The Virtual Cocaine Lab

Virtual Books

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THE MIND PROJECT

Note: These are exact print versions of the virtual books that are located on the desk in lab.

Book 1: Nervous System Anatomy and Function

Book 1: Ch. 1: Nervous System Anatomy

The nervous system performs many tasks: it coordinates the activity of the muscles, it monitors the organs, it processes input from the senses, and it initiates action. The brain and spinal cord comprise the central nervous system. The **central nervous system** is protected by bone (skull and vertebrae). The peripheral nervous system is not because its function is to relay information to and from the organs and the limbs. The peripheral nervous system consists of all the other structures that do not lie within the central nervous system. These other structures include motor neurons, which stimulate muscle tissue, and sensory neurons, which include those connected to pain- or temperature-sensitive receptors in the skin.

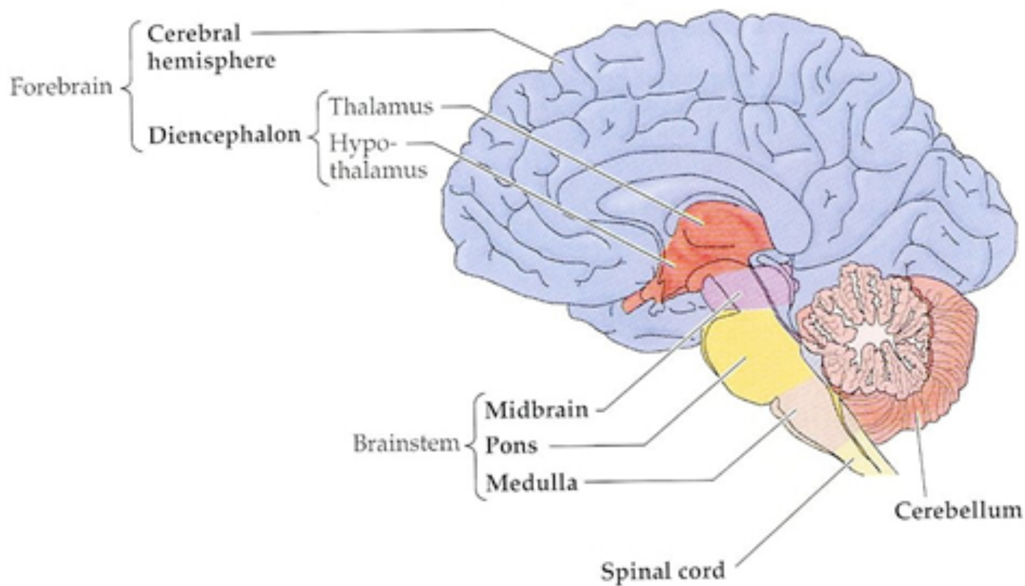
Anatomically, the brain is divided into three parts: **forebrain**, **midbrain**, and **hindbrain** (see Figure 1).

These subdivisions control different functions. Not unexpectedly, these parts come in varying sizes in different animals, depending upon specific needs or features of the animal.

Collectively, the cerebellum, medulla, and pons are called the **hindbrain** and perform “lower-level functions.” The cerebellum is the ball-like structure resting on the back of the brain. It controls fine motor movement, coordination, and posture. Closest to the spinal cord, the medulla controls breathing and heart beat. Above the medulla is the pons (or “bridge”). It relays sensory information between the cerebellum and the cerebrum. The **midbrain** (which lies between the hindbrain and the forebrain) is involved in movement and audio-visual processing. The medulla, pons, and midbrain comprise the **brainstem**.

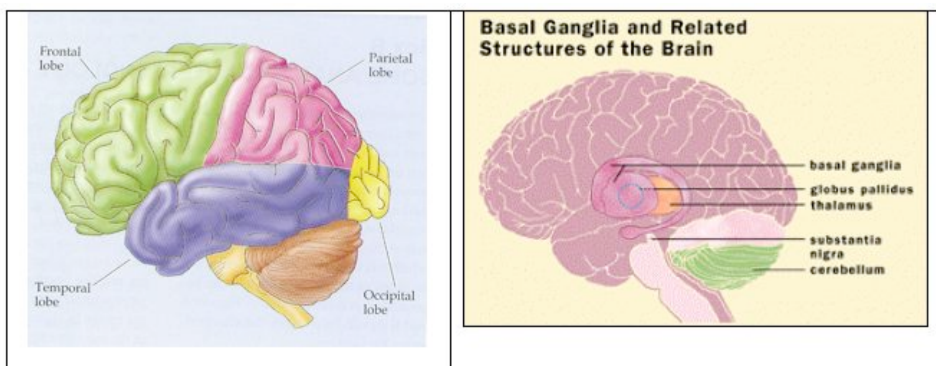
The **forebrain** consists of the diencephalon and the two cerebral hemispheres (or the cerebrum). The **diencephalon** (“in between” brain) is comprised of two prominent structures: the hypothalamus and thalamus. The hypothalamus is located at the very bottom of the brain, directly on top of the roof of the mouth. It controls a variety of involuntary functions, including: blood pressure, temperature regulation, feeding, sexual behavior, and the pituitary gland – the body's master gland. The thalamus resides on top of the hypothalamus and is the major relay system in the brain. All sensory information except smell first comes to the thalamus before being sent to other regions for processing.

Figure 1: Major divisions of the brain.



In humans, the two **cerebral hemispheres** (or **cerebrum**) are by far the largest structure of the brain. They are involved in cognition, memory, language processing, motor processing, and sensory information processing. They also contain the **basal ganglia** — the group of nuclei that is central to the control of movement. Parkinson's disease and Huntington's disease are diseases that result from damage to the basal ganglia.

Figure 2: Four lobes and basal ganglia.

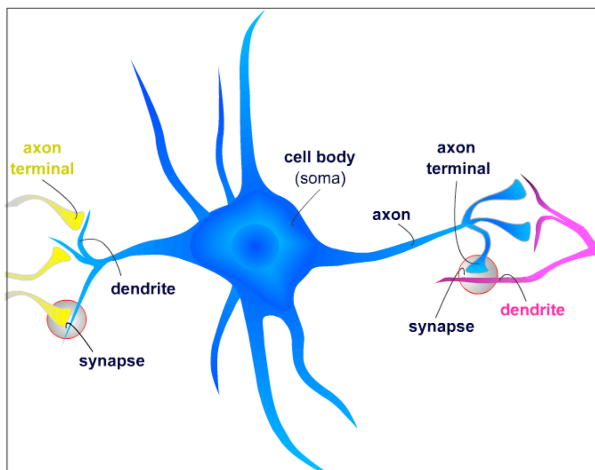


Each cerebral hemisphere is composed of four lobes: frontal, parietal, temporal, and occipital (see Figure 2). The **frontal lobes** are considered the site of executive decisions, planning, and personality. They are also the primary seat of motor information processing. The **parietal lobes** are involved with attention and somatosensory (i.e., "body senses") information processing. The **temporal lobes** are involved in recognition and auditory information processing. The **occipital lobes,** which have no cognitive function, contain the primary visual information processing centers.

Book 1: Ch. 2 Neuronal Signaling

Neurons are the basic information processing structures in the brain. A "typical" neuron has four distinct parts (or regions). The first part is the **cell body** (or soma). This is not only the metabolic "control center" of the neuron, it is also its "manufacturing and recycling plant." (For instance, it is within the cell body that neuronal proteins are synthesized.)

Fig. 3: Structure of a neuron.



The second and third parts are **processes** — structures that extend away from the cell body. Generally speaking, the function of a process is to be a conduit through which signals flow to or away from the cell body. Incoming signals from other neurons are (typically) received through its **dendrites**. The outgoing signal to other neurons flows along its **axon**. A neuron may have many thousands of dendrites, but it will have only one axon. The fourth distinct part of a neuron lies at the end of the axon, the **axon terminals**. These are the structures that contain neurotransmitters. **Neurotransmitters** are the chemical medium through which signals flow from one neuron to the next at chemical synapses.

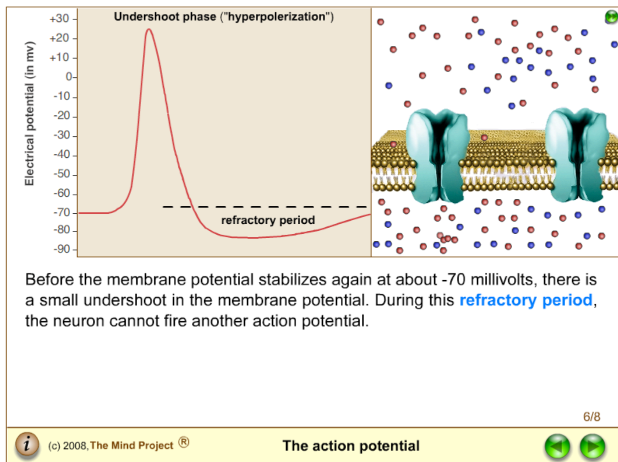
To support the general function of the nervous system, neurons have evolved unique capabilities for **intra-cellular signaling** (communication within the cell) and **inter-cellular signaling** (communication between cells). To achieve long distance, rapid communication, neurons have evolved special abilities for sending electrical signals (**action potentials**) along axons. This mechanism, called **conduction**, is how the cell body of a neuron communicates with its own terminals via the axon. Communication between neurons is achieved at **synapses** by the process of **neurotransmission**.

Conduction

To begin conduction, an **action potential** is generated near the cell body portion of the axon. An action potential is an electrical signal very much like the electrical signals in

electronic devices. But whereas an electrical signal in an electronic device occurs because electrons move along a wire, an electrical signal in a neuron occurs because **ions** move across the neuronal membrane. How this occurs is illustrated in the following animation.

Animation 1: Generation of the neural impulse



Neurotransmission

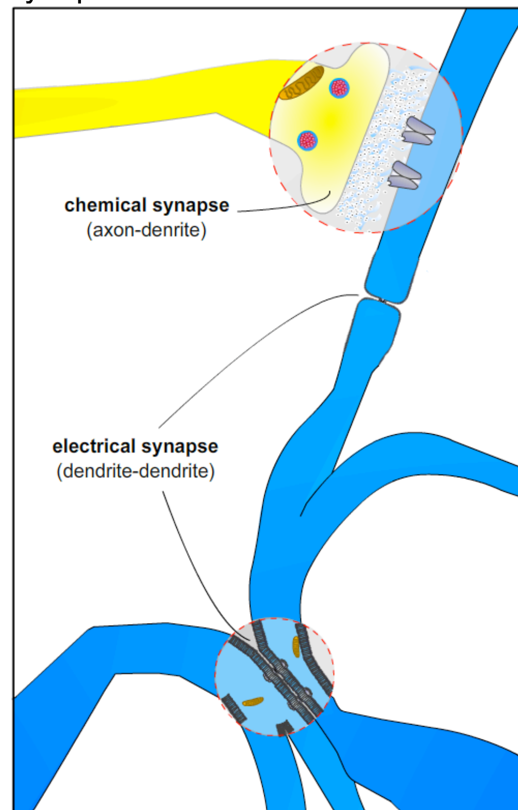
Neurotransmission is communication of information between neurons as accomplished by the movement of chemicals or electrical signals across a synapse. There are two kinds of synapses (see **Figure 4**).

At **electrical synapses**, two neurons are physically connected to one another through gap junctions. Gap junctions permit changes in the electrical properties of one neuron to effect the other, and vice versa, so the two neurons essentially behave as one.

At **chemical synapses**, two neurons are not physically connected to one another. As such, the arrival of an action potential in the presynaptic neuron triggers the release of chemical neurotransmitters. It is through chemical neurotransmitters that the presynaptic neuron communicates with the postsynaptic neuron. In mammals, most neurons communicate through chemical means. There are two models for how this occurs; namely, **“classic” chemical neurotransmis-sion** and **volume neurotransmission**. Here only the first will be discussed.

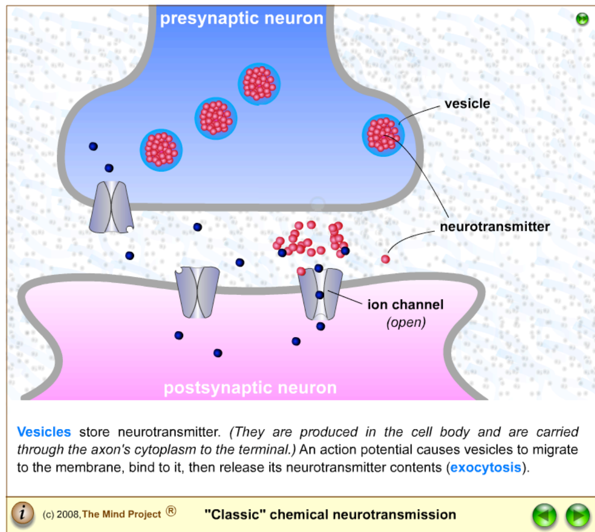
In **“classic” chemical neurotrans-mission**, the presynaptic neuron and the postsynaptic neuron are separated by a small gap — the **synaptic cleft**. The synaptic cleft is filled with extracellular fluid (the fluid bathing all the cells in the brain). Although very small, typically on the order of a few nanometers (a billionth of a meter), the

Fig. 4: Two types of synapses.

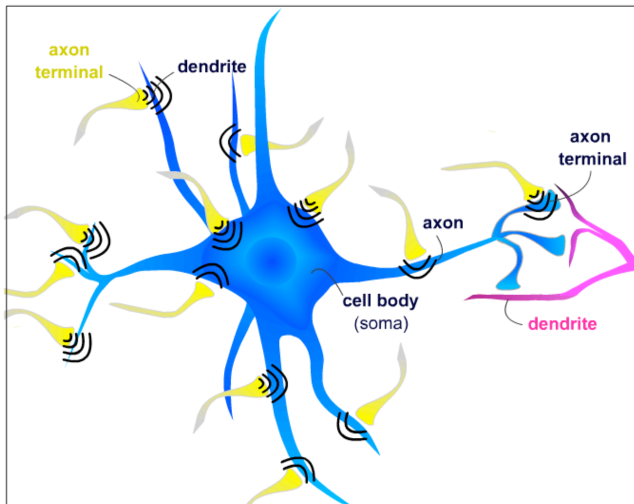


synaptic cleft creates a physical barrier for the electrical signal carried by one neuron to be transferred to another neuron. In electrical terms, the synaptic cleft would be considered a “short” in an electrical circuit. The function of neurotransmitter is to overcome this electrical short. How this occurs is illustrated in the following animation.

Animation 2: "Classic" chemical neurotransmission.



The entry of ions through ion channels produces a local partial depolarization (change in voltage) of the membrane. The postsynaptic neuron can have thousands of chemical synapses producing local partial depolarizations of its membrane (see **Figure 5** below).



When the sum of all its partial depolarizations exceeds the threshold, the postsynaptic neuron will fire an action potential, thus completing neuronal signaling. It then becomes the presynaptic neuron to all the neurons it is connected to via synapses at its axon terminals (see **Conduction** above).

Chemical neurotransmission is term-inated by removal of neurotransmitter from the cleft. Besides through diffusion, this can occur in different ways. Some types of neurotransmitter are removed by **degradative enzymes** found in the synaptic cleft. The function of these enzymes is to break down or deactivate a neurotransmitter so that it can no longer bind to a receptor. However, for most types of neurotransmitter, removal from the cleft is accomplished through a special protein on the presynaptic neuron called a **transporter**. A transporter protein acts as a pump. It binds neurotransmitter in a way that is similar to the way a receptor does, but then it moves the neurotransmitter back into the neuron, a process called **[re]uptake**. Thus, for most neurons, the same neuron that initiates neurotransmission by releasing neurotransmitter also terminates neuro-transmission by removing neuro-transmitter. Once inside the neuron, neurotransmitter molecules are either re-packaged into vesicles for use again or deactivated and broken down via degradative enzymes found in the cell.

Book 1: Ch. 3: Rats vs. Humans

Rats are often used in research because of the similarities they have with humans. In rats, as in humans, the nervous system is divided into the central nervous system and peripheral nervous system. Rats and humans share all of the major subdivisions of the brain and their general functions. And rats and humans also share similar dopamine neuron systems. **Dopamine** is an important neurotransmitter involved in movement and motivated behavior (see **Book 2**).

However, important differences exist between rats and humans. For example, humans have a considerably larger cerebral cortex, while rats have a more prominent olfactory bulb. This latter region of the forebrain is important for smell, a sense that is extremely keen in rats, but less so in humans. Another important difference is the physical relationship between the brain and the spinal cord. As an animal that stands upright, the brain and spinal cord in a human form a right angle, with the spinal cord extending down from the base of the brain. Because rats “stand” on all four paws, the relationship is more linear.

Book 2: Dopamine and Cocaine

Book 2: Ch. 1: The Dopamine Neuron

A **dopamine neuron** is a neuron that uses the neurotransmitter dopamine for chemical neurotransmission. As will be discussed, dopamine neurons are important for motor control, motivated behavior, and in mediating the effects of drugs of abuse such as cocaine.

By binding to receptors, neurotransmitters act as a chemical messenger or link connecting the action potential from one neuron with a membrane potential in another. But unlike receptors found in "classic" chemical neurotransmission, dopamine receptors function a bit differently. In "classic" chemical neurotransmission, neurotransmitter binds to receptors that open ion channels. This allows ions to pass through the neuronal membrane. In this way, the chemical signal, the neurotransmitter, is **transduced** into an electrical signal, because ion flow will generate a change in voltage at the postsynaptic membrane. If this voltage change reaches threshold, the target neuron will fire an action potential. In contrast, the dopamine receptor is called a **G-protein coupled receptor**. Instead of directly opening an ion channel, dopamine binding to its receptor activates a G-protein that in turn activates a second messenger inside the target neuron. The second message can cause several changes in the postsynaptic neuron. These changes include opening and closing ion channels, but they also include **gene transcription** (the synthesis of RNA from DNA) and **protein synthesis** (the translation of RNA into amino-acids sequences to form proteins).

Chemical neurotransmission is terminated by removal of neurotransmitter from the cleft. For dopamine, like most neurotransmitters, this is done through transporter proteins on the presynaptic neuron. Once inside the neuron, dopamine is either re-packaged into vesicles for use again or degraded via degradative enzymes. These enzymes maintain intracellular levels of dopamine at safe levels. Interestingly, high concentrations of dopamine appear to be toxic, so only by degrading dopamine or repackaging it into vesicles is the dopamine neuron protected. Also, similar to the receptors found in the postsynaptic neuron, the presynaptic dopamine neuron contains dopamine receptors itself. These **autoreceptors** function as a "thermostat," either shutting down the dopamine neuron when it is too active or speeding it up when too lethargic.

It is worth noting that the synapse between a dopamine neuron and its target appears to function rather differently than in the "classic" view. In the "classic" view of chemical neurotransmission, because transporters or degradative enzymes prevent neurotransmitter from escaping the synaptic cleft, the action of neurotransmitter is confined to the synaptic cleft. But at a dopamine chemical synapse however, dopamine released into the cleft readily diffuses out. For this reason, dopamine is often called an **extrasynaptic messenger**. Unlike "classic" chemical neurotransmission, the target of dopamine release is *not* restricted to the postsynaptic neuron. Instead, the target is *any* neuron with a dopamine receptor close enough to the dopamine synapse to be exposed to an effective dopamine concentration. Hence, in addition to "classic" chemical synapses at their terminals, dopamine neurons also form **en passant** ("in passing") synapses. These synapses allow a dopamine neuron to affect a target neuron without terminating the axon. The ability of dopamine to escape the synapse readily is why this neurotransmitter can be measured chemically in the brain without a sensor or probe that is small

enough to be placed inside the synaptic cleft. To date, no such probe or sensor exists.

Book 2: Ch. 2: Dopamine Neuron Systems

In addition to extensive overlap in brain anatomy, organization, and function, rats and humans also share similar dopamine neuron systems. A **neuronal system** describes the origins, projections, and terminations of a collection of like neurons. Thus, a dopamine neuron system is defined by the incoming or **afferent** neurons, the locations of dendrites, cell bodies, axons and terminals, and finally the outgoing or **efferent** neurons.

The brain contains several **dopamine neuron systems**. One important group originates in the hypothalamus. Consistent with the function of the hypothalamus, these dopamine neurons are involved in sexual behavior and the regulation of the pituitary gland. Although many of these dopamine neurons both begin and terminate within the hypothalamus, others project to and terminate in the spinal cord. This later subset of hypothalamic dopamine neurons would be said to be **descending** in anatomical terms, going from the forebrain to the spinal cord.
Another important group of dopamine neurons originates in the midbrain. These dopamine neurons are **ascending** because they project to and terminate in the forebrain. The ascending dopamine neurons originate in two regions of the midbrain, the **substantia nigra** and **ventral tegmental area** (see **Figure 1**).

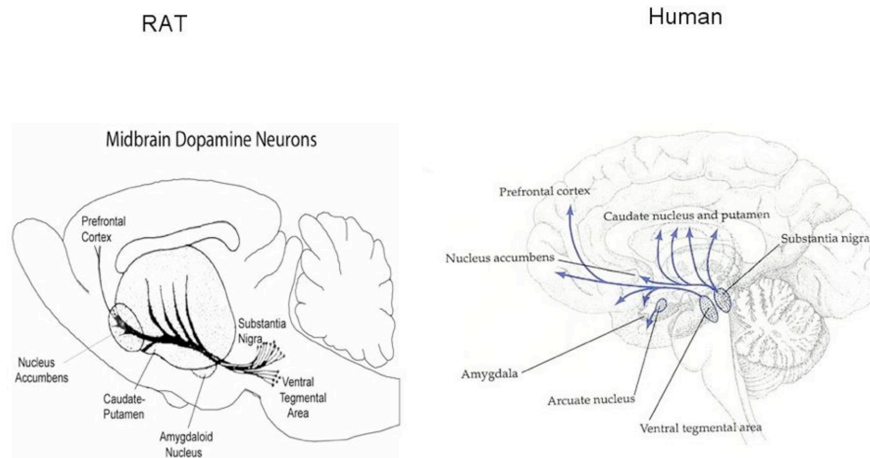


Figure 1. Comparative Anatomy of Rat and Human Brain

Book 2: Ch. 3: Approaches for Assessing Dopamine Function

Several approaches have been used to assess the role of dopamine in behavior (e.g., lesioning of dopamine neurons, use of dopamine receptor drugs, etc.). This chapter will consider only certain monitoring techniques.

One type of monitoring approach is to use a **microelectrode** (a microscopic probe typically made of glass or metal) to record the **firing rate**. The firing rate is the frequency of action potentials a dopamine neuron generates over a period of time. This technique is called **electrophysiology**. Because the cell body is physically the largest portion of the neuron, it generates the largest electrical signals. Hence, electrophysiological recordings are usually performed in a region of the brain containing neuron cell bodies. For midbrain dopamine neurons, this region would be either the

substantia nigra or ventral tegmental area. On the downside, to use electrophysiology one must assume that an action potential occurring at the cell body always causes dopamine release at the axon terminal. This may not always be true. For instance, dopamine autoreceptors can regulate dopamine release at the terminal independent of control by the cell body.

Other monitoring techniques have been developed to measure dopamine directly in **terminal fields**, which are those regions of the brain where dopamine neurons make synapses (or “terminate”). For instance, the striatum is the terminal field of midbrain dopamine neurons originating in the substantia nigra. One widely used monitoring technique is **microdialysis**. In general, dialysis is a procedure in which some but not all molecules move across a membrane. Molecules are typically excluded based on size. Such a membrane is said to be **semi-permeable**. Based on this principle, a dialysis machine is used to filter the blood of patients with kidney problems.

In microdialysis of the brain, a probe is implanted in a terminal field so dopamine can pass from extracellular fluid across a dialysis membrane and into the center of the probe. By pumping artificial extracellular fluid through the inside of the probe, dopamine is collected and measured outside of the animal using very sensitive and selective instrumentation.

Although microdialysis is used in animal experiments of many kinds, even to deliver drugs to specific brain regions via a procedure called reverse dialysis, it has two main disadvantages. First, samples are usually collected every few minutes. This is slow relative to many behaviors. Second, microdialysis probes are relatively large, about 300 microns (1000 microns = 1 millimeter) in diameter. Thus, a probe can cause damage to the region where dopamine is monitored.

Another technique for directly monitoring dopamine in terminal fields uses a **chemical microsensor**. A common type is made from a carbon fiber. Carbon is a biologically inert chemical, so it causes a minimal reaction when implanted in the brain. And carbon fibers can be made very small, 5 microns, which is 60 times smaller than the diameter of a microdialysis probe. Hence, a carbon-fiber microelectrode causes less damage than a microdialysis probe. The carbon fiber also provides an excellent surface for **electrochemistry** (the transfer of electrons between molecules), which is how chemical microsensors measure dopamine. Two events must occur before dopamine is monitored by a chemical microsensor. First, dopamine must come in contact with the carbon fiber, or at least within a few nanometers. Second, to pull off electrons from dopamine, the carbon fiber must be made positive electrically, just like the positive end of a battery. Electrons are small charged particles found in all molecules. The removal of electrons from a chemical is called **oxidation**. The rate of electrons flowing to the carbon fiber during oxidation is related to the concentration of dopamine near the microsensor. Consequently, monitoring dopamine using a chemical microsensor is called **electrochemical measurement**.

In addition to small size, electrochemical microsensors also have the important advantage of making dopamine measurements very quickly, even several times a second. Thus, chemical microsensors are a powerful tool for measuring dopamine changes during behavior. The downside is that many chemicals besides dopamine can be oxidized. This means that knowing what is measured by the chemical microsensor is an important consideration.

Book 2: Ch. 4: Dopamine Neurons and Motivated Behavior

Motivated behavior

Motivated behavior is behavior directed toward receiving a reward or goal. The reward may be natural (e.g., food, sex, etc.) or artificial (e.g., drugs of abuse). There are two components or phases of motivated behavior. The **appetitive phase** consists of those behaviors related to “approaching” the goal. In sexual behavior, for instance, the appetitive phase consists of behaviors that establish, maintain, or promote sexual interaction. Generally speaking, appetitive behaviors allow an animal to come into contact with its goal. The **consummatory phase** represents the actual “consuming” of the goal. In the case of sexual behavior, the consummatory phase is sexual intercourse. Collectively, appetitive and consummatory aspects characterize a sexual encounter, which is a motivated behavior. Addicted behavior is motivated behavior too.

The neurobiology of motivation is a field that seeks to identify the neural substrates (the brain regions, neuronal systems, neurotransmitters, receptors, etc.) that mediate motivated behavior. As described below, the classic experiment of intracranial self-stimulation demonstrated the existence of a **brain reward system**. The **nucleus accumbens** plays a central role in this system. Through dopamine neurons, it links motivational information processed in the cortex with emotional information processed in the limbic system, and then sends this combined information to regions of the brain controlling motor output, hormone release, and the fight-or-flight response. Thus, dopamine neurons terminating in the nucleus accumbens play an important role in motivated behavior. Not unexpectedly, the activity of these dopamine neurons changes during motivated behavior. And such changes can be monitored while an animal engages in motivated behavior.

Intracranial self-stimulation

One of the earliest experiments identifying a relationship between nucleus accumbens dopamine neurons and motivated behavior was **intracranial self-stimulation**. During this experiment, a stimulating electrode is implanted in the ventral tegmental area to activate dopamine neurons artificially using electrical pulses. The stimulating electrode and the instrument generating the electrical pulses are connected to the lever of a bar press machine. When the animal presses the lever, electrical pulses are delivered to the stimulating electrode. Thus, the animal controls stimulation of its dopamine neurons. (This type of control is called **contingent**. When a scientist controls the stimulation, the control is called **non-contingent**.) To obtain the “rewarding” electrical stimulation, rats lever press at astonishing rates, sometimes as fast a five times per second. They will also lever press continuously for hours.

Early studies with intracranial self-stimulation were very informative. Indeed, the highest rates of lever pressing during intracranial self-stimulation occurred with the stimulating electrode activated dopamine neurons directly. Collectively, such experiments led neuroscientists studying the neurobiology of motivated behavior to conclude that dopamine was the neural substrate of reward. In this view, dopamine is released when the animal consumes the reward, and the amplitude of dopamine release reflects the magnitude of the reward (or how good does this reward make it feel). Thus, **dopamine is said to act as the neural substrate of reward during the consummatory phase of motivated behavior**. Moreover, all rewards, whether natural (e.g., food, sex, etc) or artificial (e.g., electrical stimulation or drugs of abuse), were thought to be mediated by dopamine

release.

Recent results challenge the traditional view that dopamine is the neural substrate of reward. One of the key considerations with this new evidence is this: To understand fully the role of dopamine in motivated behavior, one must be able to monitor dopamine very quickly because behavior can be very fast as well. For example, microdialysis clearly shows that dopamine release increases when animals lever press for a rewarding electrical stimulation. But rats will bar press at rates upwards of 5 per second during intracranial self-stimulation. Microdialysis can in no way tell us what happens to dopamine release with each bar press. Nor can it tell us what happens to dopamine levels just before the animal bar presses. Both the bar press and the time leading up to it constitute the appetitive phase of this motivated behavior. Because of faster sampling rates, chemical microsensors *can* do both these things. One type of chemical microsensor technique, **fast-scan cyclic voltammetry** (or **voltammetry**), can measure dopamine 10 times per second.

When **voltammetry** was used to monitor dopamine release, some very unusual findings were obtained. For example, when the electrical stimulus was applied by the experimenter, the same stimulus that animals will lever press for, dopamine levels increase in the nucleus accumbens. This result suggested that each lever press during intracranial self-stimulation appeared to be rewarding *in the same way* as other rewarding stimuli. During training of lever press behavior, when the animal learns to associate the lever with the rewarding electrical stimulation, voltammetry showed that dopamine is also released during intracranial self-stimulation. However, in well trained animals, intracranial self-stimulation did not release dopamine; that is, animals lever pressed and received electrical stimulation but dopamine release did not increase.

Moreover, the same record of lever press activity, when replayed to the same and other animals, caused dopamine release. Remarkably, these animals received the same number and timing of the electrical stimulation as during intracranial self-stimulation, but in this case, dopamine release was observed. Remarkably, non-contingent but not contingent electrical stimulation caused dopamine release. What this interesting experiment demonstrates is that dopamine is not absolutely necessary for the consumption of an award. Instead, it appears to play a role perhaps related to learning of the cues associated with reward. In intracranial self-stimulation, the cue would be the lever press. This type of learning is called associative learning.

Book 2: Ch. 5: Dopamine Neurons and Drugs of Abuse

In the United States, one of the more prominent drugs of abuse is **cocaine**. Cocaine has multiple effects on the body, both peripherally outside the brain and centrally within the brain. Cocaine was originally used in medicine as a local anesthetic during eye surgery. The effect as a local anesthetic is independent of any action cocaine has on the brain. Cocaine also has potent effects directly on the cardiovascular system, which consists of the heart and all of the blood vessels. In general, cocaine increases blood pressure by stimulating the beating of the heart and by causing blood vessels to constrict. Large doses of cocaine can result in cardiac failure. The actions of cocaine on the brain are generally thought to be mediated by three neurotransmitters: dopamine, norepinephrine, and serotonin.

Two classic experiments are used to demonstrate that cocaine and other drugs of abuse are rewarding: **conditioned place preference** and **drug self-administration**. In conditioned place preference, an animal is released into a chamber that is demarcated into different quadrants. Over a period of time, the animal, when venturing into a specific quadrant, is injected with the test substance. After sufficient time, the animal learns the association between a quadrant and the drug injection. The animal is then allowed to enter the chamber but without any drug injection. If a drug is considered rewarding, the animal will tend to localize in the quadrant causing the drug injection. If a drug is considered **aversive**, the animal will tend to localize in the other quadrants. If a drug is considered neutral, no pattern of localization will occur.

Drug self-administration is analogous to intracranial self-stimulation, except that instead of a lever press delivering a rewarding electrical stimulation, an injection of a drug is administered. The drug is typically delivered intravenously. The goal of **cocaine self-administration** training is to teach a laboratory rat to press a lever in order to obtain an injection of cocaine. Cocaine will be injected into the jugular vein by a cannula, a small hollow tube inserted into the blood vessel. The jugular vein will carry the cocaine to the heart, which will then pump it to the brain in a matter of **30 seconds**. Lever pressing rates for drug self-administration are considerably lower than for intracranial self-stimulation or even lever pressing for food reward. The reason for this is that drugs have a long period when they are active in the brain, so they do not need to be administered as often.

Two regimens are used to train animals to self-administer drugs: **manual shaping** and **autoshaping.** In manual shaping, the experimenter guides the animal to lever press for the drug using the technique of successive approximation. In other words, the researcher reinforces behavior increasingly similar to the wanted behavior. In contrast, autoshaping lets chance do most of the work. Both manual shaping and autoshaping are time-consuming processes, often taking several days to train an animal to self-administer a drug.

The pharmacological effect of cocaine on dopamine neurons is that cocaine blocks the dopamine transporter. This prevents dopamine from being transported back into the presynaptic neuron from the synaptic cleft. Norepinephrine neurons and serotonin neurons have their own specific transporters too. Cocaine binds to and blocks the action of the norepinephrine and serotonin transporters as well. Thus, by preventing the removal of released neurotransmitter, cocaine increases the extracellular levels of dopamine, norepinephrine, and serotonin in the brain.

Book 3: Lab Procedures

Book 3: Ch. 1: The Hypothesis and Overview

Introduction

This experiment will investigate how cocaine acts on dopamine neurons in the brain. Cocaine is a drug of abuse that increases extracellular dopamine levels in the nucleus accumbens by blocking the dopamine transporter. The nucleus accumbens links the limbic system (the brain network controlling motivation and emotion) to behavior. Addictive behavior is a kind of motivated behavior. Motivated behavior is divided into two components. The *appetitive* component consists of those behaviors related to "approaching" the goal (e.g., pressing a bar). The *consummatory* component represents the actual "consuming" of the goal (e.g., cocaine reaches the brain).

An important question in the neurobiology of cocaine concerns the role of dopamine: If cocaine increases brain dopamine when consumed, does dopamine only play a role during the consummatory phase of cocaine use? If so, is dopamine solely related to the reward or pleasurable aspects of cocaine consumption? Or, similar to other motivated behaviors like sex and intracranial self-stimulation, is dopamine involved in the appetitive phase when the rat is seeking cocaine?

Objective, hypothesis, and prediction

The overall objective of this experiment is to investigate the role of dopamine neurons in cocaine use. Three important questions will be asked:

(1) What happens to brain dopamine levels when cocaine is used?
(2) When do changes in brain dopamine levels occur when cocaine is used?
(3) How do changes in brain dopamine levels affect behavior?

With most research, it is often best to focus on one or two questions at a time by simplifying the experiment. Therefore, in this experiment, we shall investigate what cocaine does to dopamine neurons during the appetitive phase of motivated behavior and what this action of dopamine neurons means for behavior. Thus, we will be examining how cocaine affects the behaviors of the animal directed towards obtaining cocaine. These behaviors have collectively been called '**cocaine seeking**'. Since we shall be monitoring cocaine levels continuously, we should be able to observe what happens to dopamine levels when cocaine reaches the brain.

The hypothesis to be tested by our experiment is the following:

> **Hypothesis**: Dopamine is involved in cocaine seeking.

To test our hypothesis, we will make a prediction regarding the outcome of our experiments. If the outcome of the experiment agrees with our prediction, then our hypothesis is supported. The prediction we shall make is this:

> **Prediction**: Dopamine release will increase during cocaine seeking.

If our prediction is verified, ***then*** there are two other questions our experiment can answer:

(1) *When* during cocaine seeking is dopamine release increased?
(2) Does the increase in dopamine release cause a subsequent change in behavior?

Technical issues

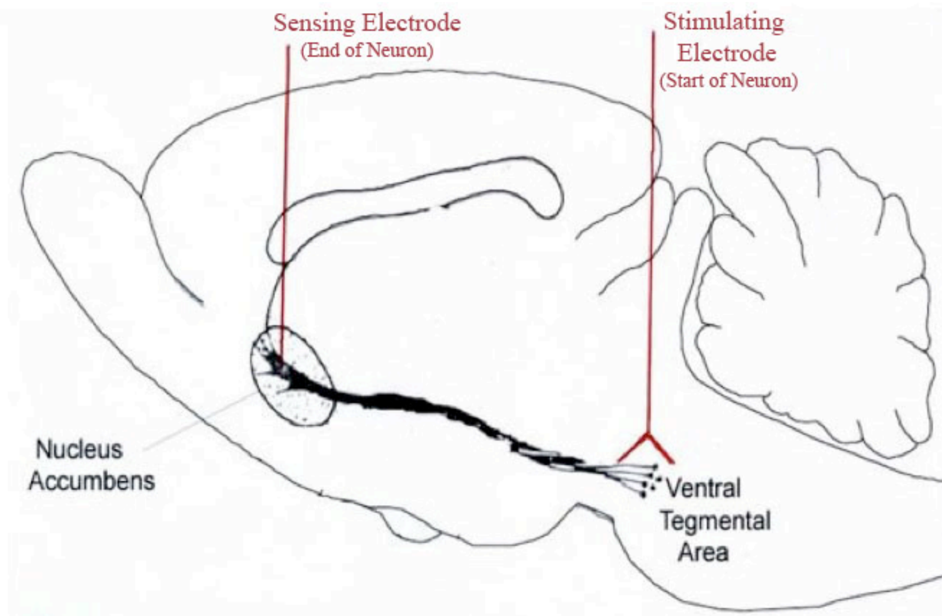
Determining whether dopamine is involved in cocaine seeking requires us to consider several technical issues. The first is that we need a laboratory animal to engage in cocaine seeking. This issue is not too difficult, because suitably trained rats will self-administer for cocaine.

Perhaps the most challenging technical issue is the actual monitoring of dopamine during cocaine seeking. Several measurement requirements must be considered: **temporal resolution** (How fast is the measurement?), **selectivity** (What is being measured?), and **spatial resolution** (Over what area of the brain is the measurement collected?). As described below, the chemical microsensor technique that will be used in our experiment, **fast-scan cyclic voltammetry at a carbon-fiber microelectrode**, has suitable measurement characteristics for monitoring dopamine during cocaine seeking. .

First, the monitoring of dopamine must be sufficiently fast to capture changes during the cocaine seeking portion of cocaine self-administration. Rats self-administer cocaine at a rate of about once every 10 min on average. *(NOTE: Our virtual rats will lever press more frequently.)* At this rate, even slow monitoring techniques could assess dopamine changes before and after a lever press for cocaine. However, the goal of this experiment is to monitor dopamine *during* the behavior(s) just prior to a lever press for cocaine self-administration. During this time, dopamine neurons may be responding to external and internal cues to direct the rat to move towards the lever and to press it. Thus, a fast measurement technique will be necessary to monitor dopamine levels during the time just before the lever press. Fortunately, **fast-scan cyclic voltammetry**, which monitors dopamine several times a second, is well suited for these measurements. Therefore, voltammetry has the appropriate temporal resolution to capture changes in brain dopamine levels during cocaine seeking.

Second, there are many chemicals in the brain that, like dopamine, are **electroactive** (which means they can be measured electrochemically). These electroactive substances could potentially interfere with the measurement of dopamine using voltammetry and the chemical microsensor. Fortunately, fast-scan cyclic voltammetry is a unique type of voltammetry that collects a “chemical signature“ (called a **voltammogram**) to identify the chemical being measured by the microsensor. Thus, the measurement of dopamine in the rat brain by fast-scan cyclic voltammetry is selective. However, in the present experiment, the substance measured during cocaine seeking will be chemically compared to dopamine that is released by the direct activation of dopamine neurons with electrical pulses applied to a stimulating electrode implanted in the ventral tegmental area (see **Figure 1**). Decades of research have established that this type of electrical stimulation elicits “authentic dopamine” in the rat brain. Moreover, for direct comparison, the chemical signature for this authentic dopamine will be collected by the same microsensor in the same area of the brain as the chemical signature recorded during cocaine seeking behavior.

Figure 1: Placement of Electrodes



Finally, it is necessary to consider where in the brain dopamine will be monitored and the extent of damage the implanted microsensor will cause. While several regions could potentially be sampled, for three main reasons, the best place to record dopamine is the **nucleus accumbens**. First, this region is part of one of the major midbrain dopamine systems, the mesolimbic dopamine system, which originates in the ventral tegmental area and terminates in the nucleus accumbens. Second, this region links reward-related information processed in the limbic system and cortex with motor output. Thus, the nucleus accumbens occupies a key role in the brain circuitry supporting motivated behavior. Third, it is well established that cocaine self-administration will increase dopamine release in the nucleus accumbens as measured by microdialysis. However, the probe associated with this technique is rather large compared to the size of the nucleus accumbens. And the microdialysis probe is known to cause damage to the adjacent tissue. Fortunately, the carbon-fiber microelectrode used with fast-scan cyclic voltammetry is considerably smaller. Hence, its tip can be readily implanted within the nucleus accumbens to measure dopamine only in this region. Also, because of smaller size, the carbon-fiber microelectrode causes considerably less tissue damage when implanted.

Experimental design

The experimental design will be divided into three parts:

PART 1: Training a rat to self-administer cocaine.

In this experiment, any animal you will work with has already been trained to self-administer for cocaine.

PART 2: Preparation and surgery for voltammetry measurements of dopamine.

In preparation for brain surgery, a rat must be selected, weighed, and anesthetized properly. You will complete all the steps leading up to surgery. Professor Neuro will complete the surgery procedure while you observe. When surgery is completed, the rat will have the hardware implanted that will allow the recording of dopamine

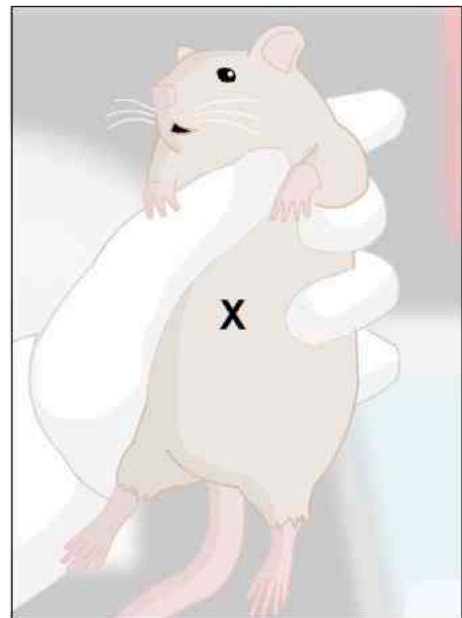
levels and the administration of cocaine.

PART 3: Monitoring dopamine during cocaine seeking.
Two weeks after surgery, the rat is ready to take part in the experiment. You will perform the experiment on a rat that has already recovered. Just before the rat is allowed to engage in cocaine seeking, a microdrive armed with a microsensor will be loaded into the surgically implanted hub. The microsensor will then be lowered into the nucleus accumbens. The electrically evoked dopamine signal will be transmitted to the computer by a wireless piece of equipment in a backpack using radio waves or **telemetry**. In this way, dopamine can be monitored in the brain of the animal without being connected or "hardwired" to the equipment. The computer will register a lever press and send a signal to the wireless backpack to administer cocaine.

The wireless telemetry and drug delivery systems used in this "virtual" lab were not used in the actual experiments on which this one was based. However, both systems are being developed and employed now. Hence, this "virtual" lab represents some of the latest technology using voltammetry and chemical microelectrodes to measure dopamine during cocaine self-administration in rats.

Completing the experiment will require that you perform specific tasks in each of the lab areas and record your observations or responses in your lab book.

Book 3: Ch. 2: Prep Area Lab Procedures



1) Put on non-sterile surgical gloves.
2) Select a rat.
3) Weigh rat and then place the rat in the prep tray.
4) Determine how much anesthesia to draw.
   a) per weight dose, e.g., for a pre-mixed cocktail of ketamine and xylazine, a common anesthetic of laboratory rodents, 1 ml/kg (inject 0.4 ml into a 400 g rat)
   b) too much anesthesia, rat dies
   c) too little anesthesia, need to inject again, but secondary doses are tricky and rats may also die even with normal amount
5) Grab a vile of anesthesia and a syringe.
   a) anesthesia is premixed
   b) place both objects in the prep tray
6) Inject the needle into the vile and draw the correct amount of anesthesia.
7) Properly inject the anesthesia.

a) inject into peritoneal cavity
   (1) if too high (in thoracic cavity), causes pneumothorax, deflating lungs and killing animal
   (2) if too low (in bladder), causes complications and perhaps death

b) properly dispose of needle in sharps container

8) Place the rat in the prep tray.
   a) rat needs to be on a heating pad as anesthesia prevents thermoregulation
(1) rat can die from being too hot or too cold

Book 3: Ch. 3: Experiment Area Lab Procedures

If this experiment were to be completed in real time instead of through the virtual world, one would have to perform certain tasks before beginning the actual experiment. As the experimenter, you would be responsible for verifying that the hardware is attached properly, secure, and that the animal is healthy. If this is not done, hardware failure will prevent dopamine measurement or cocaine injection. If a rat is unhealthy, it could die from cocaine injection, and the data would be skewed.

A recovered rat will be placed in the experiment area and the telemetry unit will be attached to the animal. The jugular injection line is flushed with saline to verify that the line is not clogged. The microdrive is then loaded with the dopamine microsensor to verify the microsensor functions correctly. The protective cap covering the brain is removed from the cemented hub and the microdrive is then attached to the hub, which was implanted during surgery into the rat's brain. The connections are then attached between the telemetry unit and the reference electrode (which supports the electrochemical measurements of dopamine), the stimulating electrode, the microsensor (carbon-fiber microelectrode), and the jugular cannula. So as not to break the microsensor, the microsensor is lowered at the appropriate speed to the correct depth in the nucleus accumbens – the depth that provides measurement of dopamine.

The rat must now be allowed to habituate to the experiment area in order to feel comfortable with its surroundings and not be stressed. A stressed animal may behave erratically and have an adverse reaction to cocaine. The microsensors must then be allowed to come to equilibrium in the brain so the microsensors will not perform erratically. A test stimulation will be performed to check the functionality of the microsensor and have it replaced if necessary, and verify the microsensor has been placed in a dopamine-rich area so that appropriate readings will take place.

Next, the stimulating electrodes must be checked to make sure there is not a bad connection, a bad stimulation unit, or the stimulating electrode has been moved. Wireless communication must also be checked to verify there is no problem with the battery, the telemetry pack, or the computer.

The next step is to turn on the video camera in order to record the rat's behavior. It is now time to begin the experiment and to monitor dopamine during cocaine self-stimulation. The following steps will take place during the lab experiment.

a) start session by pressing the on button
b) session starts with extension of lever and display of data on monitor
c) every bar press results in
   i) six second injection of cocaine
   ii) illuminating the light above lever for 20 seconds
   iii) auditory tone for 20 seconds
   iv) during this 20 seconds, additional lever presses will not result in additional injections of cocaine
d) session ends
   i) lever is retracted
   ii) typical session duration is 120 min *(NOTE: Our's will be much, much shorter.)*
e) repeat test stimulation
   i) check for functioning microsensor

Once you have completed the experiment, it is time for you to write up your analysis in your lab report.