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(IJITR) INTERNATIONAL JOURNAL OF INNOVATIVE TECHNOLOGY AND RESEARCH
Volume No.6, Issue No.6, October - November 2018, 8792-8807

Micro-Stimulation Of Neurons

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Abstract: **This project encompasses the basic understanding of neuropharmacology. Neuropharmacology is the study of how drugs affect cellular function in the nervous system, and the neural mechanisms through which they influence behavior. There are two main branches of neuropharmacology: behavioral and molecular. Behavioral neuropharmacology focuses on the study of how drugs affect human behavior (neuropsychopharmacology), including the study of how drug dependence and addiction affect the human brain.Molecular neuropharmacology involves the study of neurons and their neurochemical interactions, with the overall goal of developing drugs that have beneficial effects on neurological function. Both of these fields are closely connected, since both are concerned with the interactions of neurotransmitters, neuropeptides, neurohormones, neuromodulators, enzymes, second messengers, co-transporters, ion channels, and receptor proteins in the central and peripheral nervous systems. Studying these interactions, researchers are developing drugs to treat many different neurological disorders, including pain, neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, psychological disorders, addiction, and many others.**

Before understanding the effect of drugs we studied firing rates of neurons and neural networks. Initially we started off understanding firing rates and at what frequencies spikes will be recorded on the SpikerBox. We found that at low frequencies we can observe spikes. Later we started stimulating muscles in the cockroach's leg using music. Upon varying bass, treble, etc we noticed the difference in spikes and recorded the same. After understanding the spikes we injected Nicotine and Mono Sodium Glutamate with control (water) at intervals of 2-4 minutes to observe which drug would have an effect on the neurons. MSG is present in 80% networks however for insects we found Nicotine stimulates and MSG does not.

I. INTRODUCTION

Neural Coding is a Neuroscience- related field concerned with characterizing the relationship between the stimulus and the individual or ensemble neuronal responses and the relationship among the electrical activity of the neurons in the ensemble.Based on the theory that sensory and other information is represented in the brain by networks of neurons, it is thought that neurons can encode both digital and analog information.

Neurons are remarkable among the cells of the body in their ability to propagate signals rapidly over large distances. They do this by generating characteristic electrical pulses called Action Potentials: voltage spikes that can travel down nerve fibers. Sensory neurons change their activities by firing sequences of action potentials in various temporal patterns, with the presence of external sensory stimuli, such as light, sound, taste, smell and touxch. It is known that information about the stimulus is encoded in this pattern of action potentials and transmitted into and around the brain. Although action potentials can vary in duration, amplitude and shape, they are typically treated as identical stereotyped events in neural coding studies.

The study of Neural Coding involves measuring and characterizing how stimulus attributes, such as light or sound intensity, or motor actions, such as the direction of an arm movement, are represented by neuron action potentials or spikes. In order to describe or analyze neuronal firing, statistical methods and methods of probability theory and stochastic point processes have been widely applied. With the development of large-scale neural recording and decoding technologies, researchers have begun to crack the neural code and already provided the first glimpse into the real-time neural code as memory is formed and recalled in the hippocampus, a brain region known to be central for memory formation.Neuroscientists have initiated several large-scale brain decoding projects.

The link between stimulus and response can be studied from two opposite points of view. Neural encoding refers to the map from stimulus to response. The main focus is to understand how neurons respond to a wide variety of stimuli, and to construct models that attempt to predict responses to other stimuli. Neural decoding refers to the reverse map, from response to stimulus, and the challenge is to reconstruct a stimulus or certain aspects of that stimulus, from the spike sequences that it evokes.

A sequence, or 'train', of spikes may contain information based on different coding schemes. In motor neurons, for example, the strength at which an innervated muscle is flexed depends solely on the 'firing rate', the average number of spikes per unit time (a 'rate code'). At the other end, a complex 'temporal code' is based on the precise timing of single spikes. They may be locked to an external stimulus such as in the visual and auditory system or be generated intrinsically by the neural circuitry.

Whether neurons use rate coding or temporal coding is a topic of intense debate within the neuroscience community, even though there is no clear definition

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of what these terms mean. In one theory, termed "neuroelectrodynamics", the following coding schemes are all considered to be epiphenomena, replaced instead by molecular changes reflecting the spatial distribution of electric fields within neurons as a result of the broad electromagnetic spectrum of action potentials, and manifested in information as spike directivity. The rate coding model of neuronal firing communication states that as the intensity of stimulus increases, the frequency or rate of action potentials, or "spike firing", increases. Rate coding is sometimes called frequency coding. Rate coding is a traditional coding scheme, assuming that most, if not all, information about the stimulus is contained in the firing rate of the neuron. Because the sequence of action potentials generated by a given stimulus varies from trial to trial, neuronal responses are typically treated statistically or probabilistically. They may be characterized by firing rates, rather than as specific spike sequences. In most sensory systems, the firing rate increases, generally non-linearly, with increasing stimulus intensity. Any information possibly encoded in the temporal structure of the spike train is ignored. Consequently, rate coding is inefficient but highly robust with respect to the ISI 'noise'.

During rate coding, precisely calculating firing rate is very important. In fact, the term "firing rate" has a few different definitions, which refer to different averaging procedures, such as an average over time or an average over several repetitions of experiment. In rate coding, learning is based on activity-dependent synaptic weight modifications.

Rate coding was originally shown by ED Adrian and Y Zotterman in 1926. In this simple experiment different weights were hung from a muscle. As the weight of the stimulus increased, the number of spikes recorded from sensory nerves innervating the muscle also increased. From these original experiments, Adrian and Zotterman concluded that action potentials were unitary events, and that the frequency of events, and not individual event magnitude, was the basis for most inter-neuronal communication.

In the following decades, measurement of firing rates became a standard tool for describing the properties of all types of sensory or cortical neurons, partly due to the relative ease of measuring rates experimentally. However, this approach neglects all the information possibly contained in the exact timing of the spikes. During recent years, more and more experimental evidence has suggested that a straightforward firing rate concept based on temporal averaging may be too simplistic to describe brain activity.

The Spike-count rate, also referred to as temporal average, is obtained by counting the number of spikes that appear during a trial and dividing by the duration of trial. The length T of the time window is set by experimenter and depends on the type of neuron recorded from and the stimulus. In practice, to get sensible averages, several spikes should occur within the time window. Typical values are T = 100 ms or T = 500 ms, but the duration may also be longer or shorter.

The spike-count rate can be determined from a single trial, but at the expense of losing all temporal resolution about variations in neural response during the course of the trial. Temporal averaging can work well in cases where the stimulus is constant or slowly varying and does not require a fast reaction of the organism — and this is the situation usually encountered in experimental protocols.

Real-world input, however, is hardly stationary, but often changing on a fast time scale. For example, even when viewing a static image, humans perform saccades, rapid changes of the direction of gaze. The image projected onto the retinal photoreceptors changes therefore every few hundred milliseconds.

Despite its shortcomings, the concept of a spike-count rate code is widely used not only in experiments, but also in models of neural networks. It has led to the idea that a neuron transforms information about a single input variable (the stimulus strength) into a single continuous output variable (the firing rate). There is a growing body of evidence that in Purkinje neurons, at least, information is not simply encoded in firing but also in the timing and duration of non-firing, quiescent periods.

The time-dependent firing rate is defined as the average number of spikes (averaged over trials) appearing during a short interval between times t and t+ t, divided by the duration of the interval. It works for stationary as well as for time-dependent stimuli. To experimentally measure the time-dependent firing rate, the experimenter records from a neuron while stimulating with some input sequence. The same stimulation sequence is repeated several times and the neuronal response is reported in a Peri-Stimulus-Time Histogram (PSTH). The time t is measured with respect to the start of the stimulation sequence. The t must be large enough (typically in the range of one or a few milliseconds) so there are sufficient number of spikes within the interval to obtain a reliable estimate of the average. The number of occurrences of spikes nK(t;t+ t) summed over all repetitions of the experiment divided by the number K of repetitions is a measure of the typical activity of the neuron between time t and t+ t. A further division by the interval length t yields time-dependent firing rate r(t) of the neuron, which is equivalent to the spike density of PSTH.

For sufficiently small t, r(t) t is the average number of spikes occurring between times t and t+ t over multiple trials. If t is small, there will never be more than one spike within the interval

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between t and t+ t on any given trial. This means that r(t) t is also the fraction of trials on which a spike occurred between those times. Equivalently, r(t) t is the probability that a spike occurs during this time interval.

As an experimental procedure, the time-dependent firing rate measure is a useful method to evaluate neuronal activity, in particular in the case of time-dependent stimuli. The obvious problem with this approach is that it cannot be the coding scheme used by neurons in the brain. Neurons cannot wait for the stimuli to repeatedly present in an exactly same manner before generating response.

Nevertheless, the experimental time-dependent firing rate measure can make sense, if there are large populations of independent neurons that receive the same stimulus. Instead of recording from a population of N neurons in a single run, it is experimentally easier to record from a single neuron and average over N repeated runs. Thus, the time-dependent firing rate coding relies on the implicit assumption that there are always populations of neurons.

NEUROPHARMACOLOGY

Neuropharmacology is the study of how drugs affect cellular function in the nervous system, and the neural mechanisms through which they influence behavior. There are two main branches of neuropharmacology: behavioral and molecular. Behavioral neuropharmacology focuses on the study of how drugs affect human behavior (neuropsychopharmacology), including the study of how drug dependence and addiction affect the human brain.Molecular neuropharmacology involves the study of neurons and their neurochemical interactions, with the overall goal of developing drugs that have beneficial effects on neurological function. Both of these fields are closely connected, since both are concerned with the interactions of neurotransmitters, neuropeptides, neurohormones, neuromodulators, enzymes, second messengers, co-transporters, ion channels, and receptor proteins in the central and peripheral nervous systems. Studying these interactions, researchers are developing drugs to treat many different neurological disorders, including pain, neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, psychological disorders, addiction, and many others.

Neuropharmacology did not appear in the scientific field until, in the early part of the 20th century, scientists were able to figure out a basic understanding of the nervous system and how nerves communicate between one another. Before this discovery, there were drugs that had been found that demonstrated some type of influence on the nervous system. In the 1930s, French scientists began working with a compound called phenothiazine in the hope of synthesizing a drug that would be able to combat malaria. Though this drug showed very little hope in the use against malaria-infected individuals, it was found to have sedative effects along with what appeared to be beneficial effects toward patients with Parkinson’s disease.

This black box method, wherein an investigator would administer a drug and examine the response without knowing how to relate drug action to patient response, was the main approach to this field, until, in the late 1940s and early 1950s, scientists were able to identify specific neurotransmitters, such as norepinephrine (involved in the constriction of blood vessels and the increase in heart rate and blood pressure), dopamine (the chemical whose shortage is involved in Parkinson’s disease), and serotonin (soon to be recognized as deeply connected to depression). In the 1950s, scientists also became better able to measure levels of specific neurochemicals in the body and thus correlate these levels with behavior.The invention of the voltage clamp in 1949 allowed for the study of ion channels and the nerve action potential. These two major historical events in neuropharmacology allowed scientists not only to study how information is transferred from one neuron to another but also to study how a neuron processes this information within itself.

Neurochemical interactions:

To understand the potential advances in medicine that neuropharmacology can bring, it is important to understand how human behavior and thought processes are transferred from neuron to neuron and how medications can alter the chemical foundations of these processes.

Neurons are known as excitable cells because on its surface membrane there are an abundance of proteins known as ion-channels that allow small charged particles to pass in and out of the cell. The structure of the neuron allows chemical information to be received by its dendrites, propagated through the perikaryon (cell body) and down its axon, and eventually passing on to other neurons through its axon terminal.

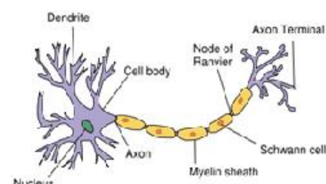

Figure 1.1 Labeling of different parts of a neuron

These voltage-gated ion channels allow for rapid depolarization throughout the cell. This depolarization, if it reaches a certain threshold, will cause an action potential. Once the action potential reaches the axon terminal, it will cause an influx of calcium ions into the cell. The calcium ions will then cause vesicles, small packets filled with

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neurotransmitters, to bind to the cell membrane and release its contents into the synapse.

This cell is known as the pre-synaptic neuron, and the cell that interacts with the neurotransmitters released is known as the post-synaptic neuron. Once the neurotransmitter is released into the synapse, it can either bind to receptors on the post-synaptic cell, the pre-synaptic cell can re-uptake it and save it for later transmission, or it can be broken down by enzymes in the synapse specific to that certain neurotransmitter. These three different actions are major areas where drug action can affect communication between neurons.

There are two types of receptors that neurotransmitters interact with on a post-synaptic neuron. The first types of receptors are ligand-gated ion channels or LGICs. LGIC receptors are the fastest types of transduction from chemical signal to electrical signal. Once the neurotransmitter binds to the receptor, it will cause a conformational change that will allow ions to directly flow into the cell. The second types are known as G-protein-coupled receptors or GPCRs. These are much slower than LGICs due to an increase in the amount of biochemical reactions that must take place intracellularly. Once the neurotransmitter binds to the GPCR protein, it causes a cascade of intracellular interactions that can lead to many different types of changes in cellular biochemistry, physiology, and gene expression. Neurotransmitter/receptor interactions in the field of neuropharmacology are extremely important because many drugs that are developed today have to do with disrupting this binding process.

Molecular Neuropharmacology:

Molecular Neuropharmacology involves the study of neurons and their neurochemical interactions, and receptors on neurons, with the goal of developing new drugs that will treat neurological disorders such as pain, neurodegenerative diseases, and psychological disorders (also known in this case as Neuro-psycho-pharmacology). There are a few technical words that must be defined when relating neurotransmission to receptor action:

1. Agonist — a molecule that binds to a receptor protein and activates that receptor
2. Competitive antagonist — a molecule that binds to the same site on the receptor protein as the agonist, preventing activation of the receptor
3. Non-competitive antagonist — a molecule that binds to a receptor protein on a different site than that of the agonist, but causes a conformational change in the protein that does not allow activation.

The following neurotransmitter/receptor interactions can be affected by synthetic compounds that act as one of the three above. Sodium/potassium ion channels can also be manipulated throughout a neuron to induce inhibitory effects of action potentials.

GABA:

The GABA neurotransmitter mediates the fast synaptic inhibition in the central nervous system. When GABA is released from its pre-synaptic cell, it will bind to a receptor (most likely the $GABA_A$ receptor) that causes the post-synaptic cell to hyperpolarize (stay below its action potential threshold). This will counteract the effect of any excitatory manipulation from other neurotransmitter/receptor interactions.

This $GABA_A$ receptor contains many binding sites that allow conformational changes and are the primary target for drug development. The most common of these binding sites, benzodiazepine, allows for both agonist and antagonist effects on the receptor. A common drug, diazepam, acts as an allosteric enhancer at this binding site.[4] Another receptor for GABA, known as $GABA_B$, can be enhanced by a molecule called baclofen. This molecule acts as an agonist, therefore activating the receptor, and is known to help control and decrease spastic movement.

Dopamine:

The dopamine neurotransmitter mediates synaptic transmission by binding to five specific GPCRs. These five receptor proteins are separated into two classes due to whether the response elicits an excitatory or inhibitory response on the post-synaptic cell. There are many types of drugs, legal and illegal, that effect dopamine and its interactions in the brain. With Parkinson's disease, a disease that decreases the amount of dopamine in the brain, the dopamine precursor Levodopa is given to the patient due to the fact that dopamine cannot cross the blood–brain barrier and L-dopa can. Some dopamine agonists are also given to Parkinson's patients that have a disorder known as restless leg syndrome or RLS. Some examples of these are ropinirole and pramipexole.[5]

Psychological disorders like that of attention deficit hyperactivity disorder (ADHD) can be treated with drugs like methylphenidate (also known as Ritalin), which block the re-uptake of dopamine by the pre-synaptic cell, thereby providing an increase of dopamine left in the synaptic gap. This increase in synaptic dopamine will increase binding to receptors of the post-synaptic cell. This same mechanism is also used by other illegal and more potent stimulant drugs such as cocaine.

Serotonin:

The serotonin neurotransmitter has the ability to mediate synaptic transmission through either GPCR's or LGIC receptors. Depending on what part of the brain region serotonin is being acted upon, will depend on whether the output is either increasing or decreasing post-synaptic responses. The most popular and widely used drugs in the

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regulation of serotonin during depression are known as SSRI's or selective serotonin reuptake inhibitors. These drugs inhibit the transport of serotonin back into the pre-synaptic neuron, leaving more serotonin in the synaptic gap to be used.

Before the discovery of SSRIs, there were also very many drugs that inhibited the enzyme that breaks down serotonin. MAOIs or monoamine oxidase inhibitors increased the amount of serotonin in the pre-synaptic cell, but had many side-effects including intense migraines and high blood pressure. This was eventually linked to the drug's interacting with a common chemical known as tyramine found in many types of food.

Ion channels:

Ion channels located on the surface membrane of the neuron allows for an influx of sodium ions and outward movement of potassium ions during an action potential. Selectively blocking these ion channels will decrease the likelihood of an action potential to occur. The drug riluzole is a neuroprotective drug that blocks sodium ion channels. Since these channels cannot activate, there is no action potential, and the neuron does not perform any transduction of chemical signals into electrical signals and the signal does not move on. This drug is used as an anesthetic as well as a sedative.

Behavioral neuropharmacology:

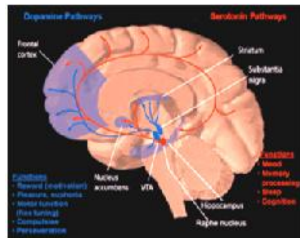

Figure 1.2 Dopamine and serotonin pathway

One form of Behavioral Neuropharmacology focuses on the study of drug dependence and how drug addiction affects the human mind. Most research has shown that the major part of the brain that reinforces addiction through neurochemical reward is the nucleus acumens. The image to the right shows how dopamine is projected into this area. Chronic alcohol abuse can cause dependence and addiction.

Parkinson's disease:

Parkinson's disease is a neurodegenerative disease described by the selective loss of dopaminergic neurons located in the substantianigra. Today, the most commonly used drug to combat this disease is levodopa or L-DOPA. This precursor to dopamine can penetrate through the blood–brain barrier, whereas the neurotransmitter dopamine cannot. There has been extensive research to determine whether L-dopa is a better treatment for Parkinson's disease rather than other dopamine agonists. Some believe that the long-term use of L-dopa will compromise neuroprotection and, thus, eventually lead to dopaminergic cell death. Though there has been no proof, in-vivo or in-vitro, some still believe that the better long-term use of dopamine agonists be better for the patient.

Alzheimer's disease:

While there are a variety of hypotheses that have been proposed for the cause of Alzheimer's disease, the knowledge of this disease is far from complete to explain, making it difficult to develop methods for treatment. In the brain of Alzheimer's patients, both neuronal nicotinic acetylcholine (nACh) receptors and NMDA receptors are known to be down-regulated. Thus, four anticholinesterases have been developed and approved by the U.S. Food and Drug Administration (FDA) for the treatment in the U.S.A. However, these are not ideal drugs, considering their side-effects and limited effectiveness. One promising drug, nefiracetam, is being developed for the treatment of Alzheimer's and other patients with dementia, and has unique actions in potentiating the activity of both nACh receptors and NMDA receptors.

NEURAL CODING

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Overview:

Neurons are remarkable among the cells of the body in their ability to propagate signals rapidly over large distances. They do this by generating characteristic electrical pulses called action potentials: voltage spikes that can travel down nerve fibers. Sensory neurons change their activities by firing sequences of action potentials in various temporal patterns, with the presence of external sensory stimuli, such as light, sound, taste, smell and touch. It is known that information about the stimulus is encoded in this pattern of action potentials and transmitted into and around the brain.

Although action potentials can vary somewhat in duration, amplitude and shape, they are typically treated as identical stereotyped events in neural coding studies.

If the brief duration of an action potential (about 1ms) is ignored, an action potential sequence, or spike train, can be characterized simply by a series of all-or-none point events in time. The lengths of interspike intervals (ISIs) between two successive spikes in a spike train often vary, apparently randomly.The study of neural coding involves

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measuring and characterizing how stimulus attributes, such as light or sound intensity, or motor actions, such as the direction of an arm movement, are represented by neuron action potentials or spikes. In order to describe and analyze neuronal firing, statistical methods and methods of probability theory and stochastic point processes have been widely applied.

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Encoding and decoding:

The link between stimulus and response can be studied from two opposite points of view. Neural encoding refers to the map from stimulus to response. The main focus is to understand how neurons respond to a wide variety of stimuli, and to construct models that attempt to predict responses to other stimuli. Neural decoding refers to the reverse map, from response to stimulus, and the challenge is to reconstruct a stimulus, or certain aspects of that stimulus, from the spike sequences it evokes.

Coding schemes:

A sequence, or 'train', of spikes may contain information based on different coding schemes. In motor neurons, for example, the strength at which an innervated muscle is flexed depends solely on the 'firing rate', the average number of spikes per unit time (a 'rate code'). At the other end, a complex 'temporal code' is based on the precise timing of single spikes. They may be locked to an external stimulus such as in the visualand auditory system or be generated intrinsically by the neural circuitry.

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Spike-count rate:

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encountered in experiment.

For example, even when viewing a static image, humans perform saccades, rapid changes of the direction of gaze. The image projected onto the retinal photoreceptors changes therefore every few hundred milliseconds.

Despite its shortcomings, the concept of a spike-count rate code is widely used not only in experiments, but also in models of neural networks. It has led to the idea that a neuron transforms information about a single input variable (the stimulus strength) into a single continuous output variable (the firing rate).

There is a growing body of evidence that in Purkinje neurons, at least, information is not simply encoded in firing but also in the timing and duration of non-firing, quiescent periods.

Time-dependent firing rate:

The time-dependent firing rate is defined as the average number of spikes (averaged over trials) appearing during a short interval between times t and t+ t, divided by the duration of the interval. It works for stationary as well as for time-dependent stimuli. To experimentally measure the time-dependent firing rate, the experimenter records from a neuron while stimulating with some input sequence. The same stimulation sequence is repeated several times and the neuronal response is reported in a Peri-Stimulus-Time Histogram (PSTH). The time t is measured with respect to the start of the stimulation sequence. The t must be large enough (typically in the range of one or a few milliseconds) so there are sufficient number of spikes within the interval to obtain a reliable estimate of the average.

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For sufficiently small t, r(t) t is the average number of spikes occurring between times t and t+ t over multiple trials. If t is small, there will never be more than one spike within the interval between t and t+ t on any given trial. This means that r(t) t is also the fraction of trials on which a spike occurred between those times. Equivalently, r(t) t is the probability that a spike occurs during this time interval.

As an experimental procedure, the time-dependent firing rate measure is a useful method to evaluate neuronal activity, in particular in the case of time-dependent stimuli. The obvious problem with this approach is that it cannot be the coding scheme used by neurons in the brain. Neurons cannot wait for the stimuli to repeatedly present in an exactly same manner before generating response.

Nevertheless, the experimental time-dependent firing rate measure can make sense, if there are large populations of independent neurons that receive the same stimulus. Instead of recording from a population of N neurons in a single run, it is experimentally easier to record from a single neuron and average over N repeated runs. Thus, the time-dependent firing rate coding relies on the implicit assumption that there are always populations of neurons.

Temporal coding:

When precise spike timing or high-frequency firing-rate fluctuations are found to carry information, the neural code is often identified as a temporal code.A number of studies have found that the temporal resolution of the neural code is on a millisecond time scale, indicating that precise spike timing is a significant element in neural coding.

Neurons exhibit high-frequency fluctuations of firing-rates which could be noise or could carry information. Rate coding models suggest that these irregularities are noise, while temporal coding models suggest that they encode information. If the nervous system only used rate codes to convey information, a more consistent, regular firing rate would have been evolutionarily advantageous, and neurons would have utilized this code over other less robust options. Temporal coding supplies an alternate explanation for the “noise," suggesting that it actually encodes information and affects neural processing. To model this idea, binary symbols can be used to mark the spikes: 1 for a spike, 0 for no spike. Temporal coding allows the sequence 000111000111 to mean something different from 001100110011, even though the mean firing rate is the same for both sequences, at 6 spikes/10 ms. Until recently, scientists had put the most emphasis on rate encoding as an explanation for post-synaptic potential patterns.

However, functions of the brain are more temporally precise than the use of only rate encoding seems to allow. In other words, essential information could be lost due to the inability of the rate code to capture all the available information of the spike train. In addition, responses are different enough between similar (but not identical) stimuli to suggest that the distinct patterns of spikes contain a higher volume of information than is possible to include in a rate code.

Temporal codes employ those features of the spiking activity that cannot be described by the firing rate. For example, time to first spike after the stimulus onset, characteristics based on the second and higher statistical moments of the ISI probability distribution, spike randomness, or precisely timed groups of spikes (temporal patterns) are candidates for temporal codes. As there is no absolute time reference in the nervous system, the information is

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carried either in terms of the relative timing of spikes in a population of neurons or with respect to an ongoing brain oscillation.

The temporal structure of a spike train or firing rate evoked by a stimulus is determined both by the dynamics of the stimulus and by the nature of the neural encoding process. Stimuli that change rapidly tend to generate precisely timed spikes and rapidly changing firing rates no matter what neural coding strategy is being used. Temporal coding refers to temporal precision in the response that does not arise solely from the dynamics of the stimulus, but that nevertheless relates to properties of the stimulus. The interplay between stimulus and encoding dynamics makes the identification of a temporal code difficult.

In temporal coding, learning can be explained by activity-dependent synaptic delay modifications.The modifications can themselves depend not only on spike rates (rate coding) but also on spike timing patterns (temporal coding), i.e., can be a special case of spike-timing-dependent plasticity.

The issue of temporal coding is distinct and independent from the issue of independent spike coding. If each spike is independent of all the other spikes in the train, the temporal character of the neural code is determined by the behavior of time-dependent firing rate r(t). If r(t) varies slowly with time, the code is typically called a rate code, and if it varies rapidly, the code is called temporal.

Temporal coding in sensory systems:

For very brief stimuli, a neuron's maximum firing rate may not be fast enough to produce more than a single spike. Due to the density of information about the abbreviated stimulus contained in this single spike, it would seem that the timing of the spike itself would have to convey more information than simply the average frequency of action potentials over a given period of time. This model is especially important for sound localization, which occurs within the brain on the order of milliseconds. The brain must obtain a large quantity of information based on a relatively short neural response. Additionally, if low firing rates on the order of ten spikes per second must be distinguished from arbitrarily close rate coding for different stimuli, then a neuron trying to discriminate these two stimuli may need to wait for a second or more to accumulate enough information. This is not consistent with numerous organisms which are able to discriminate between stimuli in the time frame of milliseconds, suggesting that a rate code is not the only model at work.

To account for the fast encoding of visual stimuli, it has been suggested that neurons of the retina encode visual information in the latency time between stimulus onset and first action potential, also called latency to first spike. This type of temporal coding has been shown also in the auditory and somato-sensory system. The main drawback of such a coding scheme is its sensitivity to intrinsic neuronal fluctuations.In the primary visual cortex of macaques, the timing of the first spike relative to the start of the stimulus was found to provide more information than the interval between spikes. However, the interspike interval could be used to encode additional information, which is especially important when the spike rate reaches its limit, as in high-contrast situations. For this reason, temporal coding may play a part in coding defined edges rather than gradual transitions.

The mammalian gustatory system is useful for studying temporal coding because of its fairly distinct stimuli and the easily discernible responses of the organism.Temporally encoded information may help an organism discriminate between different tastants of the same category (sweet, bitter, sour, salty, umami) that elicit very similar responses in terms of spike count. The temporal component of the pattern elicited by each tastant may be used to determine its identity (e.g., the difference between two bitter tastants, such as quinine and denatonium). In this way, both rate coding and temporal coding may be used in the gustatory system – rate for basic tastant type, temporal for more specific differentiation.Research on mammalian gustatory system has shown that there is an abundance of information present in temporal patterns across populations of neurons, and this information is different from that which is determined by rate coding schemes. Groups of neurons may synchronize in response to a stimulus. In studies dealing with the front cortical portion of the brain in primates, precise patterns with short time scales only a few milliseconds in length were found across small populations of neurons which correlated with certain information processing behaviors. However, little information could be determined from the patterns; one possible theory is they represented the higher-order processing taking place in the brain.

As with the visual system, in mitral/tufted cells in the olfactory bulb of mice, first-spike latency relative to the start of a sniffing action seemed to encode much of the information about an odor. This strategy of using spike latency allows for rapid identification of and reaction to an odorant. In addition, some mitral/tufted cells have specific firing patterns for given odorants.

This type of extra information could help in recognizing a certain odor, but is not completely necessary, as average spike count over the course of the animal's sniffing was also a good identifier.Along the same lines, experiments done with the olfactory system of rabbits showed distinct patterns which correlated with different subsets of odorants, and a similar result was obtained in experiments with the locust olfactory system.

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Temporal coding applications:

The specificity of temporal coding requires highly refined technology to measure informative, reliable, experimental data. Advances made in optogenetics allow neurologists to control spikes in individual neurons, offering electrical and spatial single-cell resolution. For example, blue light causes the light-gated ion channel rhodopsin to open, depolarizing the cell and producing a spike. When blue light is not sensed by the cell, the channel closes, and the neuron ceases to spike. The pattern of the spikes matches the pattern of the blue light stimuli. By inserting channelrhodopsin gene sequences into mouse DNA, researchers can control spikes and therefore certain behaviors of the mouse (e.g., making the mouse turn left).Researchers, through optogenetics, have the tools to effect different temporal codes in a neuron while maintaining the same mean firing rate, and thereby can test whether or not temporal coding occurs in specific neural circuits.

Optogenetic technology also has the potential to enable the correction of spike abnormalities at the root of several neurological and psychological disorders.If neurons do encode information in individual spike timing patterns, key signals could be missed by attempting to crack the code while looking only at mean firing rates. Understanding any temporally encoded aspects of the neural code and replicating these sequences in neurons could allow for greater control and treatment of neurological disorders such as depression, schizophrenia, and Parkinson's disease. Regulation of spike intervals in single cells more precisely controls brain activity than the addition of pharmacological agents intravenously.

Phase-of-firing code:

Phase-of-firing code is a neural coding scheme that combines the spike count code with a time reference based on oscillations. This type of code takes into account a time label for each spike according to a time reference based on phase of local ongoing oscillations at low or high frequencies.[A feature of this code is that neurons adhere to a preferred order of spiking, resulting in firing sequence. It has been shown that neurons in some cortical sensory areas encode rich naturalistic stimuli in terms of their spike times relative to the phase of ongoing network fluctuations, rather than only in terms of their spike count.Oscillations reflect local field potential signals. It is often categorized as a temporal code although the time label used for spikes is coarse grained. That is, four discrete values for phase are enough to represent all the information content in this kind of code with respect to the phase of oscillations in low frequencies. Phase-of-firing code is loosely based on the phase precession phenomena observed in place cells of the hippocampus. (Also see Phase resetting in neurons)

Phase code has been shown in visual cortex to involve also high-frequency oscillations. Within a cycle of gamma oscillation, each neuron has its own preferred relative firing time. As a result, an entire population of neurons generates a firing sequence that has duration of up to about 15 ms.

Population coding:

Population coding is a method to represent stimuli by using the joint activities of a number of neurons. In population coding, each neuron has a distribution of responses over some set of inputs, and the responses of many neurons may be combined to determine some value about the inputs.

From the theoretical point of view, population coding is one of a few mathematically well-formulated problems in neuroscience. It grasps the essential features of neural coding and yet is simple enough for theoretic analysis. Experimental studies have revealed that this coding paradigm is widely used in the sensor and motor areas of the brain. For example, in the visual area medial temporal (MT), neurons are tuned to the moving direction.In response to an object moving in a particular direction, many neurons in MT fire with a noise-corrupted and bell-shaped activity pattern across the population. The moving direction of the object is retrieved from the population activity, to be immune from the fluctuation existing in a single neuron's signal. In one classic example in the primary motor cortex, ApostolosGeorgopoulos and colleagues trained monkeys to move a joystick towards a lit target.They found that a single neuron would fire for multiple target directions. However it would fire fastest for one direction and more slowly depending on how close the target was to the neuron's 'preferred' direction.

Kenneth Johnson originally derived that if each neuron represents movement in its preferred direction, and the vector sum of all neurons is calculated (each neuron has a firing rate and a preferred direction), the sum points in the direction of motion. In this manner, the population of neurons codes the signal for the motion. This particular population code is referred to as population vector coding. This particular study divided the field of motor physiologists between Evarts' "upper motor neuron" group, which followed the hypothesis that motor cortex neurons contributed to control of single muscles, and the Georgopoulos group studying the representation of movement directions in cortex. The Johns Hopkins University Neural Encoding laboratory led by Murray Sachs and Eric Young developed place-time population codes, termed the Averaged-Localized-Synchronized-Response (ALSR) code for neural representation of auditory acoustic stimuli. This exploits both the place or tuning within the auditory nerve, as well as the phase-locking within each nerve fiber Auditory nerve. The first ALSR representation was for

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steady-state vowels; ALSR representations of pitch and formant frequencies in complex, non-steady state stimuli were demonstrated for voiced-pitchand formant representations in consonant-vowel syllables.

The advantage of such representations is that global features such as pitch or formant transition profiles can be represented as global features across the entire nerve simultaneously via both rate and place coding.

Population coding has a number of other advantages as well, including reduction of uncertainty due to neuronal variability and the ability to represent a number of different stimulus attributes simultaneously. Population coding is also much faster than rate coding and can reflect changes in the stimulus conditions nearly instantaneously.Individual neurons in such a population typically have different but overlapping selectivities, so that many neurons, but not necessarily all, respond to a given stimulus.

Typically an encoding function has a peak value such that activity of the neuron is greatest if the perceptual value is close to the peak value, and becomes reduced accordingly for values less close to the peak value.

It follows that the actual perceived value can be reconstructed from the overall pattern of activity in the set of neurons. The Johnson/Georgopoulos vector coding is an example of simple averaging. A more sophisticated mathematical technique for performing such a reconstruction is the method of maximum likelihood based on a multivariate distribution of the neuronal responses.

Correlation coding:

The correlation coding model of neuronal firing claims that correlations between action potentials, or "spikes", within a spike train may carry additional information above and beyond the simple timing of the spikes. Early work suggested that correlation between spike trains can only reduce, and never increase, the total mutual information present in the two spike trains about a stimulus feature.However, this was later demonstrated to be incorrect. Correlation structure can increase information content if noise and signal correlations are of opposite sign. Correlations can also carry information not present in the average firing rate of two pairs of neurons.

A good example of this exists in the pentobarbital-anesthetized marmoset auditory cortex, in which a pure tone causes an increase in the number of correlated spikes, but not an increase in the mean firing rate, of pairs of neurons.

Position coding:

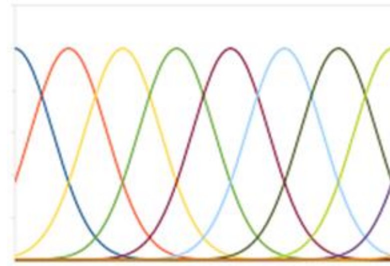

Figure 1.3 Plot of typical position coding

A typical population code involves neurons with a Gaussian tuning curve whose means vary linearly with the stimulus intensity, meaning that the neuron responds most strongly (in terms of spikes per second) to a stimulus near the mean. The actual intensity could be recovered as the stimulus level corresponding to the mean of the neuron with the greatest response. However, the noise inherent in neural responses means that a maximum likelihood estimation function is more accurate.

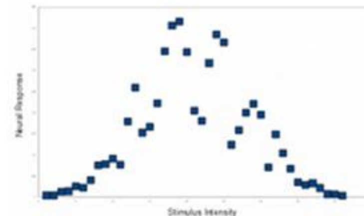

Figure 1.4 Neural responses are noisy and unreliable.

This type of code is used to encode continuous variables such as joint position, eye position, color, or sound frequency. Any individual neuron is too noisy to faithfully encode the variable using rate coding, but an entire population ensures greater fidelity and precision. For a population of unimodal tuning curves, i.e. with a single peak, the precision typically scales linearly with the number of neurons. Hence, for half the precision, half as many neurons are required. In contrast, when the tuning curves have multiple peaks, as in grid cells that represent space, the precision of the population can scale exponentially with the number of neurons. This greatly reduces the number of neurons required for the same precision.

EFFECT OF MSG AND NICOTINE

Consider two neurons:

Once a spike reaches the end the first neuron, it can cause the neuron to release "neurotransmitters" across the small distance between the two neurons called the "synapse." These neurotransmitters bind to receptors on the second neuron, which then cause the second neuron to begin firing spikes (or stop firing spikes). These receptors are very sensitive to electrical activity and certain chemicals. In fact, the very sensitivity of these receptors is how neurons, and ultimately you, learn!

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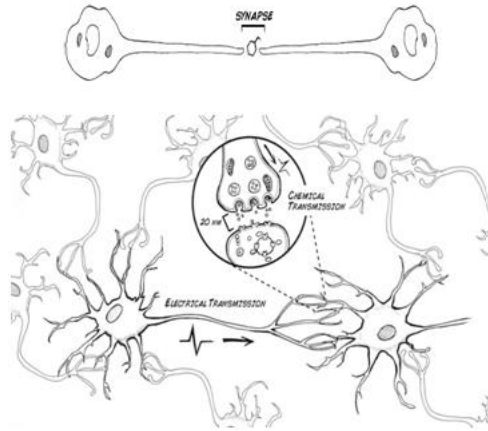
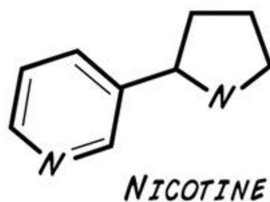


Figure 1.4: Synapse

In this experiment we will test the effect of neuroactive compounds on central nervous system neurons. Obtaining drugs that affect neurons can be quite difficult, as they are often very dangerous (like the Batrachotoxins of poison dart frogs or the tetrodotoxins of Fugu Puffer Fish, both of which block sodium channels) or are drugs of abuse (like cocaine, which allows dopamine to stay in synapses longer than normal). But, we have access to two types of drugs we can use on our insects.

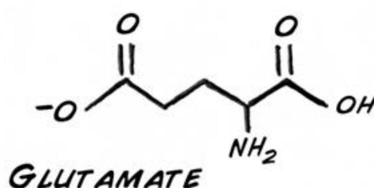
Nicotine:

Nicotine comes from the tobacco plant. Tobacco evolved nicotine to prevent insects from eating its leaves. Nicotine is a powerful acetylcholine receptor agonist; it amplifies the effect of acetylcholine binding to its receptors in synapses, causing a neuron to fire more (due to increased sodium ion influx).



Monosodium glutamate:

Monosodium glutamate itself is a neurotransmitter. Once dissolved in water, it turns into positively charged sodium ions and negatively charged glutamate ions. Glutamate is normally part of the metabolic pathway of glycolysis (breakdown of sugar) and is readily available from the foods you eat.



In fact, over 80% of the synapses in your brain use glutamate as its excitatory neurotransmitter.

II. MICRO-STIMULATION OF NEURONS TO PRODUCE MUSCULAR MOVEMENT

Microstimulation is a technique that stimulates a small population of neurons by passing a small electrical current through a nearby microelectrode. Microstimulation is used in neuroanatomical research to identify the functional significance of a group of neurons. For example, Vidal-Gonzalez et al. (2006) applied microstimulation to the rat prelimbic and infralimbicsubregions of the medial prefrontal cortex while testing the subjects in a fear-inducing low-footshock condition for various behavioral indicators of fear (such as freezing). This test allowed them to compare the relative fear behavior of rats under microstimulation in either subregion to normal rats in the same condition. The researchers concluded that the prelimbicsubregion excites fear behavior while the infralimbicsubregion inhibits fear behavior. In this instance, the correlation between stimulation and behavior helped identify the function of these two subregions in the process of fear.

Microstimulation is being explored as a method to deliver sensory percepts to circumvent damaged sensory receptors or pathways. For example, stimulation of primary visual cortex creates phosphenes (flashes of light) which can be used to restore some vision for a blind individual. Other applications include bladder prostheses; cochlear and brain-stem auditory prostheses and retinal and thalamic visual prostheses. The primate brain is thought to contain a map of the body that is used to control movement. This map is stretched across the cortex in front of the central sulcus, with the feet at the top of the brain and the face near the bottom (e.g., Fritsch and Hitzig 1870, Penfield and Boldrey 1937, Woolsey et al. 1952, Strick and Preston 1978, Huntley and Jones 1991). Many fundamental questions about this map remain unanswered. First, the somatotopic organization within the precentralgyrus is in question. A well-defined map of muscles does not appear to exist. Different body parts are represented in an intermingled fashion (Penfield and Boldrey 1937, Woolsey et al. 1952, Donoghue et al. 1992, Schieber and Hibbard 1993, Sanes et al. 1995). Though it is possible to distinguish a broad leg area, arm area, and face area, there appears to be little somatotopic organization within each of these areas. The significance of this apparent disorder is not clear. Second, when neurons at one location in the map become active, do they specify joint angle, muscle tension, force, velocity, direction, or some other movement parameter. Third, the relationship between primary motor cortex and the adjacent premotor cortex is uncertain. A traditional view is that premotor cortex instructs primary motor cortex, which in turn instructs the spinal cord (Fulton, 1935). However, both premotor and primary motor cortex project directly to the

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spinal cord in complex, overlapping patterns suggesting that a simple hierarchy may not be correct.

Stimulation of the primate brain through microelectrodes has become a widely used technique to study the behavioral function of brain areas. Microstimulation activates not only the neuronal elements near the electrode tip, but also a network of neurons sharing connections with those directly stimulated. Thus, the effect of electrical stimulation is thought to depend on the recruitment of physiologically relevant brain circuits. Stimulation has been used to uncover maps of eye movement in the frontal and supplementary eye fields, the lateral intraparietal area, and the superior colliculus. In these areas, stimulation evokes saccadic eye movements that are similar or identical to those made voluntarily. These studies typically use stimulation trains on the same time scale as a normal saccade; shorter stimulation trains result in truncated movements (Stanford et al., 1996). In the superior colliculus, stimulation trains up to 400 ms in duration, on the same time scale as a normal head movement, evoke coordinated movements of the head and eyes (Freedman et al., 1996). In the smooth pursuit area in the arcuate sulcus, stimulation trains up to 500 ms in duration are used to evoke long, smooth pursuit movements of the eyes (Gottlieb et al., 1993).

Stimulation is also used in sensory areas to influence perceptual decisions. In primary somatosensory cortex, stimulation can mimic the effect of a tactile stimulus on the finger (Romo et al., 1998). In that experiment, the duration of the stimulation train was set to 500 ms, matching the duration of the externally applied tactile stimulus. In visual areas MT and MST, stimulation can influence the monkey's perceptual judgements. These studies used stimulation trains of 1000 ms, estimated to match the time scale of the monkey's perceptual decision.

Stimulation applied to the lateral hypothalamus of rats and primates evokes feeding behaviors, and stimulation applied to the posterior hypothalamus evokes mating behaviors. When the stimulation train stops, the evoked behavior stops. These stimulation trains are typically 10–30 s long, and sometimes as long as 3 min, again roughly matching the time course of the behaviors under study.

Stimulation is thus used to study a range of brain areas, evoking meaningful, coordinated movements and behavioral repertoires and influencing perceptual decisions. In most cases, the duration of the stimulation train is chosen to match the time course of the behavior being studied.

Electrical stimulation has also been used to investigate primary motor and premotor cortex. Most previous studies of motor and premotor cortex used short stimulation trains, usually less than 50 ms, that evoked brief muscle twitches.

RATE CODING

Objectives:

To observe Rate Coding in Neurons.

To distinguish between different types of stimuli.

Prerequisites:

Full knowledge of SpikerBox

Part 1: Observing Spikes

In this exercise, we have used several methods to record data from our cockroach leg, and measured the spikes caused by different stimuli. Now that we have successfully witnessed spikes, you are ready to record, quantify, and graphically present electrophysiology data. The method we have used to compare differences in spiking from different stimuli is called "**rate coding**" and is one of the most reliable tools in neuroscience. Rate coding is simply measuring the number of spikes that occur during a set period of time. However, even though it is simple, rate coding can be used to answer complex questions about how neurons respond to stimuli.

Set-Up:

1. We needed a cockroach leg set prepared in the same way as the first lab, Getting Started With the SpikerBox. We placed one electrode in the coxa and one in the femur (see diagram below).

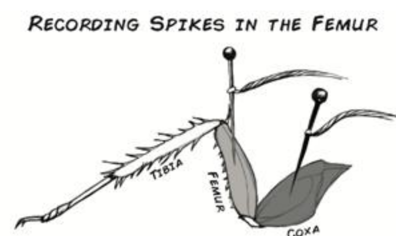

Figure 4.1: Recording Spikes

2. We plugged in the audio adapter cable into the SpikerBox and our computer.
3. Note: for the SpikerBox to record properly, one's computer's audio input jack must be audio only, not a combined audio In/Out jack. To make sure everything is working properly, we had to listen to the sounds coming from the SpikerBox speaker.
4. Audacity was turned on and a new window was opened. We ensured the setup described in the previous exercise was completed.
5. To record from the SpikerBox, we simply clicked the Red Circle at the top of the screen and stopped recording at any time by pressing the Yellow Square. If we began to record again, a new track appeared below the previous recordings. Each track was renamed by selecting the Audio Track button next to our waveform.

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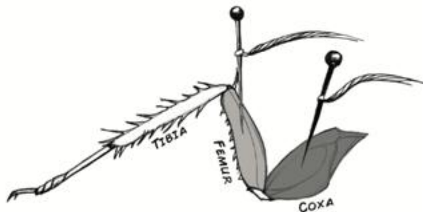
To delete a track, the X button in the upper left of each track was selected.

6. All the cell phones and Wi-Fi were turned off. Signal interference from these devices is significant.
7. When we saved, Audacity saved our project in two forms that may be confusing. The first file saved was a folder ending in _data and the second was a file ending in .aup. The .aup file must be in the folder holding the _data folder. In other words, the .aup file was kept in the parent directory of the _data folder.

Part 2: How the nerves of the femur respond to stimulation

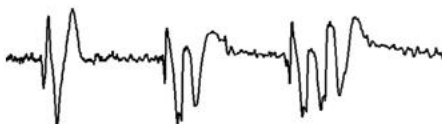
We can now begin experimenting with our cockroach leg! With our cockroach leg, we compared how neurons in the leg communicated with no stimulation, a light touch from a toothpick, and a strong blow of air through a straw.

Procedure:



1. Firstly we had to ensure that the setup was functional by poking the leg a few times.
2. When we got stable recordings, we isolated the leg from any wind and began the actual recording. Notes were taken whenever anything happened to the leg.
3. The spontaneous spiking patterns from the leg for 5 minutes were recorded.
4. A toothpick was taken to stimulate the hairs on the tibia of the leg. Several variations of stimulation including constant pressure or repetitive poking was done, until we found one that gave us consistent reactions.

ExpectedResult:



Experimental Result:

Figure 4.2: Spikes observed

Discussion:

1. If you chose a higher threshold, fewer spikes will exceed the threshold, and therefore the counts for every treatment will be lower. Choosing a lower threshold would mean more spikes would exceed the threshold, and therefore counts would be higher. Choose too high a threshold, and you will have no data, choose too low a threshold, and every spike will exceed it and you will have nothing but noise.

2. The spike rate increased more when the tibia was stimulated, than the femur. Neurons in the tibia respond more strongly to stimulation.

3. Neurons fired rapidly when a stimulus began. However, when the stimulus was applied over time they did begin to respond less to it. This is because the neurons become accustomed to the stimulus and then reduced their firing rate, a phenomena know as neural adaptation or sensory adaptation.

4. Spikes might appear smaller if the neuron firing was far away from the recording electrode. In low-stimulus treatments, fewer neurons fired over all. Therefore it was more common for far away neurons to fire, and for these spikes to be registered as “smaller” when recorded by the SpikerBox.

5. Different strengths of stimuli can indicate different types of environmental changes. For example a predator rapidly approaching might create a strong air current, while a soft breeze would create only a weak air current. Being able to discriminate between these types of air currents would be help insects distinguish between real threats, and background air movement.

Micro-stimulation of Neurons and Muscles

Electricity plays a critical role how our nervous and muscle systems work. In this experiment we stimulate a cockroach's leg muscles by using the music output of your mobile phone or computer.

Objectives:

- To explain how a nervous system controls muscle movement.
- To describe how distinct movements are caused by specific spiking patterns.
- To design an experiment to map ideal frequencies for stimulation.

Equipment:

SpikerBox

Laptop Cable

Stimulation Cable

External speaker

Cockroach

Ice water or Freezer

Dissection Scissors

Toothpick

External Speaker(Optional)

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Additional Software:

For iPhone or iPad, these free apps can be found at the iTunes store:

- AudioSigGen
- FreqGen

  If one is using a PC, one can use this online software:

- Rhintech

  Additionally, one can simply download various frequencies as MP3s and play them through any MP3 player. Here is a website from which you can download free frequencies appropriate for this exercise:

- TestSound

Procedure:

Part 1: Cockroach Leg Microstimulation Preparation

1. Cockroach leg was prepared and the micro stimulation electrode was attached.

Figure 4.3: Dancing Cockroach

2. The Microstimulation Electrode was plugged into an ESG such as a computer or MP3 player.
3. The cockroach leg was stimulated with music.
4. Change the frequencies and amplitude and observe for a response.

Observation:

	$\frac{1}{4}$ VOLUME	$\frac{1}{2}$ VOLUME	$\frac{3}{4}$ VOLUME	FULL VOLUME
20Hz	+	+	+	+
100Hz	+	+	+	+
200Hz	+	+	+	+
400Hz	+	+	+	+
600Hz	+	+	+	+
1000Hz		+	+	+
2000Hz		+	+	+
3000Hz			+	+
5000Hz				+

Result:

Frequency: Low frequencies (below 600) work best

Volume: At low frequencies, you can stimulate the leg at low volumes. At high frequency, more volume is required to produce response.

Discussion:

1. Cockroach legs responded most strongly to low frequencies. In order to achieve movement at higher frequencies, you had to increase the volume.

2. Spiking patterns that resulted in movements typically were high frequency (lots of little spikes together). These were usually associated with leg contractions.

NEUROPHARMACOLOGY: EFFECT OF MSG AND NICOTINE

To do this experiment, we use a cercal preparation (the cerci are the long antenna-like structures on the rear of an insect) on a cockroach to test the effects of neuromodulating agents that are found in common products.

Equipment:

Spikerbox

Smartphone Cable

Laptop Cable

Cockroaches

Ice water or Freezer

Dissection Scissors

Toothpick

3" Petri Dish

Corrugated Cardboard (cut to fit into petri dish)

Plastic Pipettes

1 cc/ 1 ml syringes

Procedure:

Part 1: Preparation of Neuroactive Solutions

Saline Solution :

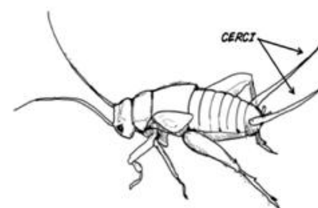
1. Simple saline solution by combining 1.5 g table salt (NaCl) and 1.25 g baking soda in 250 mL of distilled water was prepared.

Nicotine:

1. A cigarette or small cigar was taken, and all the shredded tobacco leaves were removed, and placed in a small container (a clear pill bottle, for example).
2. The container was filled with saline solution, capped, shaken, and allowed to sit for a couple days to extract the nicotine.
3. Over time the solution turned yellowish-brown.

Part 2: Cockroach Cercal Ganglion Preparation

For this exercise, we used measurements from the cockroach nervous system to determine the effect of a drug on spiking patterns and rates.



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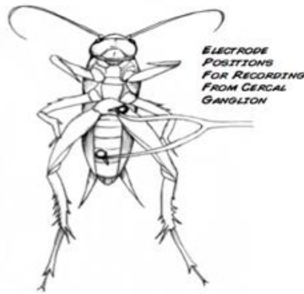

1. We placed the cockroaches in ice water for 5 minutes.
2. The cockroach was placed on the cork bed of the SpikerBox, ventral (belly) side up.
3. SpikerBox recording electrode was inserted through the abdomen of the cockroach into the cereal ganglion. The cereal ganglion is the most posterior ganglion in the ventral nerve cord (VNC). The ground electrode was stuck into middle of the abdomen. Now, using a small syringe, we injected a few drops of saline solution into the abdomen of the cockroach.
4. Using a toothpick or by blowing, we stimulated the cerci of the cricket. This generated a strong spiking pattern.
5. The SpikerBox and Audacity was turned on. SpikerBox was connected to the computer / mobile device and recordings were taken on the software.

Part 3: Testing Nicotine's effect on neuron firing

1. Using a plastic pipette, we removed any excess saline from the recording electrode.
2. Using the small syringe we inject a few drops of nicotine solution into the abdomen of the cockroach.
3. After a minute we stimulate the cerci. This took several attempts. Recordings were taken.
4. Using the same procedure, we amplify and sketch the traces of the successful stimulations.
5. After finishing with the nicotine solution, we washed out the recording electrode with saline.

Part 4: Testing the effect of MSG on neuron activity

We repeated steps 1-5 with the MSG solutions taking care to wash out residual solutions with saline in between trials. Results were recorded.

Observations:

Part 3: Testing Nicotine's effect on neuron firing

Part 4: Testing the effect of MSG on neuron activity

Discussion:

1. Nicotine increases neural firing, you can see there is an increase in the size of the spikes recorded after nicotine is introduced.
2. Nicotine is an agonist for acetylcholine, so it binds to acetylcholine receptors and increases the effect of the acetylcholine in the synapse. This causes the neuron to fire more frequently.
3. We expected to see ethanol decrease the rate of firing, but we did not see any strong effects of ethanol.
4. It might be more difficult to detect decreases in neuron firing, because if the neuron is firing at a very slow rate, you might only detect firing infrequently. Decreases in spiking would make it hard to observe an already infrequent event. Ethanol might decrease firing rate, but we could not detect it because the neuron wasn't firing very much in the first place.

III. ACKNOWLEDGEMENTS

This research project was supported by Department Of Biotechnology, RV College of Engineering. I thank myHoD Ms. VidyaNiranjan who provided insight and expertise that greatly assisted the research

I thank my project mentor and guides

Dr. LingayyaHiremath for assistance with our project and my seniors Nishita R Rao and Shreya Malikwho gave me an insight as to how the project should be taken forward

I would also like to show my gratitude to Dr. S Sridhar Vice chancellor DKNMU for recommending this paper for Publication after thorough review. I am also immensely grateful to my friends and family for their Support.

IV. REFERENCES

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