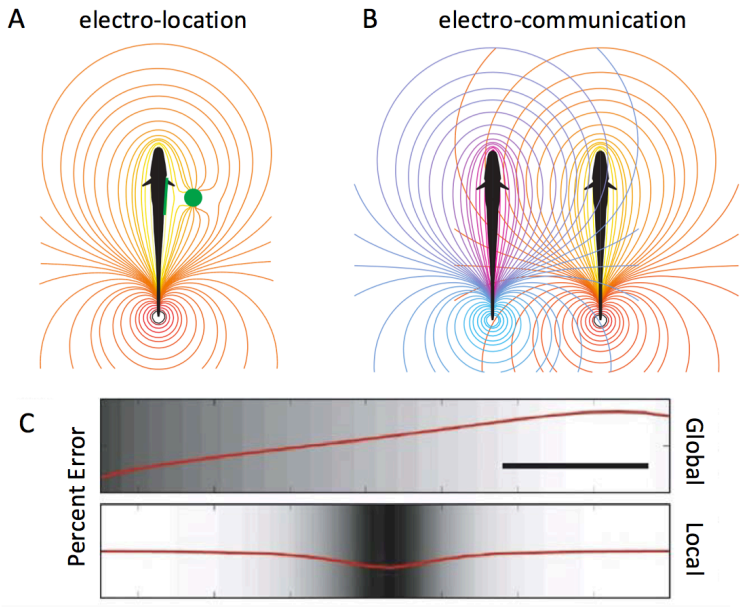

Figure 1.8 Illustrations of local and global stimuli. (A) Fish might encounter local stimuli that create small perturbations in the electric field. (B) Two fish can swim near each other, causing their electric fields to interact, creating global stimuli. (C) The effect global and local stimuli have on the receptors along the side of the fish. Scale bar: 2 cm.

Source: (A/B) Krahe & Maler 2014; (C) Fortune 2006.

1.12 Neural Pathway

The neural pathway responsible for decoding the information from the electroreceptors is well studied and is composed of two main sections – the electrosensory lateral line lobe (ELL) and the torus semicircularis (Ts) (Figure 1.9; Rose & Call 1992; Rose & Heiligenberg 1985; Krahe & Maler 2014). Information from ampullary and P-type tuberous receptors are transmitted to the brain via VIIIth nerve afferents, where they terminate in different areas within a structure known as the ELL. Pyramidal cells the ELL send afferents to the midbrain torus semicircularis (Ts). The Ts is the first area in the

central nervous system where ampullary and tuberous information interact directly (Rose & Call 1992; Fortune 2006).

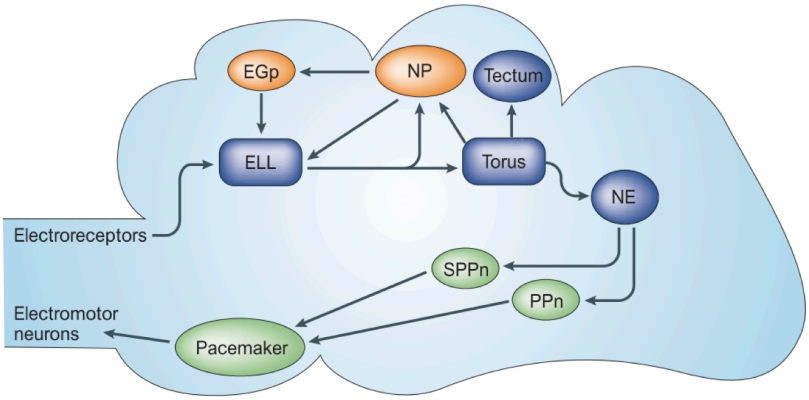

Figure 1.9 Electrosensory pathway in *Eigenmannia*. Sensory information travels from electroreceptors to the ELL and then to the Torus semicircularis.

Source: Rose 2004.

1.12.1 Electrosensory Lateral Line (ELL) Lobe

The ganglia afferents transfer the electrosensory information to the somatotopically structured ELL of the hindbrain. Here the information is filtered, but ampullary and tuberous information remain independent. There are four somatotopic maps located in the ELL: ampullary afferents terminate on pyramidal cells in the medial segment of the ELL and P-type tuberous afferents terminate on pyramidal cells and interneurons in the central-medial, central-lateral, and lateral segments of the ELL (Figure 1.10a; Heiligenberg & Rose 1985).

Tuberous afferents trifurcate after entering the brain and terminate in each of the three tuberous maps. In general, pyramidal cells in the LS map exhibit high-pass filtering, meaning it encodes higher frequency information from larger receptive fields, whereas

pyramidal cells in the CMS typically act as low-pass filters with smaller receptive fields. The CLS map includes neurons with band-pass filters and intermediate-sized receptive fields. (Figure 1.10b; Heiligenberg & Rose 1985).

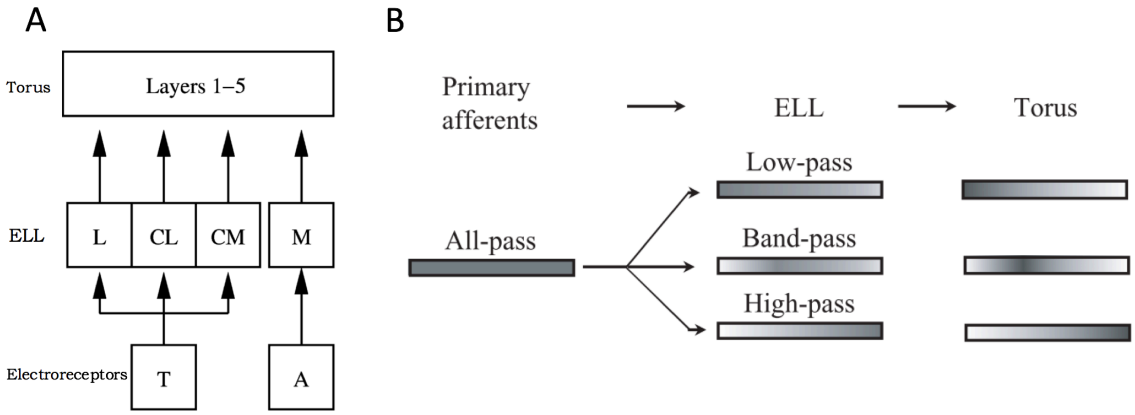

Figure 1.10 Schematics illustrating how electrosensory information is filtered and travels through the brain. (A) Tuberous (T) and ampullary (A) receptors project to the ELL, which in return projects to the Torus. (B) Information from electroreceptors is lightly filtered in the ELL, and strongly filtered in the Torus.

Source: (A) Ramcharitar et al. 2005 (B) Rose & Fortune 1999.

There are two main classes of pyramidal cells found in the ELL: E-cells and I-cells. E-cells receive P-type tuberous inputs and respond to increases in EOD amplitude, whereas I-cells respond greater to decreases in the amplitude of the EOD. I-cells are able to do so because the P-type tuberous afferents synapse to interneurons, which inhibit I-cells due to EOD amplitude increases. E-cells and I-cells are then further subdivided into deep, intermediate, and superficial types depending on morphological and molecular factors. In each of the three tuberous maps, the six types of pyramidal neurons are found in columns— a column is the basic unit of the ELL. Each column has inputs from the same receptive field, but each pyramidal neuron is able to process the information in a different way (Krahe & Maler 2014).

1.12.2 Torus semicircularis (Ts) – Where Multimodal Integration First Occurs

The pyramidal neurons of the four ELL maps project onto the dorsal torus semicircularis (Ts) of the midbrain generating a single somatotopic map (Figure 1.9 and 1.10). The Ts is a large, laminated structure composed of 12 layers with around 50 cell types (Krahe & Maler 2014; Heiligenberg & Rose 1985). Laminae 1-5 are differentiated due to ampullary and tuberous processing— each layer responds to either one, or both modalities. Integration of information is promoted by the lamination of the torus, which is organized in columns going through the lamina. Each column receives information from the same section of the skin (Rose & Call 1992).

Tuberous afferents have been found to project mainly to layers 5 and 7, and minimally to layer 3. Ampullary afferents, however, terminate in layers 1-3, and rarely in layer 5. Studies have found that lamina 4 contains neurons that respond to both modalities. What frequencies the bimodal neuron responded to depended on the layers where its dendrites were found. The dendrites mainly extend to layers 1-3 and 5, thereby integrating information coming from the same area of the skin (Rose & Call 1992).

13.1 The Hypothesis and Experimental Approach

The hypothesis is that neurons exhibit multisensory enhancement to features of stimuli that are congruent across different modalities. Specifically, we expect that neurons will exhibit non-linear facilitation to congruent multisensory stimuli. The goal of the experimental approach was to examine the activity multimodal neurons to assess how the nervous system integrates and weighs salient sensory stimuli. This experiment involved measuring the responses of Ts neurons to ampullary and tuberous stimuli that were

presented both alone and simultaneously. We expected that any shared parts of the ampullary and P-type tuberous stimuli would cause the neurons to respond more strongly than to the sum of responses to each modality when stimulated separately.

Two categories of stimuli were presented. One type was called Golden Ratio stimuli in which an ampullary and P-type tuberous sine waves with frequencies that are golden ratios of each other. This ensures that all amplitude and phase combinations are achieved during the presentation of the two sine waves. The second type of stimulus was Sum of Sines in which five sinusoids were combined in both the ampullary and P-type tuberous modalities. The frequencies of the sine waves were different between ampullary and tuberous stimuli expect for one shared frequency. The idea was that neurons would exhibit facilitated responses to this shared frequency. These Sum of Sines stimuli appear to be random to the animal, but can nevertheless be analyzed with respect to each of the individual sine waves.

Analysis of the data showed that our initial hypothesis was incorrect. Neurons did not exhibit facilitated responses to shared features across modalities. Rather, neurons appeared to use an absolute dominance approach in which activity from one modality was suppressed. Future experiments will determine if the amplitude ratios of the stimuli affect responses, and will examine how differences in shared information affects multimodal integration.

CHAPTER 2

MATERIALS AND METHODS

2.1 Animal Care and Acquisition

Weakly electric fish *Eigenmannia virescens* were used in this study. Fish were obtained from commercial aquarium fish suppliers: *Eigenmannia* were captured in South America and transferred to Central Mass Aquatics (Worcester, MA), the supplier used for these experiments. Fish were kept in tanks with up to five other fish at temperatures between 23 and 30°C and conductivity 250–650 $\mu S \cdot cm^{-1}$. All experimental procedures were approved by New Jersey Institute of Technology's Animal Care and Use Committee. Experimental procedures followed guidelines set by the Society for Neuroscience.

2.2 Experimental Setup and Procedure

The methods used were similar to previous studies (Rose & Fortune 1996). At the start of each experiment, a fish between 10cm and 20cm was picked from one of the tanks; the sex of the fish was not assessed, and we expect that fish of both sexes were used in this study. The fish was brought to the behavioral tank where it was allowed to acclimate to the temperature and conductivity of the tank for a few minutes while its EOD was measured and recorded. The fish was then injected with the nicotinic acetylcholine inhibitor, gallamine (in saline with concentration 20mg/mL, each fish was given roughly 3 µl), which also attenuated the fish's EOD. If necessary, an additional 1-2µl of Gallamine was administrated when the amplitude of the EOD increased significantly and/or when gilling resumed (normally four to five hours after the first injection) to ensure the fish was immobilized. The initial injection was done with a 10µl syringe near

the spine, while the fish was on top of a small pad. The fish was returned to the tank quickly as to put as little strain on the animal as possible.

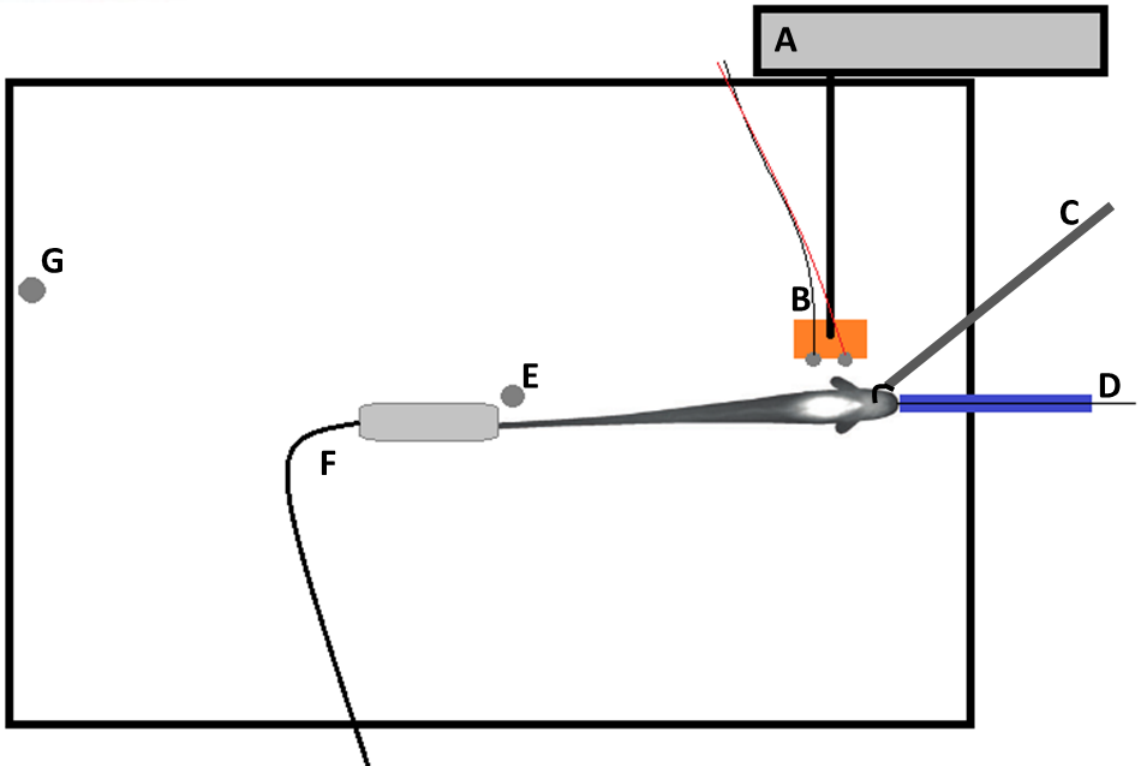

Figure 2.1 Experimental setup. (A) Manipulator that moves the local stimulus. (B) Two carbon electrodes in their holder that emit the local stimulus. (C) Metal rod that is glued to the head of the fish. (D) Mouth tube with the wire electrode that emits the S1 in the mouth. (E) Carbon electrode that emits the S1 near the tail. (F) Tail holder that has the wires inside to monitor the residual EOD. (G) Carbon electrode that grounds the tank.

After the injection, the mouth of the fish was placed on a tube. This tube delivered water over the fish's gills (Figure 2.1 D). Gallamine reduces the EOD of the fish by over 1000x; during experiments we replaced this electric field using a sinusoidal mimic. This mimic was generated by sine wave generator and delivered through a carbon electrode placed near the tail of the animal and a silver wire placed inside of the mouth tube (Figure 2.1 D and E). The residual EOD was recorded by placing two wires into a tube

surrounding the tail. This signal was monitored during experiments to determine the health of the fish (Figure 2.1 F).

During experiments, the body of the fish was submerged in the water except for the top of its head where the incision is made. For surgery, the skin was anesthetized with topical application of 2% Lidocaine solution. The surgery included removing just enough skin, roughly 6mm^2, exposing the skull. The animal was stabilized by gluing a metal rod to the skull using cyanoacrylate glue (Figure 2.1C). A small hole (roughly 2mm^2) was drilled through the skull using a dental drill directly over the tectum, and the dura mater was removed using a forceps and/or scalpel. The brain was kept moist with saline throughout the experiment.

A Flaming/Brown type micropipette puller (Model P-97, Sutter Instruments, Novato, CA) was used to create patch pipettes made from borosilicate glass capillaries (A-M systems 5960; 1 mm outer diameter, 0.58mm inner diameter) (Rose & Fortune 1996). An electrode was filled with a physiological solution (recipe listed below, Appendix A) and then placed onto an electrode holder (A&M Systems, 1.6 mm Pin Holder, Narrow, With Suction Port and Wire) that is equipped with a suction port. The suction port was connected to a 60mL syringe by a tube to allow suction or pressure to be applied. The resistances of the electrodes was then tested by lowering the tip of the electrode into the saline covering the brain; the electrodes used were between 15 and 30 MΩ, with the best results obtained from resistances in the lower 20s. A three-axis micromanipulator was used to maneuver the electrode above the brain (Rose & Fortune 1996).

The electrode was advanced by roughly 2 μm increments through the brain (Siskiyou, MC1000e microcontroller) first through the tectum and then into the top five layers of the torus (normally stopping between 700 and 1000 μm from the surface of the brain).

2.3 Neurophysiological Recordings

While slowly moving the electrode into the brain (~ 1.5 μm steps), a square wave current was applied to the electrode to measure resistance. If the resistance increased, illustrated by an increase in the voltage response, a seal on the neuron is made by adding light suction with a 60 mL syringe. Spikes or small ripples in the recording trace were also seen when the electrode came close to a neuron.

An A-M systems DC Amplifier (Neuroprobe Model 1600) amplified the neural activity. An A-M Systems audio monitor (Model 3300) was then used to listen to the neural recording. We listened for spikes or rumbling sounds indicating a neuron was near the electrode tip— neurons are always nearby, but not necessarily heard. Once a seal had been made and the neuron was spiking, a small amount of negative current was applied, usually less than -0.25nA and the square wave current shut off.

Stimuli were then played in the water. The waveforms of the stimuli were generated using custom MATLAB code and converted into analogue signals using a CED 1401 Power3. These signals were delivered to the fish via a custom-built circuit including a stimulus isolator— the tuberous stimulus was multiplied by the S1 and the ampullary stimulus was added to the S1. Two carbon electrodes placed near the left side of the fish (Figure 2.1 B) delivered the stimuli into the water. These electrodes emitted the stimulus

locally, meaning it was not distributed equally around the whole animal like a global stimulus. The carbon electrodes were attached to a manipulator that allowed the stimulus to be moved between the head and tail of the fish to ensure it was near the receptive field of the neuron (Figure 2.1A).

The A-M Systems Neuroprobe Amplifier (Model 1600) amplified the electrical activity of the neuron. The amplified signals were digitized using a CED 1401 and recorded using Spike2 software. Spike2 showed, in real time, the residual EOD of the fish, the signals in the tank, the ampullary and tuberous stimuli output of the CED 1401 Power3, the current being applied to the neuron, and the action potentials created by the neuron. Data were exported to MATLAB for analysis.

At the end of the experiment, the fish was given 2-phenoxyethanol (an anesthetic) and either decapitated and its head was placed in 10% formalin so the brain could be extracted and stained, or perfused trans-cardially to remove blood and improve fixation of tissue.

2.4 Stimuli

Two types of stimuli were used in this experiment to determine how a neuron responds to ampullary and tuberous stimuli when played individually and played simultaneously. These include golden ratio stimuli and Sum of Sines stimuli.

For tuberous stimuli, signals were generated and *multiplied* with the EOD. For ampullary stimuli, signals were generated and *added* to the EOD. For convenience, the signals show below for tuberous and ampullary stimuli are those before they were multiplied/added to the EOD.

2.4.1 Golden Ratio Stimuli

The first type of stimulus was created so that the sine waves that comprised the ampullary and tuberous signals were golden ratios of each other. MATLAB scripts created multiple stimuli with different core frequencies (Table 2.1). The ampullary stimulus was the core frequency, and the tuberous stimulus was created by multiplying the core frequency by the golden ratio: $\phi = \frac{1+\sqrt{5}}{2}$ (Figure 2.2).

Table 2.1 The Core Frequencies and Lengths of the Golden Ratio Stimuli

Frequency (Hz)	**Length of stimulus (seconds)**
1	32
2	16
4	8
8	4
16	2
32	1

In the golden ratio stimuli, two sinusoidal stimuli, one that would stimulate tuberous receptors and the other that stimulates ampullary receptors, were simultaneously delivered. The frequencies of these stimuli were at different frequencies determined by the golden ratio, which ensured that all amplitude and phase combinations were achieved during the stimulus presentation. If a neuron responded to both ampullary and tuberous stimuli, theoretically, it would spike for specific combinations of the ampullary and

tuberous signals, such as a positive tuberous slope and ampullary slope being zero (Figure 2.2).

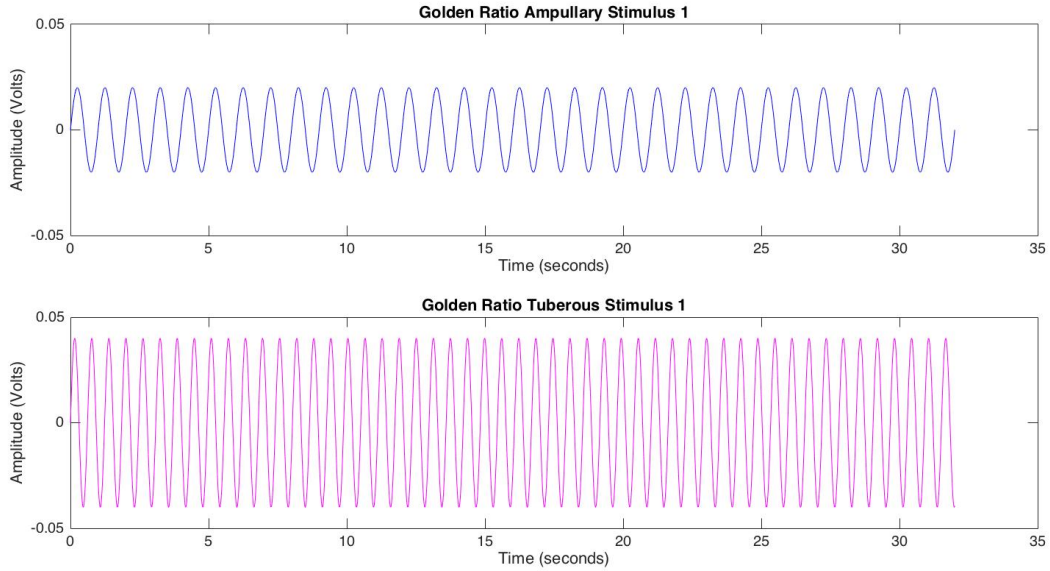

Figure 2.2 Golden Ratio Stimulus. The ampullary signal (top, blue) has a frequency of one and the tuberous signal (bottom, pink) has a frequency of one multiplied by the golden ratio.

2.4.2 Sum of Sine Waves Stimuli

In the Sum of Sines stimuli, five sine waves, each at a different frequency (frequencies between 0.3 and 31 Hz) were summed in each of a tuberous and an ampullary stimulus (Figure 2.3). This type of stimulus is pseudorandom, but we can analyze the data with respect to each of the component sine waves.

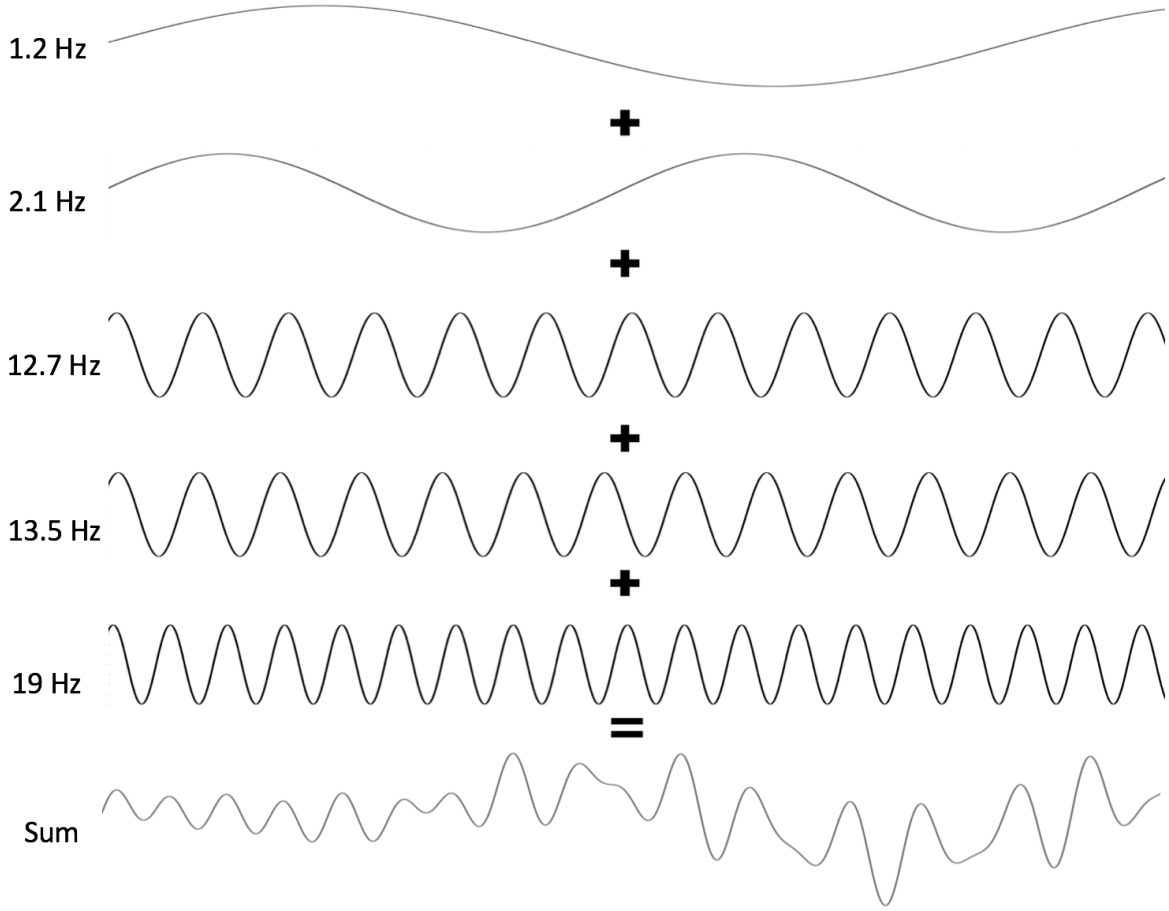

Figure 2.3 A depiction showing how five sines waves are added together to form a pseudorandom stimulus. The frequencies of each sine wave is shown on the left. These are the frequencies used to create the tuberous part of the 2.1 Sum of Sines stimulus.

This stimulus structure is useful with respect to our prediction that multisensory neurons should ignore information that is uncorrelated and should have facilitated responses to information that is correlated between the two sensory modalities. To test if neurons were sensitive to features that were shared between ampullary and tuberous stimuli, one of the frequencies in the sum of sine waves was shared between the two modalities while all of the others were not shared (Table 2.2). For example, if the tuberous stimulus was composed of 1.1, 2.5, 6, 15.5, and 21 Hz sine waves, the ampullary stimulus might contain 0.9, 2.5, 6.3, 14, and 19.9 Hz – the 2.5 Hz stimulus

being identical in the two stimuli. We might expect that a multisensory neuron would respond to frequencies in both the ampullary and tuberous signals when presented alone, but perhaps there will be a supralinear response to the frequency that is shared between the stimuli when both are presented simultaneously.

Table 2.2 The Sine Wave Frequencies that Comprised Each Sum of Sines Stimulus

Common Frequency (Hz)	**The Five Frequencies Comprising the Stimulus (Hz)**	
	Tuberous	**Ampullary**
1	0.1 0.6 0.9 1 3.3	0.2 0.4 0.5 1 2
2.1	1.2 2.1 12.7 13.5 19	2 2.1 7.8 21.7 30.8
4.5	2.3 4.5 14.1 16 17.2	3.6 4.5 8.7 10.6 25.9
6	1.7 6 7.5 12.8 17.7	1 4.6 6 20.7 28.5
8.2	5.1 8.2 12.9 17.2 17.7	3.1 8.2 10.6 20.9 28.5
12.5	1.7 4.3 12.5 17.2 18.2	1 6.8 10.6 12.5 29.5
25.6	4.5 5.1 17.9 20.9 25.6	3.1 7.3 12.9 25.6 28.9

The stimuli were composed of five parts: 1) 1.8 seconds no signal in the water, 2) the tuberous stimulus played alone, 3) both stimuli played together, 4) the ampullary stimulus played alone, and 5) 1.8 more seconds of 'silence'. The tuberous and ampullary parts were each ten seconds long and were repeated simultaneously during part 3 – the multisensory part - so that the same 'noisy' Sum of Sines was played to the animal twice in each stimulus (Figure 2.4). The sequence of sections 2, 3, and 4 were varied to avoid potential effects due to the order of the stimuli.

Variations of the Sum of Sines stimulus were used. The first variation has an inverted ampullary signal— the sine waves comprising the ampullary signal were negative (Figure 2.4b). This was done because both ampullary and tuberous neurons in the ELL are found in two types known as "E" and "I". In short, E and I neurons respond 180 degrees out of phase with each other. The inversion of the ampullary stimulus controls for the potential confound that the neurons in the midbrain may receive either E or I information from both ampullary and tuberous neurons in the ELL.

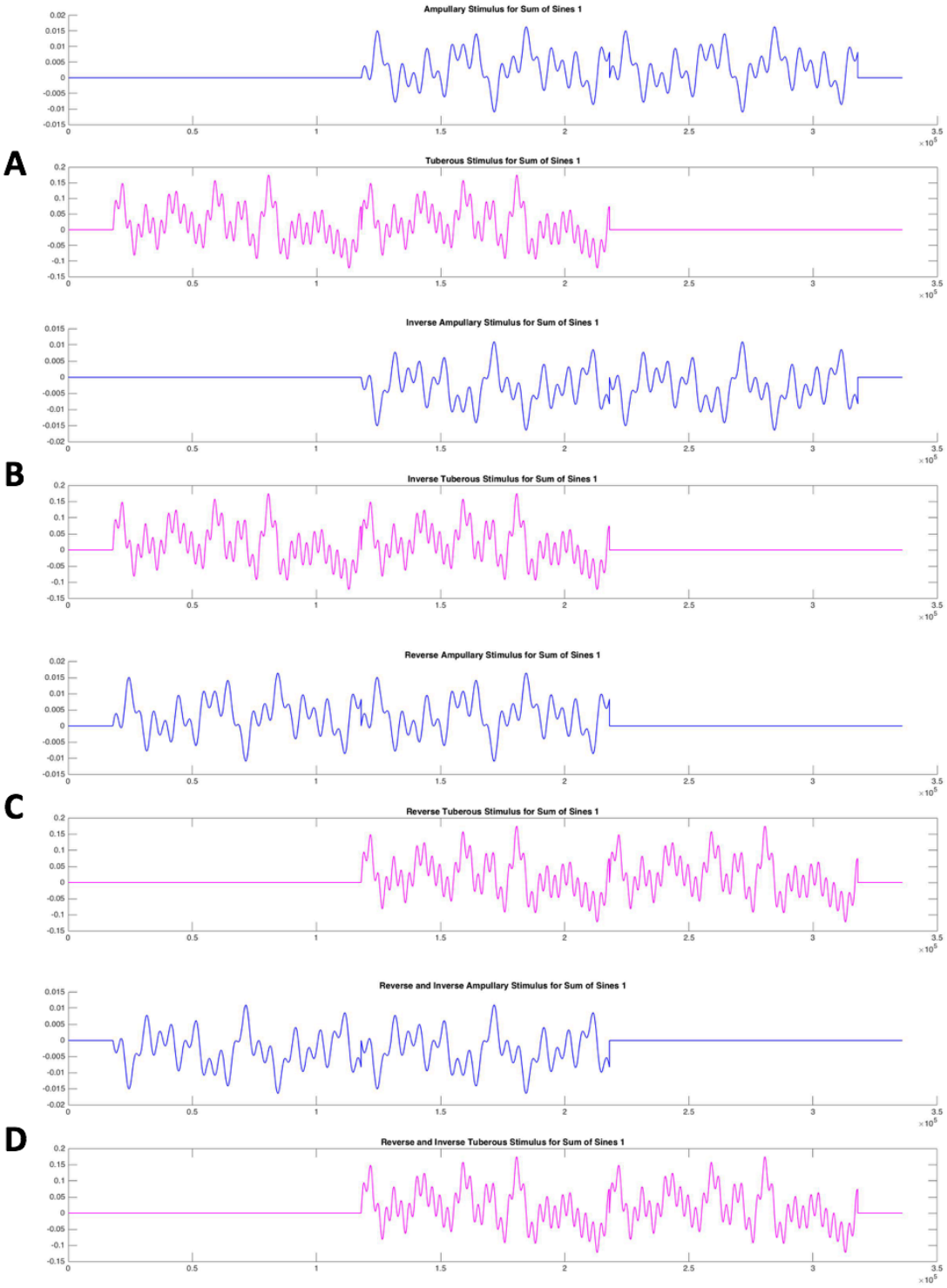

Figure 2.4 The Sum of Sines 1 stimulus shown in all four variations. (A) The 'normal' variation where only the tuberous signal (magenta) is played first, followed by the ampullary (blue). (B) The inverse of the 'normal' variation where the ampullary signal (blue) is inverted. (C) The backwards version where only the ampullary signal (blue) is played first, followed by the tuberous (magenta). (D) The backwards version of the inverse, where the ampullary signal (blue) is inverted and played first.

2.5 Data Analysis

Neurophysiological recordings were filtered in Spike2 using a second-order high-pass IIR filter with a cutoff between 100 and 150 Hz. Spike times were obtained using a user-set threshold. The data was then exported to MATLAB for analysis.

2.5.1 Sum of Sines— Single Sine Response Histogram

A custom MATLAB script was written to analyze the response of each neuron to the individual sine waves found in the stimuli. The spike trains were divided into epochs—the length of which was determined by the period of each sine wave frequency. These epochs were then used to create a histogram to illustrate the response of the neuron during that specific time interval.

2.5.2 Sum of Sines— Stimulus-Response Coherence

Coherence measures the shared power found between the stimulus frequencies and the response of the neuron. To calculate the coherence, the spike train was converted into an analogue signal by introducing an alpha function at each spike time. The coherence between the original stimulus and this reconstructed spike train was computed using the Matlab function mscohere. Based on previous reports, we interpreted coherence values of greater than 0.1 at stimulus frequencies to indicate a response.

2.5.3 Sum of Sines— Vector Strength

Vector strength is an estimate of how phase-locked the response of a neuron is to a stimulus frequency. The value was calculated using this equation:

$$VS = \sqrt{SinSum^2 + CosSum^2} \Big/ total\ spikes\ ,$$

where SinSum is created by summing the sine of each spike, and CosSum is generated by summing the cosine of each spike.

CHAPTER 3

RESULTS

3.1 Neurophysiological Recordings

We recorded 31 neurons in the Ts that responded to at least one modality – ampullary or tuberous. Out of the 31, only 14 neurons responded to tuberous stimuli (found between 470 and 1130 microns from the surface of the brain), 11 neurons responded to only ampullary stimuli (found between 400 and 930 microns from the surface of the brain), and 6 neurons were found to be strongly multimodal responding to both ampullary and tuberous stimuli when played separately (found at 365, 470, 600, 620, 630, and 730 microns from the surface of the brain). Two of the multimodal neurons responded only to ampullary features when the stimuli were played simultaneously; the other four multimodal neurons responded only to tuberous features when both modalities were presented together.

All three filtering categories of neurons showed high-pass, band-pass, and low-pass filtering properties in the range of 0.1 to 31 Hz in both ampullary-only and tuberous-only neurons. The multimodal neurons appeared to only be high-pass or all-pass filters (Table 3.1).

Table 3.1 Filtering in Multimodal Neurons

Multimodal Neuron	Ampullary Stimuli	Tuberous Stimuli
1) Mainly Ampullary	High-pass filter	High-pass filter
2) Mainly Ampullary	All-pass filter	All-pass filter
1) Mainly Tuberous	High-pass filter	Band-pass filter
2) Mainly Tuberous	High-pass filter	All-pass filter
3) Mainly Tuberous	High-pass filter	All-pass filter
4) Mainly Tuberous	High-pass filter	High-pass filter

3.2 Results from an Ampullary-only Neuron

Each stimulus was made from summing five sine waves of varying frequencies together. The stimuli were therefore pseudorandom (Figure 3.1a). In the data below, there is a slight increase in firing rate when the ampullary stimulus is introduced (Figure 3.1a blue). Figure 3.1b shows the relations between the stimulus and spiking activity.

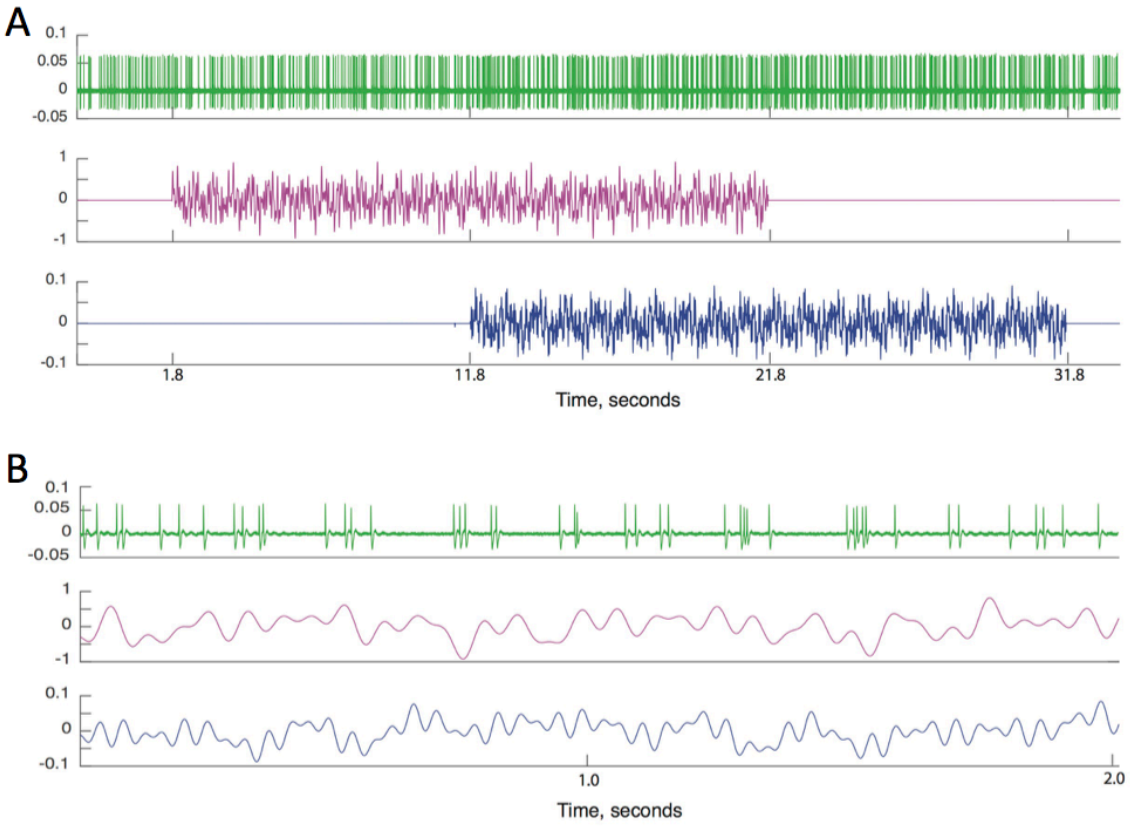

Figure 3.1 Response of an ampullary-only neuron to a Sum of Sines stimulus. (A) A figure showing the spikes (green) responding, or not responding to the stimulus. The upper stimulus is tuberous (dark pink) and the lower stimulus is ampullary (dark blue). (B) A shorter window taken from the middle of the stimulus where both stimuli were played together. Each spike (green) can be identified, and the frequency and amplitude changes in each stimulus (pink is tuberous and blue is ampullary) can be seen.

Raster plots and histograms were made for each individual sine wave by cutting the neuron's response into chunks that were the length of one period of each sine wave

frequency in the stimulus. In this way, the response of the neuron to many cycles of each sine wave frequency can be visualized independently (Figure 3.2 c). Higher frequency sine waves have more epochs during the stimulus period because their duration is shorter. Vector strength (Figure 3.2 a orange) and stimulus-response coherence (Figure 3.2 a blue) were calculated for the response to each sine wave frequency. A coherence of greater than 0.1 at a specific frequency is considered to be a significant response (McGillivray et al. 2012). The coherence measure estimates how much power is shared between the stimulus and the spikes generated by the neuron across frequencies. The vector strength measures the strength of phase-locking of activity to a specific stimulus frequency.

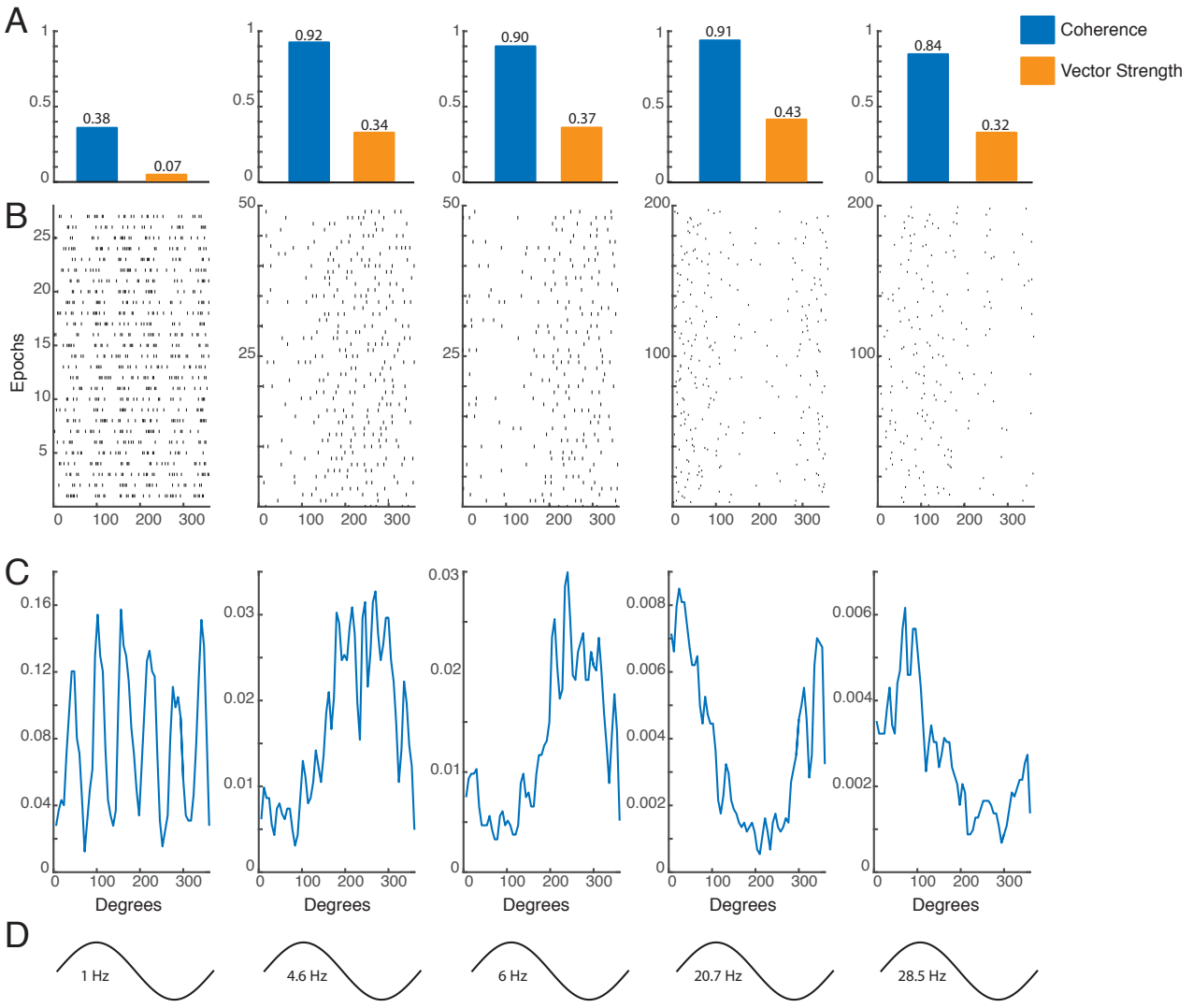

Figure 3.2 Example of the response from an ampullary-only neuron to the Sum of Sines stimulus 6 when the ampullary stimulus was played by itself. (A) This row shows the coherence (blue) and vector strength (orange) of the response to each of the five sine waves that summed to create the Sum of Sines stimulus 6. (B) Raster plots of the raw data corresponding to each frequency in the stimulus. The higher the frequency, the more repetitions are found in each stimulus (1 Hz has 10 cycles in 10 seconds, whereas 28.5 Hz has 285 cycles in 10 seconds). The y axis is repetitions and the x-axis is degrees. (C) The summed data from the raster plot, illustrating the response of the neuron. X-axis is again in degrees. (D) One period of a sine wave and the frequency that corresponds with the column of data.

3.3 Multimodal Neuron Data

Six neurons were deemed 'multimodal' as they responded to each modality with a coherence value greater than 0.1 at one or more of the stimulus frequencies. Four of these multimodal neurons responded only to the tuberous stimulus when both modalities were presented simultaneously (see Figure 3.3). For example, the neuron in Figure 3.3 responded to the ampullary stimulus when presented alone, but the coherence at the stimulus frequencies 7.3, 12.9, and 28.9 Hz are dramatically reduced when the stimuli are presented simultaneously. The coherence to the shared frequency (25.6 Hz) remains high, but is presumably driven by the tuberous stimulus. This conclusion is supported by the fact that the phase of the activity at the shared frequency matches the phase of the response to the tuberous stimulus and not the ampullary stimulus (Figure 3.4).

These results suggest that this neuron may follow an absolute dominance approach, as ampullary responses (Figure 3.3a, striped blue line) were silenced while tuberous responses remained (Figure 3.3b, striped maroon line). Note that the coherence to the tuberous stimulus appears unchanged across frequencies. This is surprising because we expected the response to the shared frequency to be facilitated under multisensory stimulation.

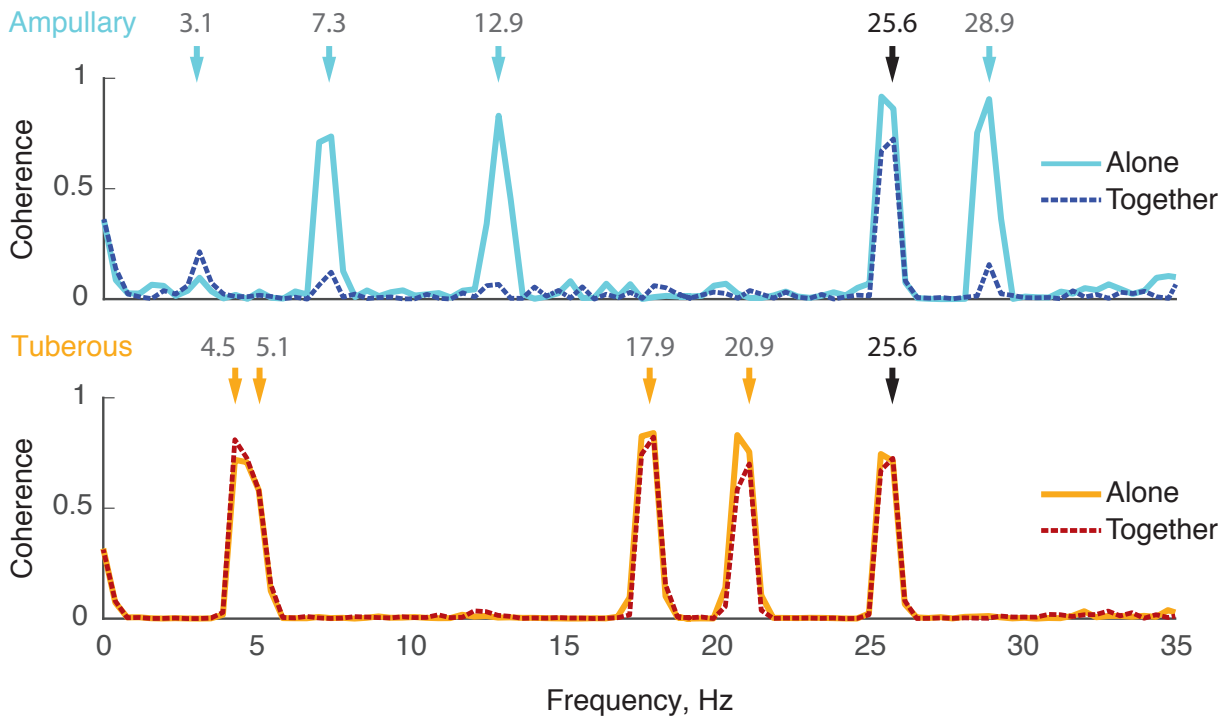

Figure 3.3 Stimulus-response coherence data from a multimodal neuron. Coherence to ampullary stimuli are indicated in blue colors and tuberous in red colors. Each of the five sine wave frequencies are indicated with the arrows (see Table 2.2). When the ampullary and tuberous stimuli were played alone, the coherence to both ampullary (top) and tuberous (bottom) stimuli were high at the stimulus frequencies. However, when presented simultaneously, coherence to the ampullary stimulus was reduced (top dashed line). The coherence at the shared frequency is presumably driven by a response to the tuberous stimulus when the two stimuli were presented simultaneously (see Figure 3.4).

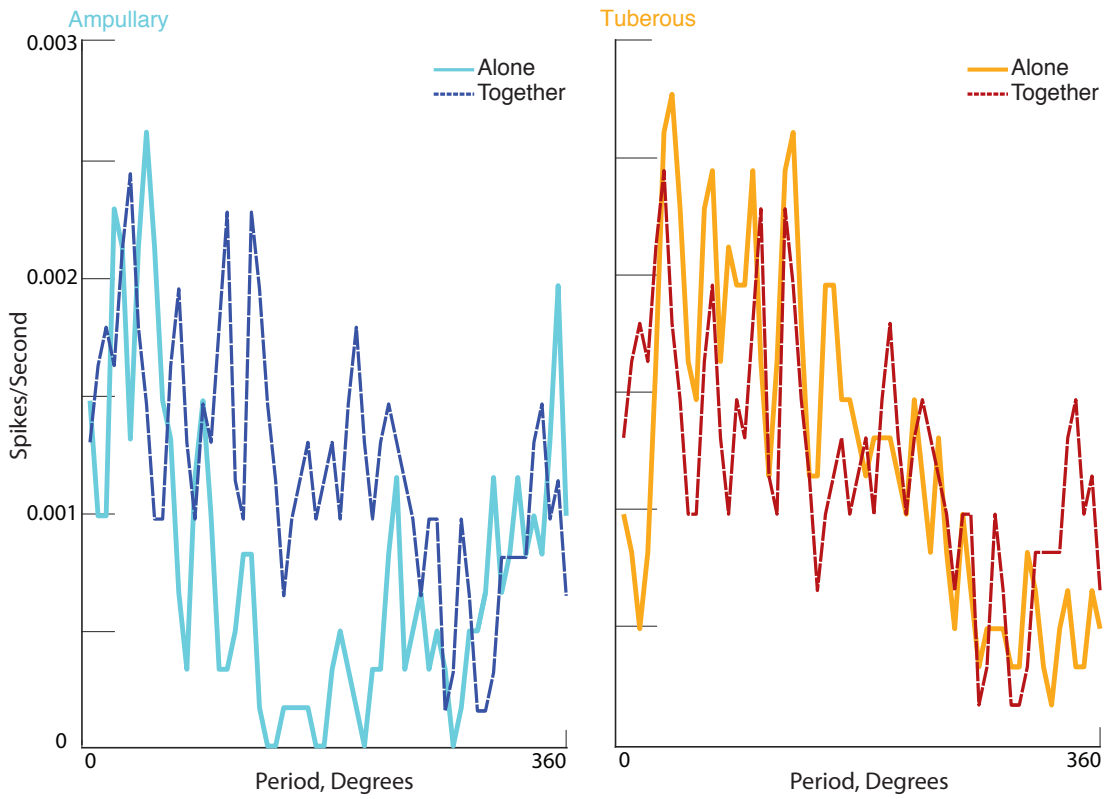

Figure 3.4 Histogram of the multimodal neuron to the common frequency of 25.6 Hz. The neuron responds to both modalities when they are played separately, and only to tuberous when they are presented simultaneously.

In two bimodal neurons, the tuberous responses were suppressed when the modalities were presented simultaneously. For example, the coherence of spiking to each of the tuberous stimulus frequencies, 4.5, 5.1, 17.9, and 20.9 Hz, were reduced when the ampullary and tuberous stimuli were presented simultaneously as compared to when the tuberous stimulus was presented alone (Figure 3.5). Taken together, these results show that either ampullary or tuberous responses can be suppressed in multisensory neurons.

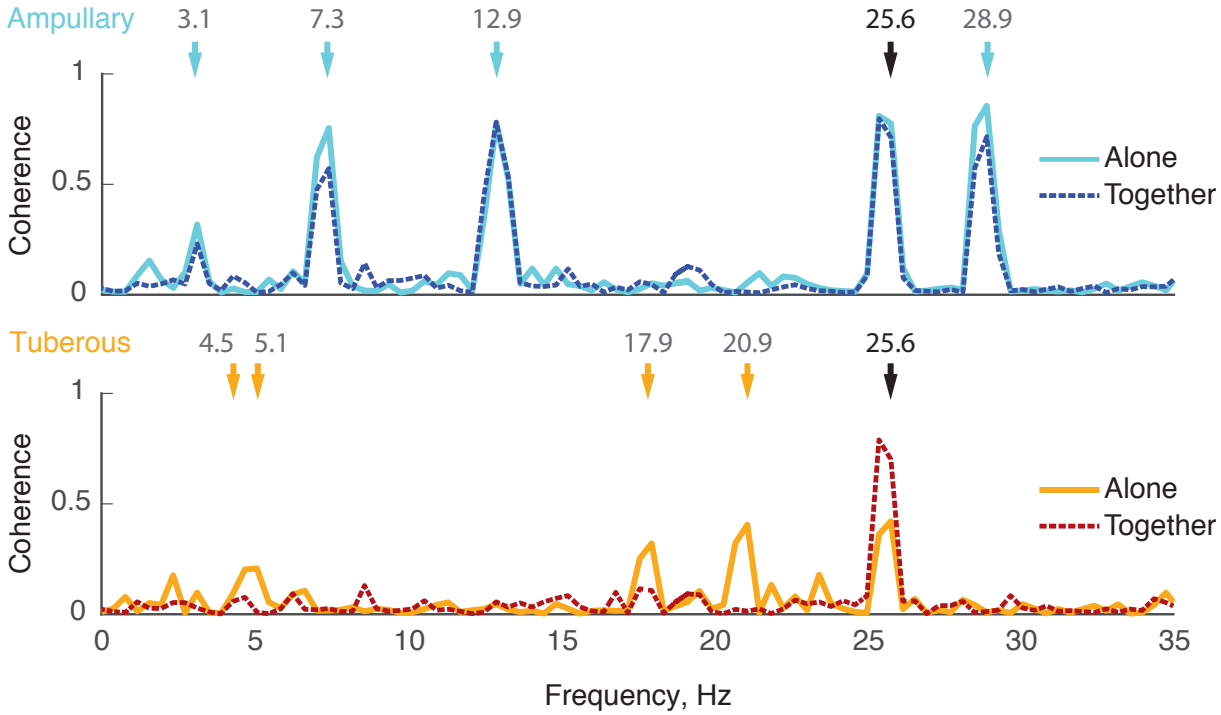

Figure 3.5 Another example of stimulus-response coherence data from a multimodal neuron. Coherence to ampullary stimuli are indicated in blue colors and tuberous in red colors. Each of the five sine wave frequencies are indicated with the arrows (see Table 2.2). When the ampullary and tuberous stimuli were played alone, the coherence to both ampullary (top) and tuberous (bottom) stimuli were above 0.1 at the stimulus frequencies. However, when presented simultaneously, coherence to the tuberous stimulus was reduced (bottom dashed line). The coherence at the shared frequency is presumably driven by a response to the tuberous stimulus when the two stimuli were presented simultaneously.

We measured the coherence at each stimulus frequency and compared the responses between stimulation regimes – stimulus alone versus stimuli presented simultaneously. For the stimulus frequencies that were not shared between the two stimuli, the coherence was significantly reduced in one modality (paired t-test, N=6, $p<0.05$). We did not find a statistically significant change in the coherence for the shared frequency (Figure 3.6).

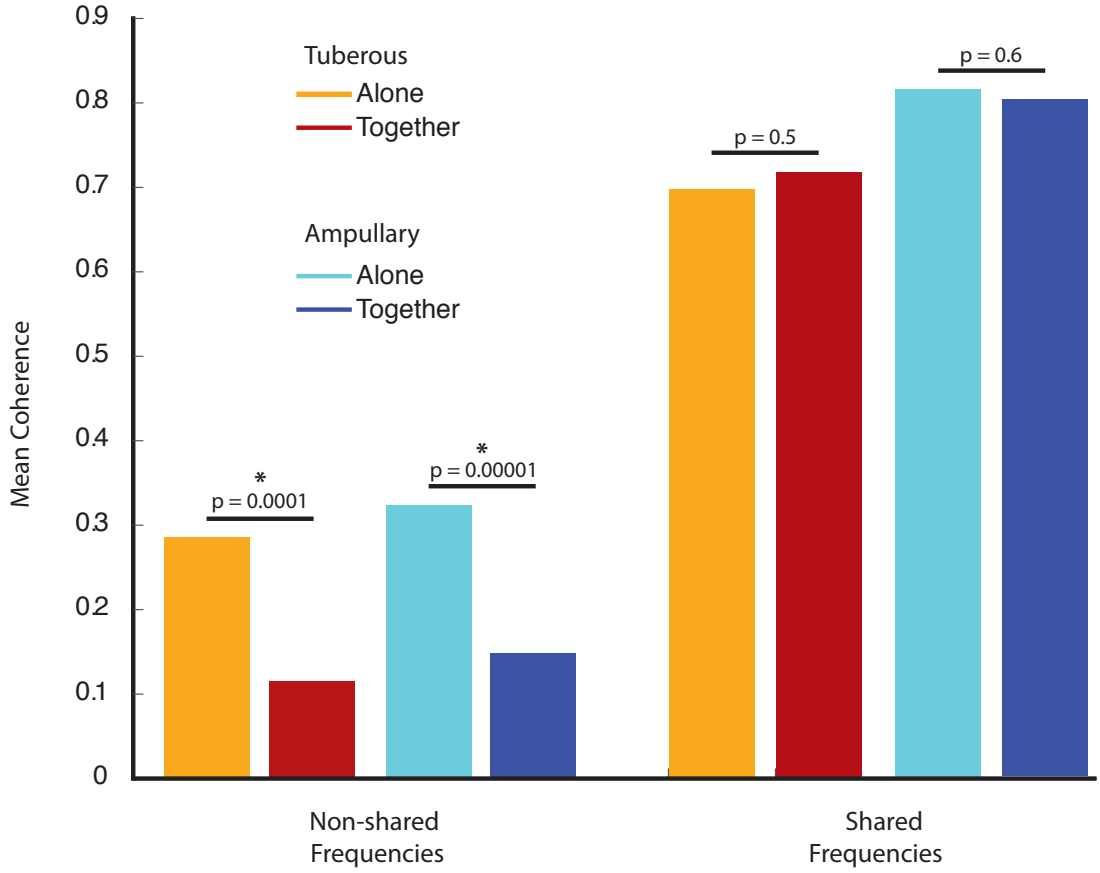

Figure 3.6 Paired t-test comparing the two types of multimodal neurons. The non-shared frequencies of the 'lessor' modality were shown to significantly decrease in response to playing the modalities together. The difference in coherence between the common frequencies was not found to be significantly different.

3.4 Another Category of Multimodal Neuron— the Silent Killers

Six of the 14 tuberous-only neurons and two of the ten ampullary-only neurons were categorized into a special group of multimodal neurons, known as *'silent killers.'* These six neurons increased spiking rates, coherence, and vector strength when one of the two (either tuberous or ampullary) was presented alone. However, when both modalities were presented simultaneously, the neuron changed its behavior – the coherence was reduced.

For example, a tuberous-only neuron changed its response when the ampullary stimulus was introduced. The neuron shown in Figure 3.7 does not respond to ampullary stimuli as seen in the very low coherence at stimulus frequencies. However, when the two modalities were played simultaneously, the tuberous responses were reduced.

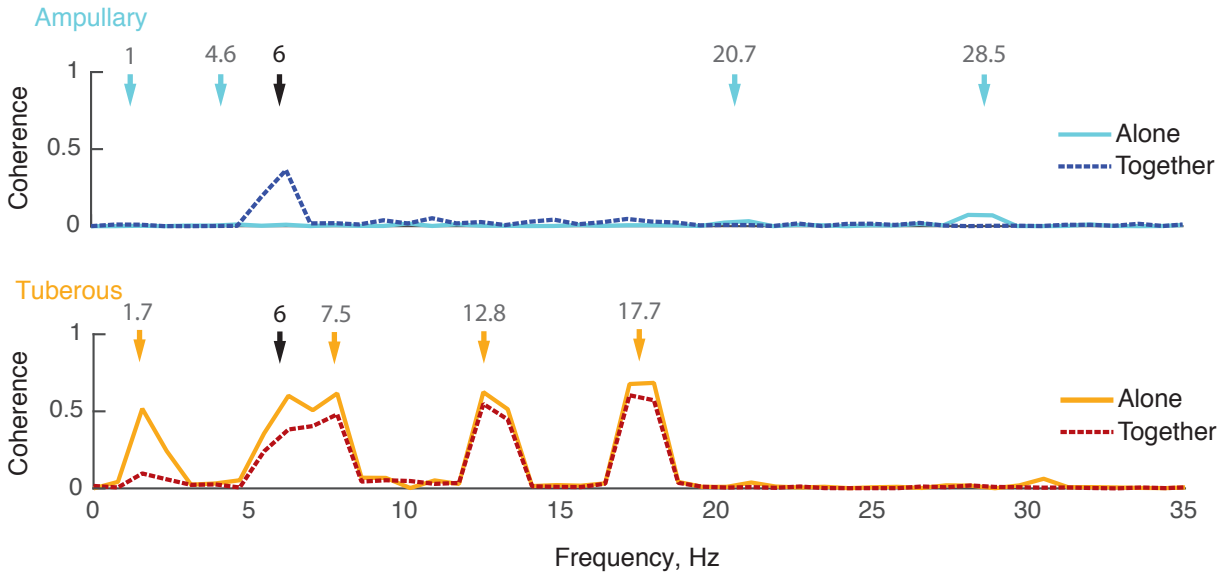

Figure 3.7 An example of a tuberous silent killer neuron. The neuron does not respond to ampullary stimuli, but when the stimuli are played together, the tuberous response to 1.7 Hz and 6 Hz is suppressed and the response to the other three frequencies is decreased.

Another example, an ampullary-only neuron responded with a stimulus-response coherence of greater than 0.5 to all five sine waves of the stimulus when the ampullary stimulus was played alone. When the tuberous stimulus was presented at the same time, however, the responses to 1 Hz and 20.7 Hz were suppressed (Figure 3.8).

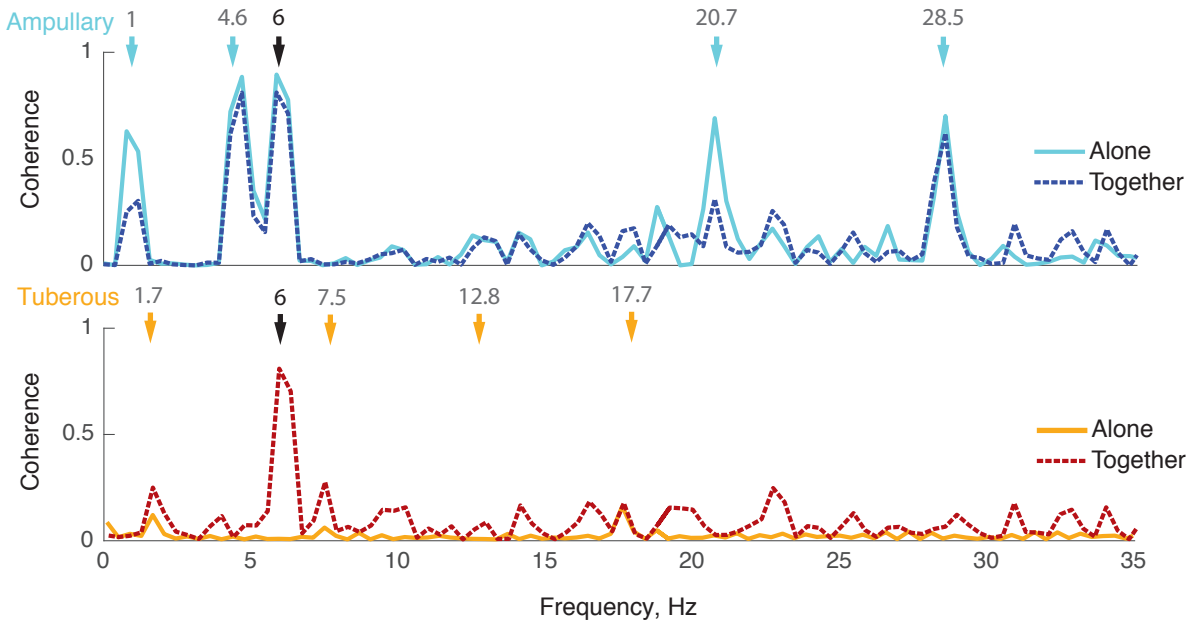

Figure 3.8 An example of an ampullary silent killer neuron. The neuron does not respond to tuberous stimuli, but when the stimuli are played together, the ampullary response to 1 Hz and 20.7 Hz is decreased.

The reduction in coherence to both the non-shared frequencies and the shared frequencies in these neurons were statistically significant (paired t-test, p=0.01, p=0.0004 respectively). This interaction suggests that the neuron receives 'silent' inputs from the apparently non-responsive modality. These could be weak excitatory inputs that are not strong enough to elicit action potentials. Alternatively, the tuberous inputs might be inhibitory, thus causing decreased responses.

CHAPTER 4

DISCUSSION

4.1 Summary of Results

We examined the idea that congruent sensory signals across sensory modalities would lead to facilitated responses in midbrain neurons. We used complex electrosensory stimuli that were composed of sinusoids at different frequencies – only one of the frequencies was shared across modalities. We predicted that the response to the shared sine wave frequency would be enhanced in multimodal stimuli.

In six multimodal neurons (Section 3.3), we found that the response to the shared frequency was unchanged in multimodal stimuli, and that responses to unshared frequencies in one modality was suppressed. These multimodal neurons appeared to use the absolute dominance approach when responding to simultaneous ampullary and tuberous stimuli— one modality was always suppressed, while the other had either the same coherence response values, or values that were slightly decreased. This would mean that there was a 'dominant' modality that was judged more salient and therefore was encoded by the neuron. Responses to the suppressed modality were significantly decreased when both modalities were played together (Paired t-test, ampullary $p = 0.00001$; tuberous $p = 0.001$). Importantly, none of the six multimodal neurons recorded from exhibited multisensory enhancement of the shared frequency when both stimuli were played simultaneously— they either had the same or a slightly decreased response.

The 'silent killers' (Section 3.4) only showed coherences above 0.1 to one modality, but nevertheless showed significant reduction in coherence when the two

modalities were played simultaneously. This reduction illustrates that the other modality had some effect on the neuron; the second modality might inhibit the neuron causing it to not respond to specific frequencies and/or cause an overall decrease in firing rate. This relationship can be beneficial to the neuron because it could reduce noise through reducing the firing rate, and it might also be a form of enhancement by increasing the signal to noise ratio. Increased signal to noise ratios is a method for improving signal detection.

4.2 Relations Between Tuberous and Ampullary Signals in *Eigenmannia*

4.2.1 Encoding of Social Signals

Eigenmannia are social fish that are most commonly found in groups of 3 to 5 fish. When *Eigenmannia* are in groups, the electric fields interact to produce AMs that are encoded by tuberous receptors. The average difference between the EOD frequencies is generally between 23 and 41 Hz, which cause modulations in firing in tuberous neurons at those rates. These modulations, at least in laboratory conditions do not stimulate ampullary receptors (Tan et al. 2005; Fortune 2006).

These relatively high frequencies, above about 20 Hz and up to about 100 Hz, are a frequency band that is encoded by tuberous electroreceptors during social interactions. In the laboratory, social stimuli do not stimulate ampullary receptors. *Eigenmannia* therefore do not experience correlations between ampullary and tuberous activity within this frequency range. Nevertheless, neurons that responded to both ampullary and tuberous stimuli in this frequency range were found, and the suppression of responses during multisensory stimulation included these frequencies (Tan et al. 2005).

Why would the effects of multisensory stimulation include frequencies in a range that is believed to always be unimodal – tuberous? In the wild fish may indeed experience correlated stimulation of ampullary and tuberous receptors in this frequency range. The reason for this difference between the laboratory and the field is that in the laboratory sinewaves with no DC offset are used, whereas the EOD of *Eigenmannia* is psuedosinusoidal and has a small DC offset. There is the possibility that the combination of DC offsets and shape of the EOD signal result in concomitant oscillations detected by ampullary receptors, leading to the possibility of correlated activity in tuberous and ampullary systems. Note that in the closely-related genus *Apteronotus*, the EOD has no DC offset. It may be that multisensory integration of ampullary and tuberous information in this frequency band may be different in species of *Apteronotus* than was observed in *Eigenmannia* (Stoddard & Markham 2008; Hagedorn & Heiligenberg 1985).

Finally, during courtship Eigenmannia produce electrical "chirps" which include interuptions in the ongoing EOD. These chirps produce both a dramatic amplitude modulation and a DC shift. These signals strongly activate both tuberous and ampullary electrosensory systems (Stoddard & Markham 2008; Hagedorn & Heiligenberg 1985).

4.2.2 Prey Capture and Lower Frequency Signals

Prey cause small perturbations in the electric field of fish due to their electrical impedance being different than that of the water. These small perturbations are often of low frequency (< 10 Hz), matching the relative rates of movements between the prey and the fish. These perturbations of the autogenous electric field are encoded by tuberous electroreceptors. Prey also stimulate ampullary receptors because the tissues are negatively charged relative to the water, and because any contractions/movements will

result in local depolarizations. The swimming motions of a *Daphnia*, for example, are on the order of a few Hz. If the *Daphnia* is moving relative to the fish, an ampullary receptor may be activated both by the oscillation due to the swimming movements of the *Daphnia* and in relation to the distance of the prey from the fish (Nelson & MacIver 1999).

These data suggest that prey capture will generate correlated information in both the ampullary and tuberous systems at low frequencies, below about 10 Hz. Interestingly, the suppression effect appears to be stronger for low frequencies than for higher frequencies. Indeed, any movement-related signals, such as tail wagging and other locomotor behaviors, generate low frequency signals (< 10 Hz) that are correlated in both ampullary and tuberous systems (Nelson & MacIver 1999).

Higher frequency signals, above 20 Hz, that the fish experience are generally created by communication signals and interactions with other conspecifics, which are almost exclusively encoded by the tuberous system. In this way, the differences in frequency could be a mechanism for segregating different categories of sensory information that have different statistics in relation to the expected correlations between ampullary and tuberous information. In other words, the nervous system should expect to see more correlations between ampullary and tuberous information at low frequencies and fewer at high frequencies. This is a question that we should explore both using neurophysiological tests, but perhaps more importantly, through behavioral measurements.

4.3 Envelopes

"Envelopes" is a term used in the electric fish community that refers to changes in the depth of modulation of the EOD amplitude modulation (AM). Envelopes can be produced in two ways: social envelopes can be created when three or more fish are close in proximity to each other and movement envelopes are modulations of AMs in relation to the distances between 2 or more fish. Movement envelopes are typically of lower frequency content and depend on the positions of each fish— how the distance and orientation changes between two or more fish. In nature, *Eigenmannia* experience both social and movement envelopes at the same time, but this information is restricted to only the tuberous system (Fortune & Rose 2000; Tan et al. 2005; Stamper et al. 2013).

It is possible that envelopes may also occur in a different way in the ampullary system. Consider that a Daphnia produces an oscillation related to its own swimming movements at frequencies around 10 Hz. If the Daphnia moves relative to the electric fish, the amplitude of the 10 Hz oscillations will be modulated at a rate that is identical to the relative velocities of the fish. This would presumably modulate the strength of the oscillation of ampullary neurons, thereby encoding this new form of envelope (Fortune & Rose 2000; Tan et al. 2005; Stamper et al. 2013; Nelson & MacIver 1999).

Because prey stimulate both the tuberous and ampullary systems in relation to the distance of the animal from the prey, the envelope of the ampullary stimulus may be correlated with tuberous information. These experiments did not examine the encoding of envelopes in the ampullary system, and therefore may be missing the critical form of multisensory integration used by these animals.

4.4 Receptive Fields and Multisensory Integration

There were two categories of neurons that showed multisensory suppression. In one type, the neurons responded to both ampullary and tuberous stimuli when they were presented by themselves. In the other type, "silent killers", neurons only responded to one of the two modalities. Are these two types of neurons or not?

The receptive fields of these neurons were not mapped. It is possible that a neuron may have different receptive fields for ampullary stimuli and tuberous stimuli. In these experiments, a local dipole was used to generate both stimulus categories and thus the region that was stimulated was identical for both ampullary and tuberous. Further, receptive fields of electroreceptors are known to have classic center-surround organization, and therefore the stimulus may have been centered for one modality and off center for the other. This may have had an impact on both the responses of the neurons to each stimulus alone and also the multisensory integration of those stimuli. Future experiments should explore the receptive field properties of these neurons.

4.5 Beyond Multisensory Integration: the Binding Problem

Multisensory integration can be used to increase the reliability of perception by reducing the effects of variability in the nervous system. Combining information, however, creates a challenge for neural computation, which is known as the Binding Problem. The Binding Problem refers to the challenge of constructing a single perceptual object from multisensory information. (Roskies 1999) How does the brain assemble a single percept of "grandmother" from her visual image and her acoustic profile?

The binding problem is divided into two components. The segregation problem refers to the mechanisms that the brain uses to segregate sensory objects. The combination problem refers to the strategies by which different modalities assembled into a unified sensation. Consider two boxes that differ in shape and color. The segregation problem deals with how the brain is able to keep those two characteristics uniquely defined in the brain— the ideas of 'red', 'blue', 'cube' and 'rectangular prism' staying separate. The combination problem then looks at how the two qualities are put back together— the brain needs to ensure that the blue cube the eyes see is the blue cube that the brain encodes and is not changed to being red (Revonsuo & Newman 1999; Zahar et al. 2009; Roskies 1999).

Keeping perceptual objects separate and combining them correctly can depend on features such as spatial agreement, properties of mechanisms in the brain, and the timing of the information. The binding problem can be used to describe different streams of information within a single modality (consider different views of the same face) or multiple modalities (consider the association between the sound the drum makes with the visual percept of the drummer's sticks; Revonsuo & Newman 1999; Zahar et al. 2009; Roskies 1999).

Can we address the binding problem via study of ampullary and tuberous systems in *Eigenmannia*? In a way, we have already started to examine the binding problem by asking neurons to recognize similar signals across modalities. However, this test uses sensory systems in which the encoding is similar if not identical. Perhaps a more interesting test would be to train animals, for example, to recognize patterns of frequency

differences between the modalities. In this way, the brain is recognizing a pattern in the differences between codes, rather than identifying similarities in codes.

4.6 Future Directions

The main result was that multimodal stimulation led to a suppression of information that was not correlated across modalities. This suppression occurred in only one modality, and which modality (ampullary or tuberous) varied between neurons. It remains unclear both how and why this suppression occurs. It remains possible that this result is an epiphenomenon related to the relative amplitudes of stimulation from each modality. Consider that each neuron has a spatial receptive field, and that the distribution of receptors within this spatial receptive field differs between modalities. So, for any stimulus configuration, it is possible that one modality will be more strongly stimulated than the other modality.

One experimental approach that can be used to examine this issue is to vary the relative amplitudes of the two stimuli. By changing the amplitude of each stimulus, we may be able to determine amplitudes of each stimulus that result in the same magnitude response. We could then titrate the amplitude to see its effects on the suppression effect. There are two likely outcomes of these experiments. We may find that the neuron maintains its preference for a particular modality without respect to the amplitudes of the stimulus. This would suggest that each neuron has a specific preference for one modality. Alternatively, we may find that the suppression effect switches modality depending on the relative amplitudes of the two stimuli. This would suggest that each

neuron may dynamically shift its properties to focus on the most reliable sensory information available to it.

Another major open question is the effects of correlated and uncorrelated information on responses in these neurons. The stimuli that we used had four uncorrelated sine waves and one shared sine wave. In other words, most of the information was uncorrelated between the two modalities. However, we might expect that under natural conditions that the level of correlation between ampullary and tuberous information might be much higher. Consider that autogenous movement will simultaneously affect both tuberous and ampullary receptors, leading to massive correlation between them. The effects that we observed, therefore, may be a result of very low correlations between the two modalities. Future experiments would vary the level and strength of correlations across modalities. The simplest experimental manipulation to test this idea would be to change the number of shared versus unshared frequencies presented across modalities. Perhaps we will discover a correlation threshold at which the multisensory enhancement that was central to our main hypothesis may emerge. In other words, we may observe non-linear facilitation when correlations across modalities are greater than those used in this study. Alternatively, we may find that the level of correlations has little or no effect on response profiles.

The second idea is to alter the Sum of Sines stimulus. In this experiment, the Sum of Sines stimuli were composed of five different sine waves, with only one of the five being common between ampullary and tuberous. The alteration would be to make stimuli with more than one common frequency, possibly use four common frequencies and only have one being different. By doing this, there will be more correlation between

the stimuli, so maybe the neuron will show multisensory enhancement, or some other type of nonlinear facilitation. The stimuli used in this experiment were pseudorandom and maybe did not emulate signals the fish would encounter in their natural environment— they might have overloaded the neurons— therefore by creating more correlated stimuli, the neurons might respond differently and possibly respond to both modalities when they are played simultaneously.

APPENDIX A

Shank Solution Recipe

The recipe creates the shank solution that is injected inside the electrode before it is inserted into the brain.

Stock Solution Recipe:

	To make 100 ml (g)	To make 50 ml (g)
K-Gluconate	2.3425	1.17
KCl	0.0149	0.0075
$MgCl_2 \cdot 6\ H_2O$	0.0203	0.0102
EGTA	0.1902	0.0951
HEPES	0.2383	0.1191
KOH	1.9 ml *	1.0 ml *

* Add KOH until the pH is approximately 7.4 (Typical amounts are listed)

Be sure that each component dissolves completely before the next is added

Shank Solution Recipe:

*take 25ml of Stock solution

*add 0.196 g Mannitol

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