[1. Data Set](#_ch46gs7nshz)

[2. Data Explorer Info](#_iy958elfx9op)

[3. Data Activity: Educator Materials](#_aa1yr6wk67nx)

[4. Data Activity: Student Handout](#_5806oyoufthp)

# **1. Data Set**

File name:

opioid crisis\_exploring neurophysiology.csv

# **2. Data Explorer Info**

**Title**

Opioid crisis: exploring the neurophysiology of opioid receptor signaling

**Summary**

Using this dataset, we explore how opioids affect the electrical activity of neurons when exposed to different opioid receptor agonists and antagonists.

**Introduction**

Using this dataset, we explore how opioids affect the electrical activity of mesolimbic pathway neurons, also known as the reward pathway, when exposed to different opioid receptor agonists and antagonists. Opioids, such as oxycodone, morphine, and fentanyl to name a few, can be prescribed by doctors to reduce pain for patients. These drugs are analgesics that reduce pain signals, but they also stimulate regions of the brain. Excessive use of opioids can also lead to addition. In fact, about one-quarter of those prescribed an opioid pain reliever misuse them, and about 8-10% develop an opioid use disorder (NIH). Alarmingly, in 2018, an average of 128 people died each day from overdosing on opioids (NIH).

The misuse of opioids overstimulates the brain’s reward pathway and can lead to drug dependency. Opioids can bind receptors that belong to three main classes – 𝛍-opioid (mu) receptors (MOR), 𝛅-opioid (delta) receptors (DOR), and 𝛋-opioid receptors (KOR) (Fields and Margolis 2015, Stein 2016). MORs are thought to be the main opioid receptor that mediates responses to opioids in the reward pathway. In the brain, MOR are found in a region called mesolimbic pathway, which is responsible for communicating our feelings of reward for food, pleasure, and goal-directed behavior among others (Fields and Margolis 2015, Wang 2019). Specifically, MORs are found on neurons of the ventral tegmental area (VTA). They can be found on dopaminergic neurons (neurons that make and secrete the neurotransmitter dopamine); or, they can be found on GABAergic neurons (neurons that make and secrete the neurotransmitter GABA). Dopaminergic neurons send dopamine to another region called the nucleus accumbens, which communicates higher cortical brain regions that signal the receipt of the reward.

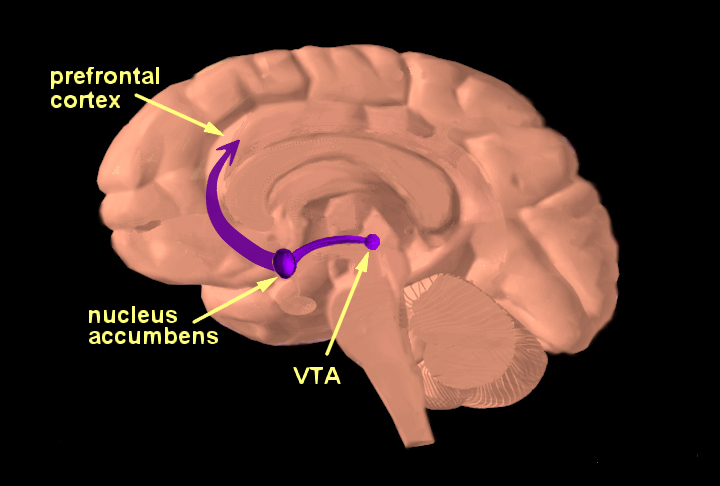


Figure 1. Brain Reward Pathway

When opioids bind MORs, they activate signaling inside a cell that decreases the activity of neurons. For example, one way opioids increase the activity of dopamine secreting neurons is by inhibiting inhibitory neurons that act on dopamine neurons. Thus, opioids disinhibit inhibitory neurons and allow more dopamine neurons to be active. Because activating the reward circuit creates feelings of pleasure, those with opioid use disorders search for stimulants that can lead to increased dopamine release in this region of the brain.

This dataset examines the work of Matsui and Williams (2011) that show how inhibitory neurons and DA neurons in the VTA interact to control dopamine release.

**About the data**

This data was extracted from Matsui and Williams (2011). For their dataset, male and female Sprague-Dawley rats were used for experiments. For electrophysiological recordings, rats were anesthetized, and their brains were quickly removed for dissection. Brains were then sliced using a vibratome, and prepared in physiological solution for recordings in the midbrain region.

For electrophysiological data, whole cell or cell attached techniques were used to record from brain slices. Drugs were applied by bath perfusion. Drugs used include the following:

* DAMGO (1µM), an agonist for 𝛍-opioid receptors (MOR)
* U69593 (1µM), an agonist for 𝛅-opioid receptors (DOR)
* DPDPE(1µM), an agonist for 𝛋-opioid receptors (KOR)
* Naloxone (1µM), an antagonist for 𝛍-opioid receptors (MOR)

Key definitions

**Agonist** - a chemical or drug binds a receptor and stimulates a response in a cell

**Antagonist -** - a chemical or drug interferes with the activity of the natural agonists leading to no response in a cell, by binding directly or indirectly the receptor

**GABA** - inhibitory neurotransmitter. When acting on neurons, GABA neurotransmitter usually decreases the activity, or excitability, or other neurons

**IPSC (inhibitory post synaptic current)** - this is the change in neuron activity when a neuron is responding to an inhibitory neurotransmitter such as GABA

**EPSC (excitatory post synaptic current)** - this is the change in neuron activity when a neuron is responding to an excitatory neurotransmitter. If EPSC is large, this can results in increased activity of the neuron

**VTA -** ventral tegmental area - brain region that is part of the mesolimbic reward pathway. Neurons in the VTA receive inputs from many areas, including the RMTg. They send outputs to the nucleus accumbens, among others.

**RMTg** - rostromedial tegmental nucleus - posterior region of the VTA that has GABAergic neurons that are thought to project to the dopamine-releasing cell bodies in other parts of the VTA - RMT is another opioid sensitive GABA relay to dopamine neurons

**Resting membrane voltage**: We call neurons that aren’t actively being excited or inhibited “at rest.” In this state, they have a baseline membrane voltage called the resting membrane voltage.

**Firing frequency (Hz)** : Neurons fire action potentials. We can determine the frequency of firing (rate) measuring the number of action potentials per section (1/second = 1Hz). Increased frequency might mean that the neuron is either excited to fire or less inhibited from firing.

The data set contains the following columns:

|  |  |
| --- | --- |
| **Column 1: Animal (treatment type)** | Describes whether rats were treated with control (saline or wash solutions), or agonist for 𝛍-opioid receptors, 𝛅-opioid receptors, and 𝛋-opioid receptors |
| **Column 2: RMTg hyperpolarization (mV)** | Describes the amount of charge decrease (compared to a baseline resting mV) in neurons in response to the application of the drug. |
| **Column 3: RMTg firing frequency (Hz)** | The firing frequency of neurons recorded from neurons in the rostromedial tegmental nucleus (RMTg) |
| **Column 4: VTA firing frequency (Hz)** | The firing frequency of neurons recorded from neurons in the ventral tegmental area (VTA) |
| **Column 5: DA neuron IPSC amplitude (% of baseline)** | Inhibitory postsynaptic current (IPSC) were recorded from dopamine (DA) neurons after pre-synaptic RMTg neurons were excited. This leads to an evoked IPSC in the dopamine neurons. Controls in this dataset are after a saline wash, MOR agonist is 1µM DAMGO, and MOR antagonist is 1µM Naloxone |

**Related activity**

This activity can be paired with the following activities:

**HHMI Biointeractive–Electrical Activity of Neurons**

<https://www.biointeractive.org/classroom-resources/electrical-activity-neurons>

<https://qubeshub.org/publications/1405/1>

**HHMI Biointeractive–Neurophysiology Virtual Lab**

<https://www.biointeractive.org/classroom-resources/neurophysiology-virtual-lab>

***Electrical Activity of Neurons*** explores how neurons communicate via electrical signaling, and how measurements of electrical activity are measured with a patch clamp electrophysiology. Watching this slideshow and accompanying videos prior to analyzing this dataset may help students with the background information needed.

**Neurophysiology Virtual Lab** may be used as a “lab” companion to better understand how neuronal activity leads to the communication of information, such as touch sensation in leeches.

**References**

CDC. "CDC." from <https://www.cdc.gov/drugoverdose/opioids/index.html>.

Fields, H. L. and E. B. Margolis (2015). "Understanding opioid reward." Trends Neurosci **38**(4): 217-225.

Matsui, A. and J. T. Williams (2011). "Opioid-sensitive GABA inputs from rostromedial tegmental nucleus synapse onto midbrain dopamine neurons." J Neurosci **31**(48): 17729-17735.

NIDA. "NIDA." from <https://www.drugabuse.gov/drug-topics/opioids/opioid-overdose-crisis>.

Stein, C. (2016). "Opioid Receptors." Annu Rev Med **67**: 433-451.

Wang, S. (2019). "Historical Review: Opiate Addiction and Opioid Receptors." Cell Transplant **28**(3): 233-238.

### **Credits**

Data curated by Jason Chan, Marian University, Indiana

Text written by Jason Chan, Marian University, Indiana

Edited by Jason Chan, Marian University

Scientific review by NAME, AFFILIATION

Figure 1: “**Brain reward pathway**” by [National Institute on Drug Abuse](http://www.publicdomainfiles.com/browse.php?q=all&s=0&o=popular&a=41&m=all), used under Public Domain License

# **3. Data Activity: Educator Materials**

This is the **teacher document** (PDF) for the activity that accompanies the data set, which should walk through a specific application of the data. It is paired with the [Student Handout](#_5806oyoufthp). When FMN folks write this document, Data Explorer may not be published yet. They can keep this more general and match it to Data Explorer later.

**Title**

Opioid crisis: exploring the neurophysiology of opioid receptor signaling

**Overview**

Of patients that are prescribed opioid medication, approximately 21-29% misuse the drug and 8-12% develop an opioid use disorder (NIH). The government has put forth initiatives to combat addiction related disorders, but in 2018, an average of 128 people died each day from overdosing on opioids in (NIH). The opioid epidemic started with over prescription of opioid medication, and the increased opioid usage and death was furthered by the production of heroin and synthetic opioid drugs. This activity explores the effect of opioids on neurons in the brain. Specifically, it focuses on how neuron communication in the brain reward center changes in response to opioids. Opioids, such as oxycodone or morphine, can become a drug of abuse because they over stimulate reward centers in the brain and can lead to addition. Through the exploration of a dataset exploring the effect of opioids on neurons of the ventral tegmental area (VTA), one the main regions in the brain’s reward pathway, students will analyze data showing that opioids indirectly increase the activity of dopamine neurons through the mu opioid receptor. To explore these concepts, students will use t-tests and ANOVA analyses to compare electrophysiological data from neurons exposed to opioid receptor agonists and antagonists.

**Key Concepts**

* Neurons can be inhibited or excited by presynaptic connections
* Dopamine producing neurons in the VTA project to the nucleus accumbens as part of the brain’s reward pathway
* Opioids act on mu-opioid receptors to disinhibit inhibitory neurons
* Opioids impact the brain’s reward pathway by allowing more DA release in the nucleus accumbens
* Naloxone is an antagonist for mu-opioid receptor that is used to reverse opioid overdose by interfering (antagonizing) opioids from activating the mu-opioid receptors

**Student Learning Targets**

* Students will describe how changes in neuronal activity contribute to communication within the reward pathway in rat models.
* Students should generate graphical representations and use statistical measures to support the hypothesis that opioids lead to increased activity of dopamine neurons in the VTA.

**Prior Knowledge**

Students should:

* Understand how neurons communicate through electrical signaling, and fire action potentials
* Understand that different neurotransmitters can either lead to neurons being excited or inhibited
* Ligands bind membrane receptors, and can lead to changes inside a cell
* Principles of synaptic transmission and communication between neurons

**Background**

Using this dataset (opioid crisis\_exploring neurophysiology.csv), students can explore how opioids affect neurons in the brain, and how opioids impact the brain’s “reward pathway.” Opioids can be classified into four categories: endogenous opioids (e.g. endorphins, which are produced by the body), opium alkaloids (e.g. morphine, which are naturally produced), semisynthetic opioids (e.g. oxycodone) and synthetic (e.g. fentanyl). Opioids are important analgesics that reduce pain signals; however, many opioids can cross the blood brain barrier to also act on the brain. Excessive use of opioids can act specifically on the brain’s mesolimbic region, also known as the reward pathway, and lead to the dependency on opioids. This can then lead to opioid use disorders, and even death due to overdose (see figure below).

**15,469**

Deaths due to Heroin

**17,087**

Deaths due to Prescription Opioids

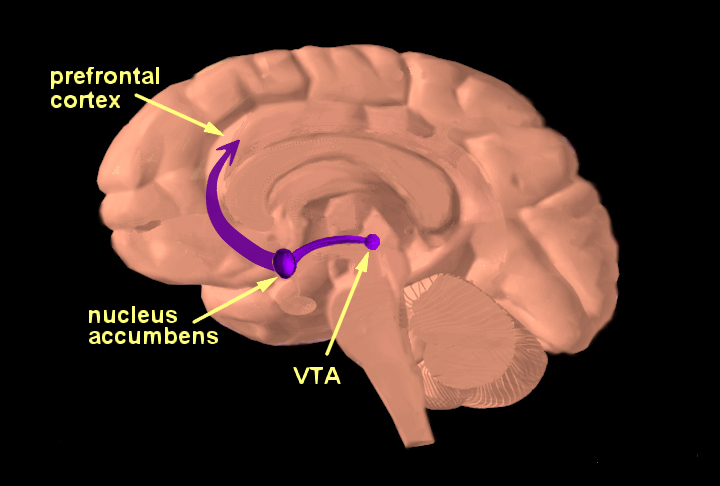
**19,413**

Deaths due to Synthetic Opioids

**In 2016, there were 42,249 deaths due to 0pioid related overdose (numbers from the National Institute of Drug Abuse)**

Opioid overdose deaths have increased six fold since 1999 and make up approximately 70% of all overdose deaths (CDC). Furthermore, 80% of heroin users first misused opioid drugs. While outside the scope of this dataset, instructors may want to use facts on the current Opioid Crisis to discuss with students. Sources include the NIH (NIDA) and the CDC (CDC).

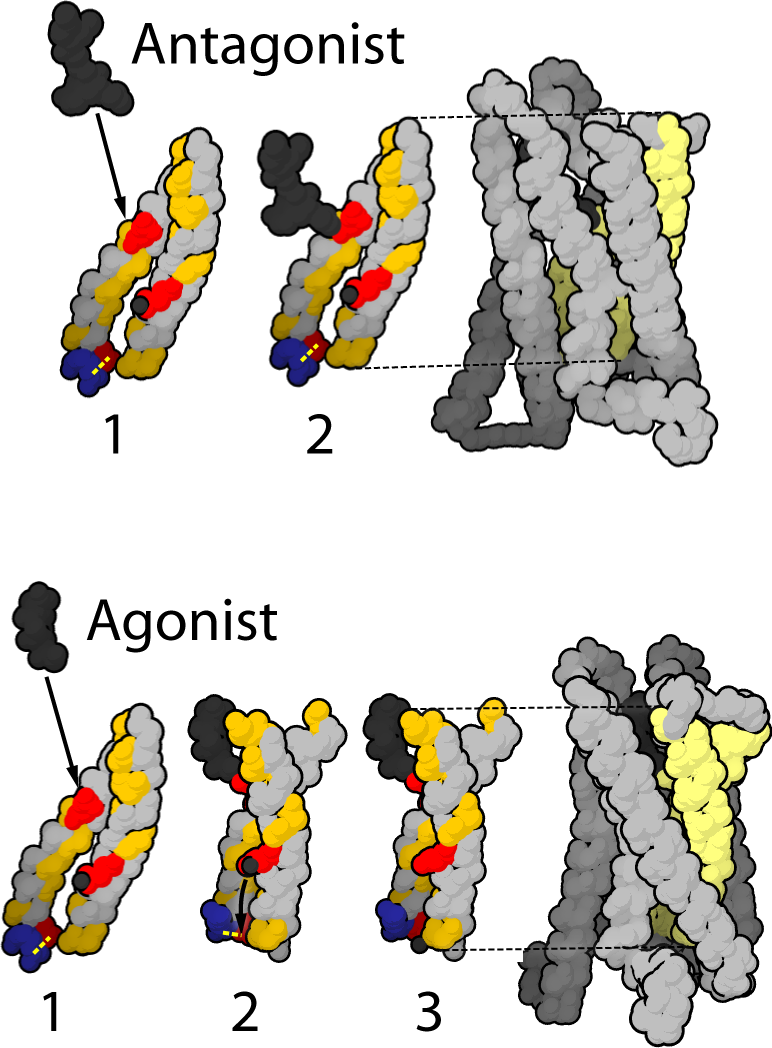
The misuse of opioids overstimulates the brain’s reward pathway and can lead to drug dependency. Opioids can bind receptors on neurons; these receptors belong to three main classes – 𝛍-opioid (mu) receptors (MOR), 𝛅-opioid (delta) receptors (DOR), and 𝛋-opioid receptors (KOR) (Fields and Margolis 2015, Stein 2016). Mu-opioid receptors are thought to be the main opioid receptor that mediates responses to opioids in the reward pathway. In the brain, mu-opioid receptors are responsible for communicating our feelings of reward for food, pleasure, and goal-directed behavior among others (Fields and Margolis 2015, Wang 2019). Specifically, Mu-opioid receptors are found on neurons of the ventral tegmental area (VTA). They can be found on dopaminergic neurons (neurons that make and secrete the neurotransmitter dopamine); or, they can be found on GABAergic neurons (neurons that make and secrete the neurotransmitter GABA). Dopaminergic neurons send dopamine to another region called the nucleus accumbens, which communicates higher cortical brain regions that signal the receipt of the reward (see figure below).



**Figure of the reward pathway**

When opioids bind mu-opioid receptors, they activate signaling inside a cell that decreases, or inhibits, the activity of neurons. For example, one way in which opioids increase the activity of dopamine secreting neurons is by inhibiting inhibitory neurons that act on dopamine neurons (referred to as disinhibition). Thus, the net result is that opioids allow more DA neurons to be active through disinhibition. Because the reward circuit generates feelings of pleasure or achievement, we search for stimulants that can lead to increased dopamine release in this region of the brain. Opioids have come to satisfy this search for many those with opioid use disorders.

To better explore the neuroscience of opioid signaling, this dataset examines the work of Matsui and Williams (2011) that show how GABAergic inhibitory neurons in the RMTg brain region act on DA neurons in the VTA to control dopamine release. By following the changes of the activity of neurons in the RMTg and VTA in response to 𝛍-opioid agonists, students can recreate the reward pathway and understand how opioids change the activity in the reward pathway.



**Figure of antagonist and agonist action on mu opioid receptors.** This model depicts how an agonist can change the structure (protein conformation) of a mu opioid receptor, which can then lead to a response inside cells. However, binding of an antagonist to the receptor may not change the receptor, and thus does not cause a cell response.

**Teaching Tips**

* This exercise follows several figures in the Matsui and Williams (2011) article. Thus, they are meant to be scaffolded in such a way as the figures in the paper. Each graph generated recreated components of the figures, and each subsequent figure builds upon the knowledge gained.
* The instructor may want to include a discussion on the opioid epidemic, and many of those resources can be found on NIH’s drugsabuse.gov site.
* If you are teaching this in a introductory neuroscience class, one will want to review/build upon excitatory and inhibitory signaling in neurons, neurotransmitters and receptors signaling, and basics of neurophysiology
* It is recommended that students are asked to generate figures in the following order

1. The effect of mu-opioid receptors on RMTg activity
2. The effect of Mmu-opioid receptors on VTA activity
3. The effect of mu-opioid receptors on RMTg induced VTA activity

* Depending on the instructor, questions on statistical tests, and explanations, can be used. Detailed discussion of statistical tests may or may not be appropriate for your use.
* The advanced questions may require a greater understanding of the underlying biochemistry of opioid signaling. HOwever, the principles can be adapted for important discussion on drugs, treating overdose, and medical ethics.

**Answer Key**

**Background questions**

1. Draw a simple circuit that shows the brain’s reward pathway.

Answer: at this point, this figure might look like the figure provided in FIgure 1. This is a question put in to see how students gain depth in their understanding, as this question is asked again later.

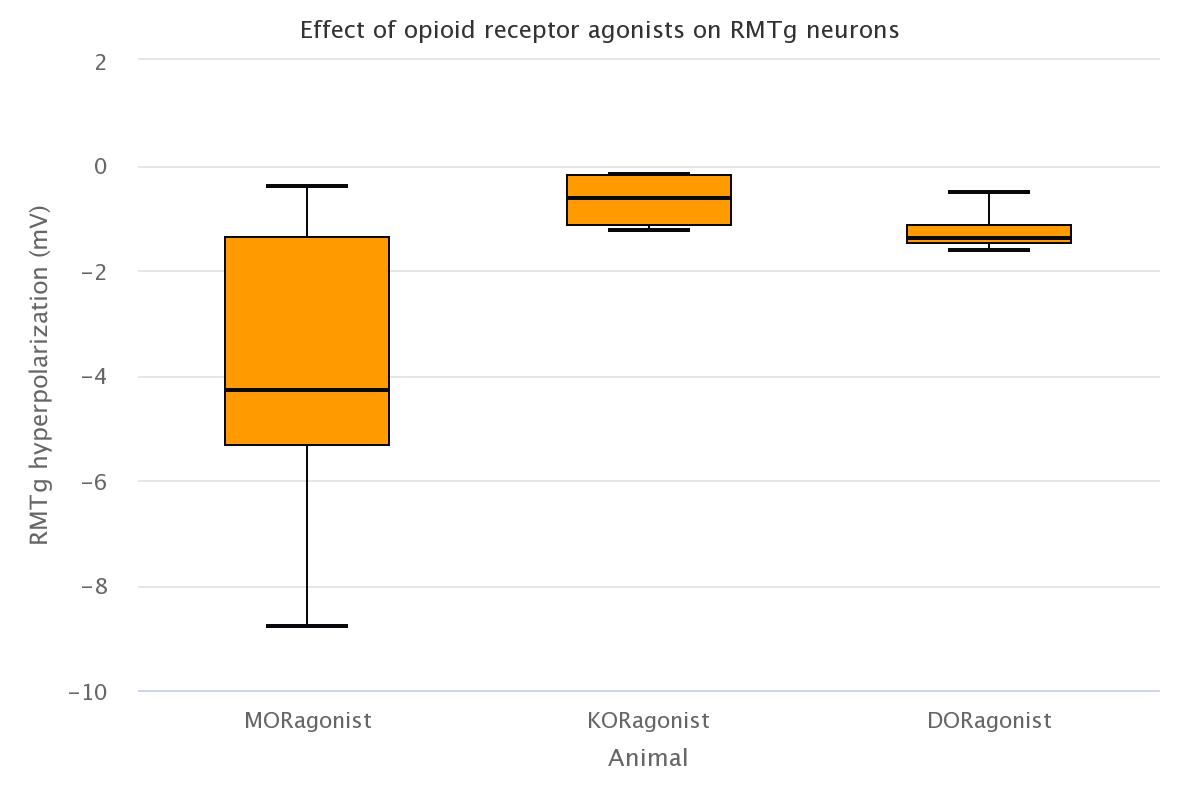
1. How does activation of the mu opioid receptors in the VTA lead to increased DA release in the nucleus accumbens (NA)?

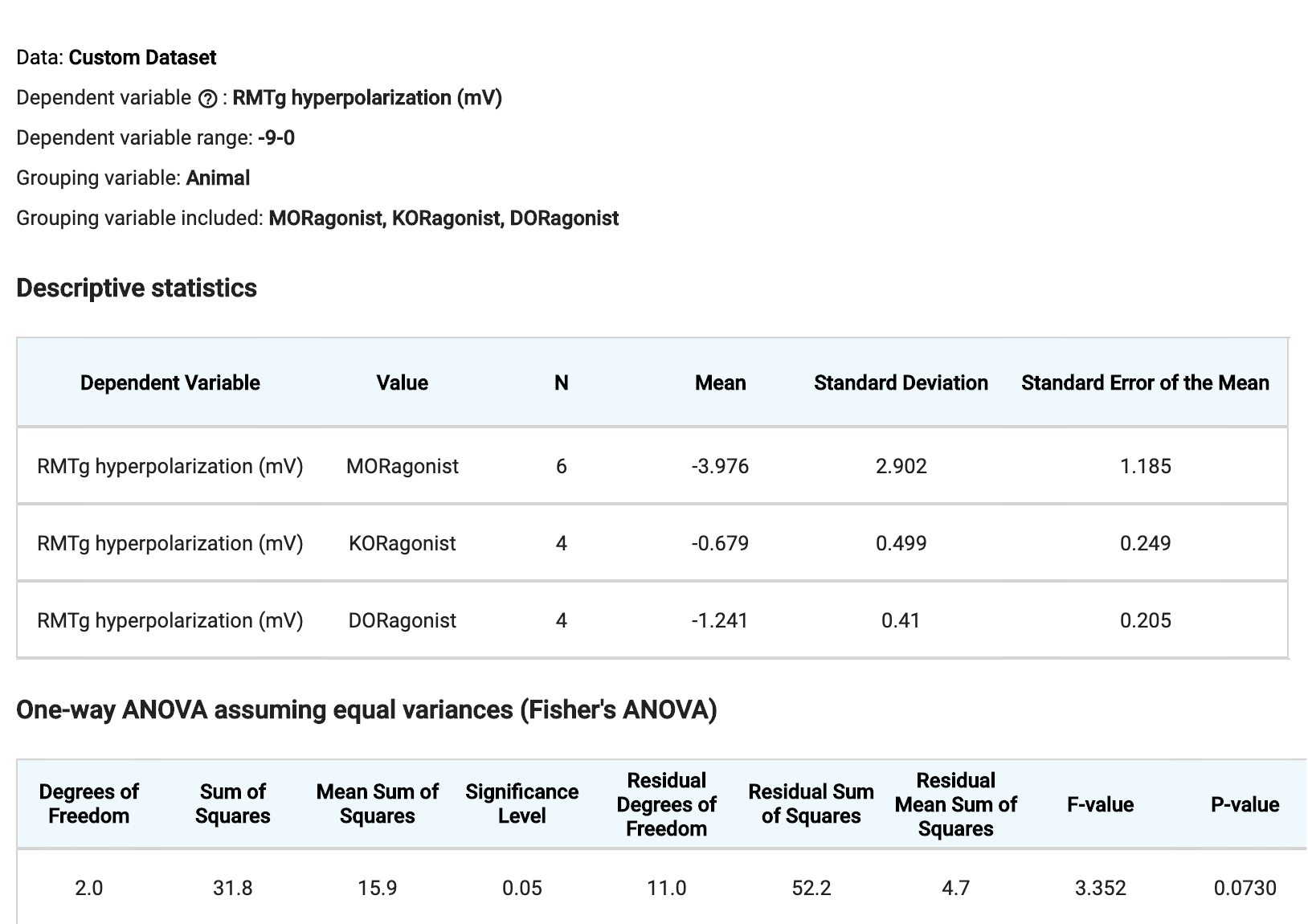
Answer: Mu opioid receptors are found on inhibitory GABA neurons, which act on dopamine neurons in the VTA. Activation of mu opioid receptors can decrease inhibitory neuron activity by GABA, thereby allowing for increased activity from dopamine neurons (disinhibition). If dopamine neurons are more active, they will also secrete more dopamine from their nerve endings in the nucleus accumbens.

**Data analysis questions**

1. Using the dataset, make a graph to visualize the level of inhibition that RMTg neurons receive in response to opioid. Scientists measured inhibition by recording from neurons in the RMTg using a technique called electrophysiology, in which they can measure changed in membrane voltage. A decrease (more negative) change in voltage is a hyperpolarization, and makes it harder for neurons to fire action potentials. Scientists measured hyperpolarization after treating RMTg neurons with different opioid receptor agonist, particularly ones that target the mu-opioid receptor (MOR), the delta-opioid receptor (DOR), and kappa-opioid receptor (KOR). Help determine which type of agonists initiates a hyperpolarization in RMTg neurons.

**Answer: Figure 1 and statistical analysis**



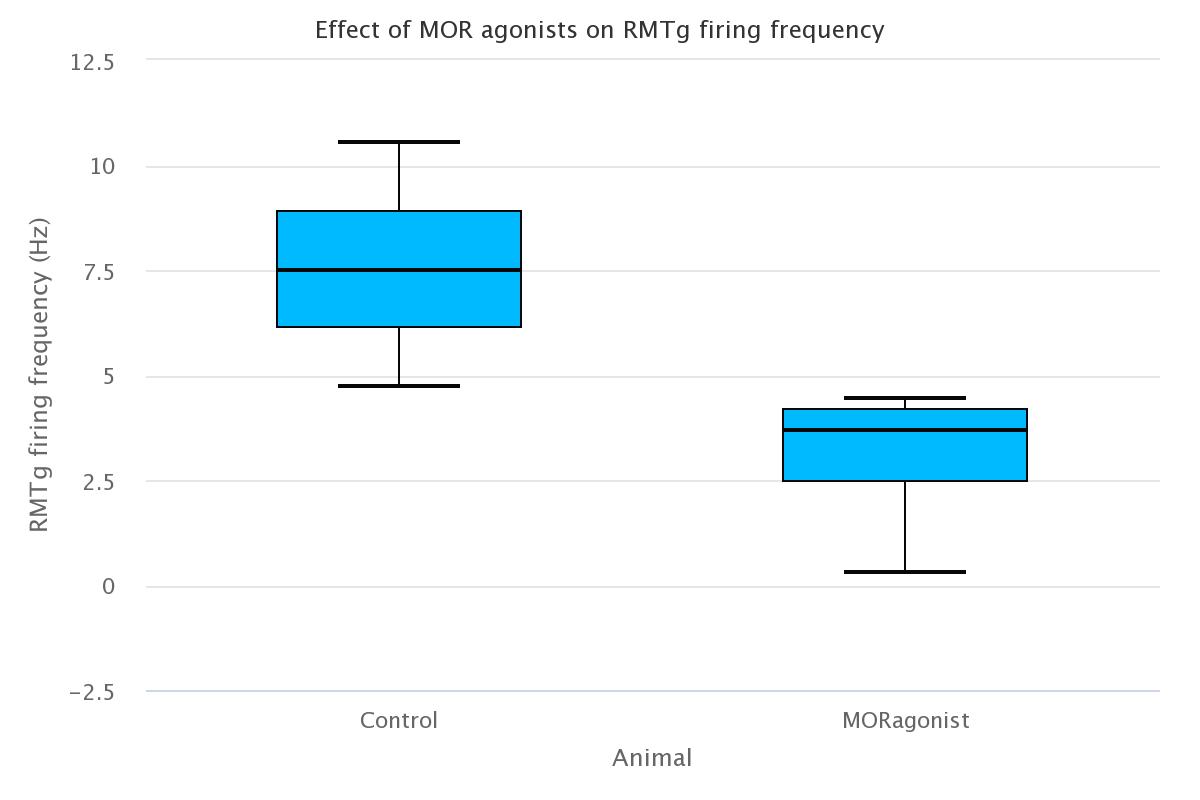


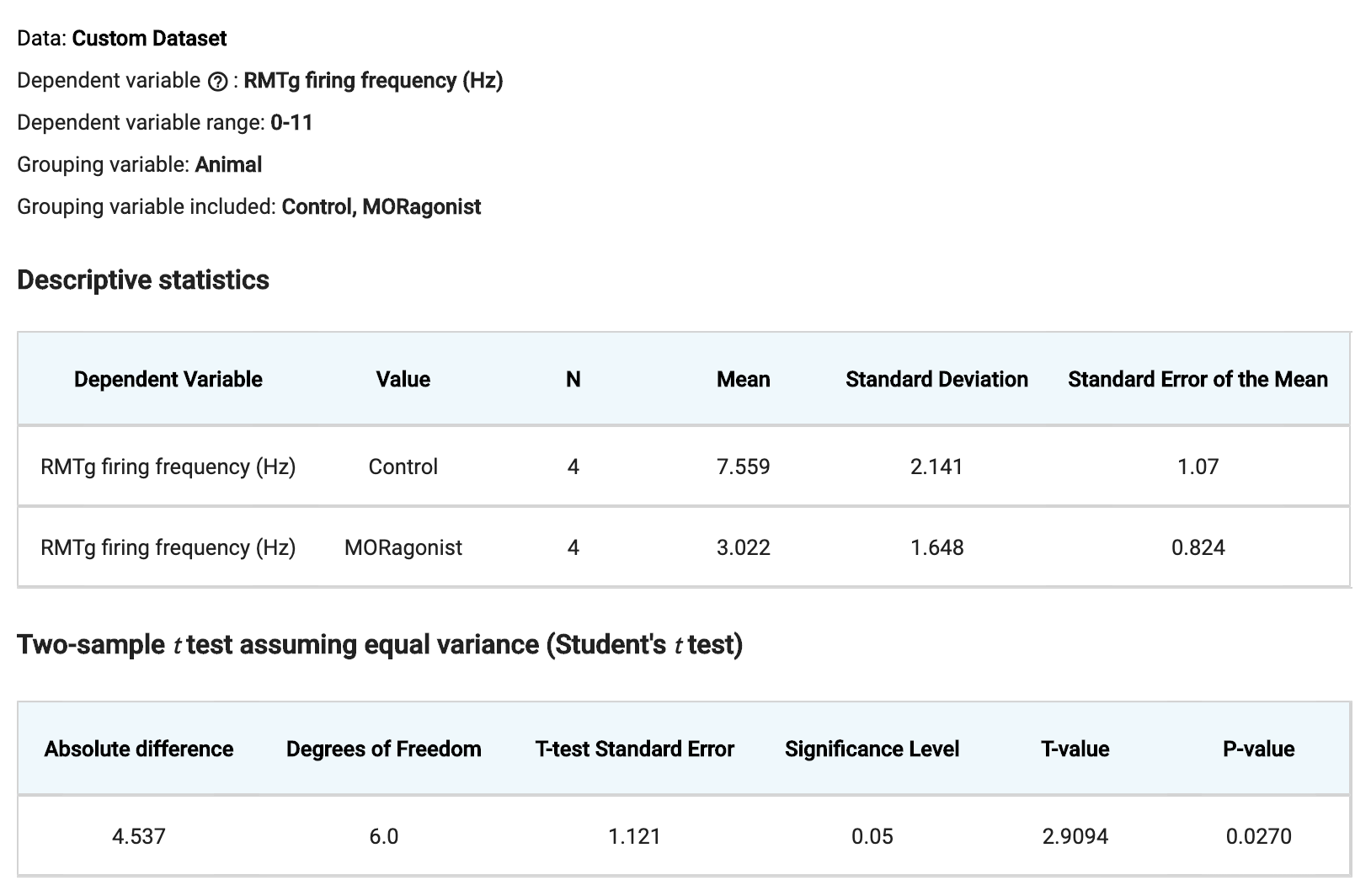
1. What do the findings indicate about the activity of RMTg neurons upon exposure to each opioid receptor agonist?

Answer: The application of the agonists MOR, but not DOR or KOR, cause RMTg neurons to hyperpolarize by a few mV. This suggests that the membrane potential of RMTg neurons, when opioids are around, may be lower. Thus, it is harder for the RMTg neurons to fire and opioids are inhibiting these neurons. If the RMTg neurons are GABAergic (inhibitory), then the net effect is that dopamine neurons are less inhibited, and have a greater chance to release dopamine into the nucleus accumbens. Instructor’s note: If students don’t get to this last point, it might be a good exercise to have them develop a hypothesis, and come up with an experiment to test their hypothesis. This should lead into the next data figure.

1. To determine whether opioids change the firing frequency of RMTg neurons, scientists used electrophysiological techniques to record how many action potentials RMTg neurons fire per second (recorded in Hz). This data is collected in the dataset under RMTg figure frequency (Hz). Using the dataset, make a graphs that visualizes the effect of mu opioid receptor agonists have on the firing frequency of RMTg neurons

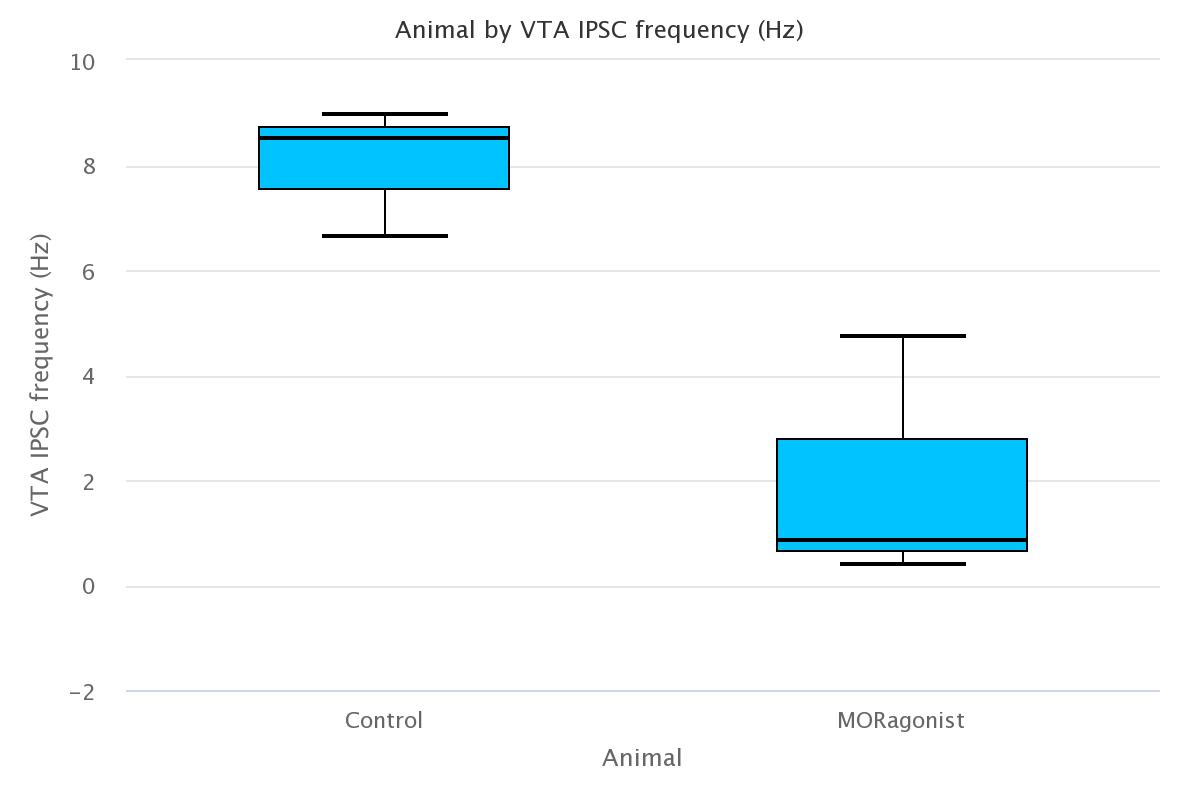
**Answer: Figure 2 and statistical analyses**

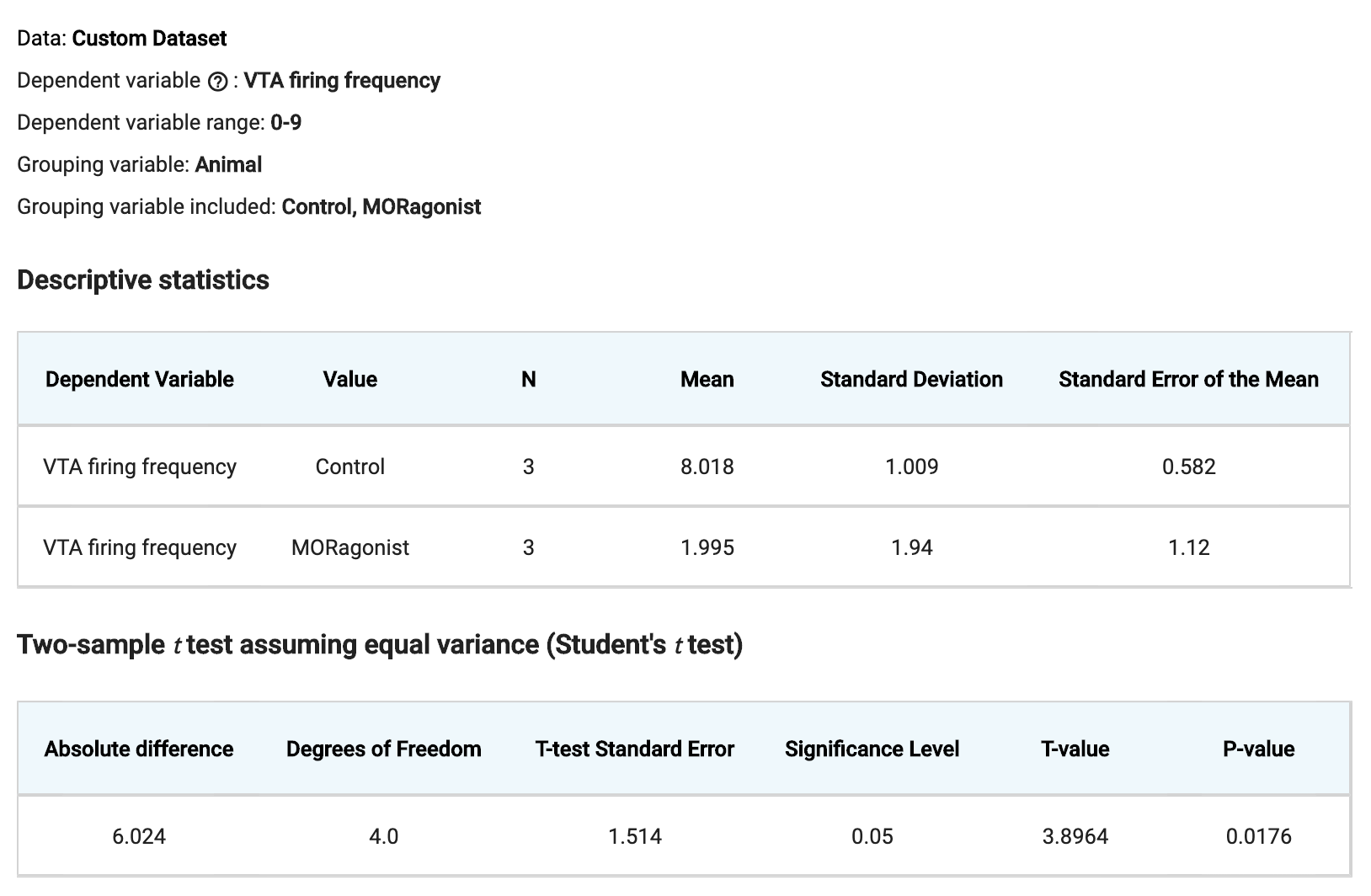




1. Our model of the brain reward system is that RMTg neurons are GABA neurons that inhibit dopamine-producing neurons in the VTA. If this is true, than mu-opioid receptor agonists should have less inhibition. To test this, scientists used electropyhsiology to record from dopamine neurons in the VTA. They measured the amount of IPSCs (inhibitory post-synaptic potentials) and this data is in the VTA IPSC frequency (Hz) column of the dataset. Create a boxplot that shows VTA IPSC frequency between control and MOR agonists.. Perform student’s t-test to determine whether changes in firing frequency is significant.

**Answer: Figure 3**





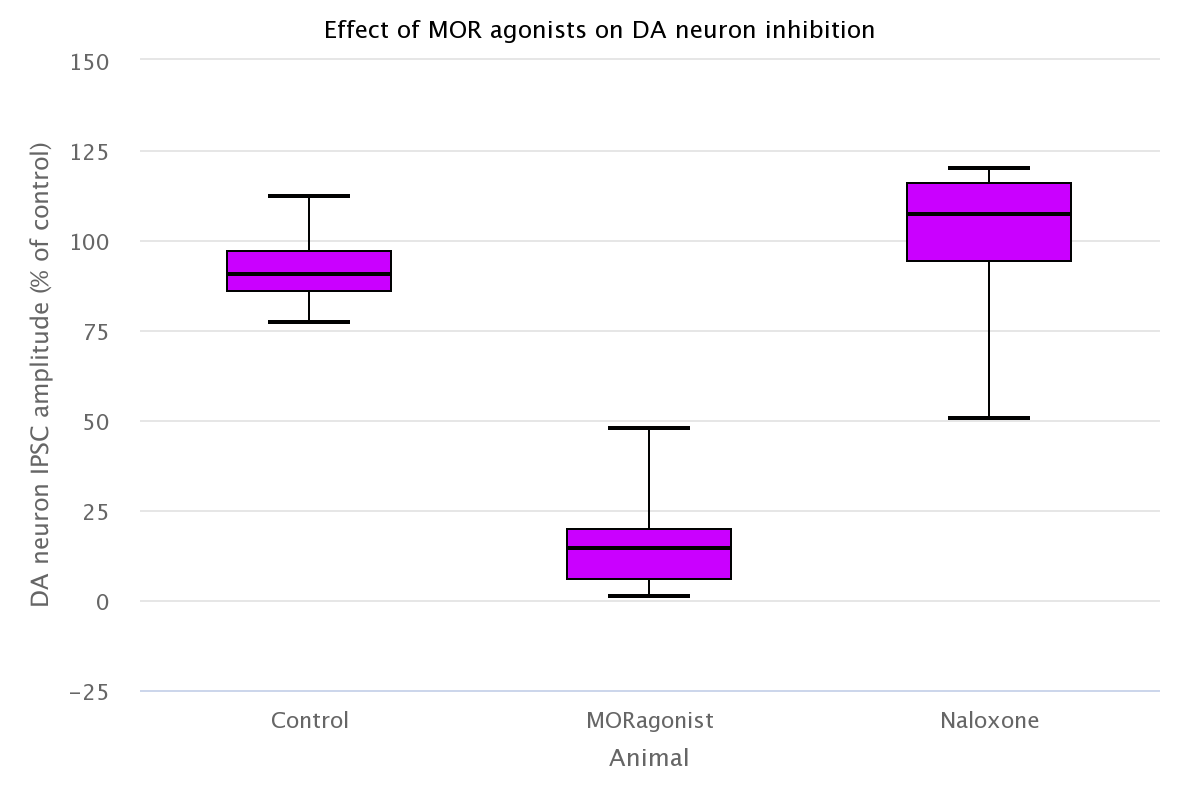
1. Explain your results. Using your data, explain how a model for how opioids affect GABA producing RMTg and dopamine producing VTA neurons in the brain’s reward pathway.

Answer: MOR agonists cause a decrease in the RMTg neuron firing frequency (3.0 +/- 0.8 Hz) compared to control (7.6 +/- 1.1 Hz). This suggests that the RMTg neurons have decreased activity. If RMTg neurons are made of GABAergic neurons that project to the VTA, we would expect that VTA neurons receive less inhibitory signals. By analyzing the effect of MOR agonists on VTA neurons, we see that the IPSC frequency is decreased with agonists (2.0 +/- 1.1 Hz) compared to controls (8.0 +/- 0.6 Hz). This indicated that they receive less inhibitory inputs. Thus, taken together, it would suggest that opioids may inhibit GABA neurons in the RMTg, and disinhibit DA neurons in the VTA.

Alternatively: MOR are found on both GABAergic neurons and DAergic neurons. MOR agonists can inhibit GABA neuron firing, but RMTg neurons might have little impact on RMTg. MOR agonists then independently reduce inhibitory postsynaptic currents (IPSCs) of DA neurons, allowing DA neurons to fire more. Follow up question: how would you design an experiment that would test this?

1. Naloxone is a drug that is sometimes given to opioid overdose victims. Naloxone is a mu-opioid receptor antagonist, meaning that it interferes with the ability for opioids to bind the receptor and cause changes in cells. Scientists tested the effect of Naloxone by adding it to dopamine neurons right after it was exposed to mu-opioid receptor agonists. First, make a prediction of what the effect of mu-opioid receptor agonists and Naloxone is on IPSC frequency of dopamine neurons in the VTA. Using the dataset, make a graph that compares the dopamine neuron IPSC amplitude when dopamine neurons are given control, mu-opioid receptor agonists, and naloxone.

**Answer: Figure 4**



1. What is the effect of mu-opioid receptor agonists on IPSC firing frequency in dopamine neurons in the VTA.

Answer: MOR causes DA neurons to have lower IPSC amplitude (Values) compare to controls (Values). This indicates that DA neurons are being inhibited less (disinhibition). Based on the decreased activity of RMTg neurons (Figure 2 generated), it might suggest that RMTg neurons that normally inhibit DA neurons, are doing so less when MOR agonists are present.

1. What happened to IPSC firing frequency in dopamine neurons in the VTA when treated with Naloxone, the antagonist for mu-opioid receptors. How does the result support your original prediction?

Answer: Naloxone treated cells showed DA neuron IPSCs similar to that of control. Thus, even in the presence of the MOR agonist (DAMGO), the DA neurons were not affected because Naloxone out competed DAMGO for MOR binding sites.

1. Re-examine your drawing of the brain’s reward circuit from question 1. Now re-draw or modify that drawing by adding the type of neuron in the RMTg and VTA. Include identifiers that indicate whether synapses are excitatory or inhibitory.

Answer: Hopefully, your students understand how neurons connect a bit more. They may end up drawing a figure closer to what is described below. Here, you can clearly see that the GABA neurons are found in the RMTg (part of the VTA), and synapse on dopamine neurons in other regions of the VTA. They may also add that this synapse can be inhibitory because the GABA neuron releases GABA which would inhibit the dopamine neuron. The dopamine neuron then synapses on neurons in the nucleus accumbens, which can propagate a reward signaling in the brain.

**Advanced questions**

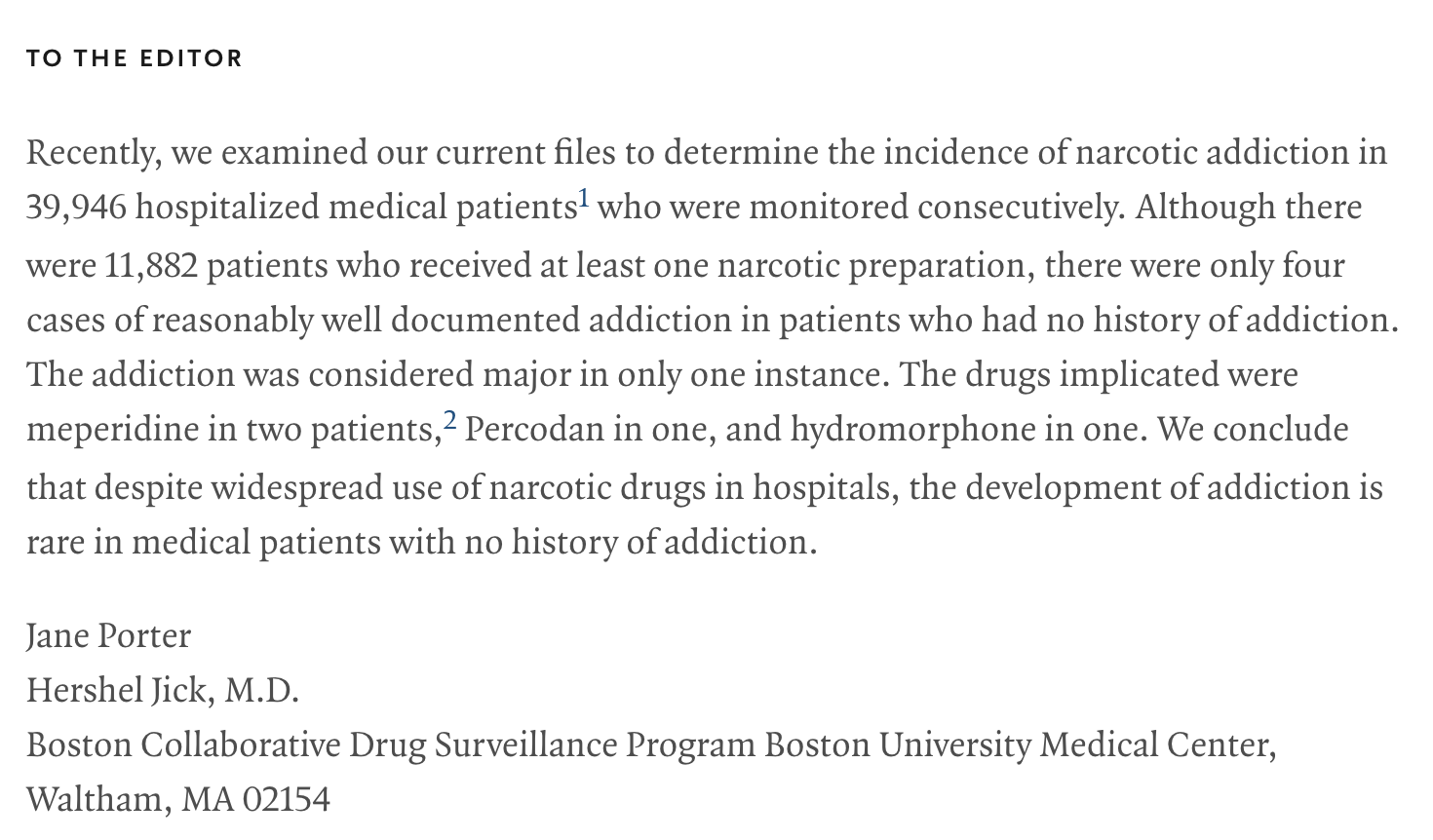
1. Opioid overdose is dangerous because it can slow breathing and lead to death. Mu-opioid receptors are also found in a brainstem region called the pre-Botzinger complex. This region of the brain controls breathing. Come up with a model to why an opioid overdose can lead to death?

Answer: Opioids can inhibit the excitatory neurons that stimulate breathing. They also inhibit pH sensitive neurons that respond to low oxygen levels. This can depress breathing and decrease responses to decreased blood oxygen.

1. Would you use Naloxone as a drug to treat people that have overdosed on opioids (use your answer in question 9 to help your answer)?

Answer: Naloxone addition to the bath solution in figure 4/question 9 returned the DA neuron IPSC back to control levels of IPSC activity. Thus, Naloxone appears to compete well with MOR agonists for MOR binding, as expected. When an overdosed patient is having trouble breathing, Naloxone treatment will remove the opioid stimulus from MOR, and allow breathing circuit to work again. Thus, it is a good idea for treatment. (in fact, it is used)

1. Ethics - The NEJM published a non-reviewed letter that concluded that despide opioid use, the “development of addiction is rare.” This led to numerous citations, including those by Purdue Pharma, maker of Oxycontin. Discuss the impact of the following NEJM letter on the opioid epidemic.



Answer: Data suggest that pharmaceutical companies, using this reference that lacked experimental design and review, pushed the use of opioid pain relievers. Furthermore, this letter did not include that opioid drugs were given at low doses, in controlled conditions. Nonetheless, companies promoted their drug, and doctors over-prescribed oxycontin and other pain relievers. This is thought to stem the opioid epidemic today. The following reference shows how often this NEJM letter was cited.

Sources: <https://www.nejm.org/doi/full/10.1056/NEJMc1700150>

Students may say that it wasn’t right for the authors to submit this letter to the NEJM, or for the NEJM to publish this letter. Ultimately, this letter lacked critical details that are normally in a controlled study. Thus, it was also not right for a big company such as Purdue Pharma to push their drug based on this letter. Purdue Pharma plead guilty of misleading doctors and patients, paying over $600 million in a lawsuit (source: <https://www.nytimes.com/2007/05/10/business/11drug-web.html>)

**References**

Cite the related research papers and publications using [Chicago Manual](http://www.easybib.com/guides/citation-guides/chicago-turabian/) of Style format. **This should include the reference for the data source listed in Data Explorer.**

**Credits**

Written by (Teacher) Jason Chan, Marian University, Indianapolis, IN

Edited by Jason Chan, Marian University

etc.

# **4. Data Activity: Student Handout**

This is the **student document** (PDF) for the activity that accompanies the data set, which should walk through a specific application of the data. It is paired with the [Educator Materials](#_aa1yr6wk67nx).

When FMN folks write this document, Data Explorer may not be published yet. They can keep this more general and match it to Data Explorer later.

**Introduction**

Of patients that are prescribed opioid medication, approximately 21-29% misuse the drug and 8-12% develop an opioid use disorder (NIH). The government has put forth initiatives to combat addiction related disorders, but in 2018, an average of 128 people died each day from overdosing on opioids in (NIH). The opioid epidemic started with over prescription of opioid medication, and the increased opioid usage and death was furthered by the production of heroin and synthetic opioid drugs. This activity explores the effect of opioids on neurons in the brain. Specifically, the dataset comes from a study that examines how the neurons of the brain’s reward center communicates and respond to opioids. Opioids, such as oxycodone or morphine, can become a drug of abuse because they over stimulate reward centers in the brain and can lead to addition. Through the exploration of a dataset exploring the effect of opioids on neurons of the ventral tegmental area (VTA), one the main regions in the brain’s reward pathway, you will analyze data showing that opioids increase the activity that mediates reward signaling. To explore these concepts, you will make graphs of electrophysiological data collected from different neurons exposed to opioid receptor agonists and antagonists.

**Background (OPTIONAL)**

Using this dataset (opioid crisis\_exploring neurophysiology.csv), you can explore how opioids affect neurons in the brain, and how opioids impact the brain’s “reward pathway.” Opioids can be classified into four categories: endogenous opioids (e.g. endorphins, which are produced by the body), opium alkaloids (e.g. morphine, which are naturally produced), semisynthetic opioids (e.g. oxycodone) and synthetic (e.g. fentanyl). Opioids are important analgesics that reduce pain signals; however, many opioids can cross the blood brain barrier to also act on the brain. Excessive use of opioids can act specifically on the brain’s mesolimbic region, also known as the reward pathway, and lead to the dependency on opioids. This can then lead to opioid use disorders, and even death due to overdose (see figure below).

**15,469**

Deaths due to Heroin

**17,087**

Deaths due to Prescription Opioids

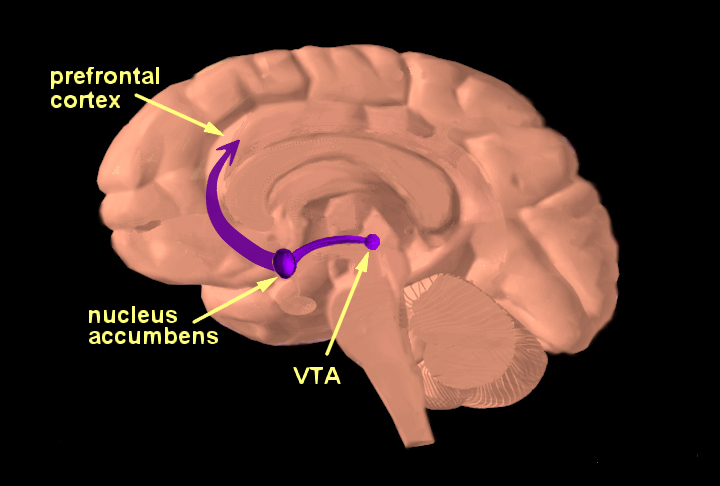
**19,413**

Deaths due to Synthetic Opioids

**In 2016, there were 42,249 deaths due to opioid related overdose (numbers from the National Institute of Drug Abuse)**

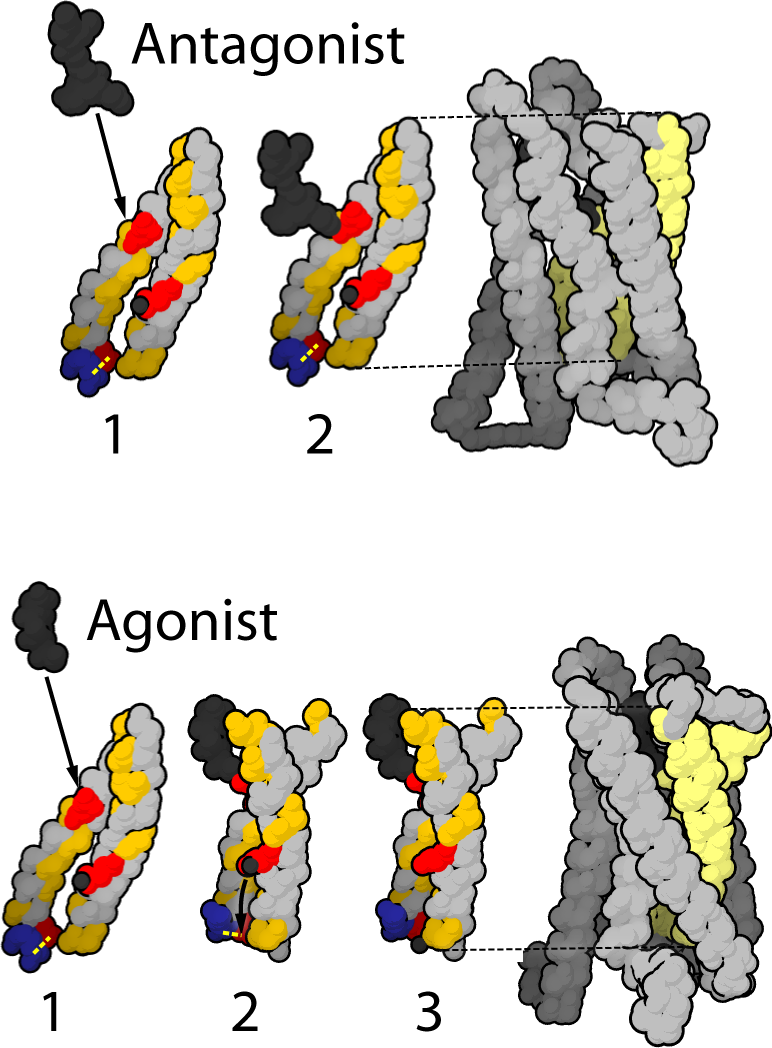
Opioid overdose deaths have increased six fold since 1999 and make up approximately 70% of all overdose deaths (CDC). Furthermore, 80% of heroin users first misused opioid drugs. While outside the scope of this dataset, instructors may want to use facts on the current Opioid Crisis to discuss with students. Sources include the NIH (NIDA) and the CDC (CDC):

The misuse of opioids overstimulates the brain’s reward pathway and can lead to drug dependency. Opioids can bind receptors on neurons; these receptors belong to three main classes – 𝛍-opioid (mu) receptors (MOR), 𝛅-opioid (delta) receptors (DOR), and 𝛋-opioid receptors (KOR) (Fields and Margolis 2015, Stein 2016). Mu-opioid receptors are thought to be the main opioid receptor that mediates responses to opioids in the reward pathway. In the brain, mu-opioid receptors are responsible for communicating our feelings of reward for food, pleasure, and goal-directed behavior among others (Fields and Margolis 2015, Wang 2019). Specifically, mu-opioid receptors are found on neurons of the ventral tegmental area (VTA). They can be found on dopaminergic neurons (neurons that make and secrete the neurotransmitter dopamine); or, they can be found on GABAergic neurons (neurons that make and secrete the neurotransmitter GABA). Dopaminergic neurons send dopamine to another region called the nucleus accumbens, which communicates higher cortical brain regions that signal the receipt of the reward (see figure below).



**Figure of the reward pathway**

To better explore the neuroscience of opioid signaling, this exercise examines data from experiments performed by Matsui and Williams (2011). They perform electrophysiological recordings on neurons of different parts of the reward pathway. Electrophysiology is a technique that allows scientists to measure the membrane electrical activity in response to stimuli, such as opioids. They analyze two main neurons types: 1) neurons that make the inhibitory neurotransmitter GABA, found in the RMTg region of the VTA and 2) neurons that make dopamine in the VTA and send reward signals to the nucleus accumbens. By following the changes of the activity of neurons in the GABA neurons in the RMTg and dopamine neurons in the VTA in response to mu-opioid agonists and antagonists, students can recreate the reward pathway and understand how opioids change the activity in the reward pathway.



**Figure of antagonist and agonist action on mu opioid receptors.** This model depicts how an agonist can change the structure (protein conformation) of a mu opioid receptor, which can then lead to a response inside cells. However, binding of an antagonist to the receptor may not change the receptor, and thus does not cause a cell response.

**Procedure/Questions**

1. Draw a simple circuit that shows the brain’s reward pathway.
2. How does activation of the mu opioid receptors in the VTA lead to increased DA release in the nucleus accumbens (NA)?

**Data analysis questions**

1. Using the dataset, make a graph to visualize the level of inhibition that RMTg neurons receive in response to opioid. Scientists measured inhibition by recording from neurons in the RMTg using a technique called electrophysiology, in which they can measure changed in membrane voltage. A decrease (more negative) change in voltage is a hyperpolarization, and makes it harder for neurons to fire action potentials. Scientists measured hyperpolarization after treating RMTg neurons with different opioid receptor agonist, particularly ones that target the mu-opioid receptor (MOR), the delta-opioid receptor (DOR), and kappa-opioid receptor (KOR). Help determine which type of agonists initiates a hyperpolarization in RMTg neurons.
2. What do the findings indicate about the activity of RMTg neurons upon exposure to each opioid receptor agonist?
3. To determine whether opioids change the firing frequency of RMTg neurons, scientists used electrophysiological techniques to record how many action potentials RMTg neurons fire per second (recorded in Hz). This data is collected in the dataset under RMTg figure frequency (Hz). Using the dataset, make a graphs that visualizes the effect of mu opioid receptor agonists have on the firing frequency of RMTg neurons
4. Our model of the brain reward system is that RMTg neurons are GABA neurons that inhibit dopamine-producing neurons in the VTA. If this is true, than mu-opioid receptor agonists should have less inhibition. To test this, scientists used electropyhsiology to record from dopamine neurons in the VTA. They measured the amount of IPSCs (inhibitory post-synaptic potentials) and this data is in the VTA IPSC frequency (Hz) column of the dataset. Create a boxplot that shows VTA IPSC frequency between control and MOR agonists.. Perform student’s t-test to determine whether changes in firing frequency is significant.
5. Explain your results. Using your data, explain how a model for how opioids affect GABA producing RMTg and dopamine producing VTA neurons in the brain’s reward pathway.
6. Naloxone is a drug that is sometimes given to opioid overdose victims. Naloxone is a mu-opioid receptor antagonist, meaning that it interferes with the ability for opioids to bind the receptor and cause changes in cells. Scientists tested the effect of Naloxone by adding it to dopamine neurons right after it was exposed to mu-opioid receptor agonists. First, make a prediction of what the effect of mu-opioid receptor agonists and Naloxone is on IPSC frequency of dopamine neurons in the VTA. Using the dataset, make a graph that compares the dopamine neuron IPSC amplitude when dopamine neurons are given control, mu-opioid receptor agonists, and naloxone.
7. What is the effect of mu-opioid receptor agonists on IPSC firing frequency in dopamine neurons in the VTA.
8. What happened to IPSC firing frequency in dopamine neurons in the VTA when treated with Naloxone, the antagonist for mu-opioid receptors. How does the result support your original prediction?
9. Re-examine your drawing of the brain’s reward circuit from question 1. Now re-draw or modify that drawing by adding the type of neuron in the RMTg and VTA. Include identifiers that indicate whether synapses are excitatory or inhibitory.

**Advanced questions**

1. Opioid overdose is dangerous because it can slow breathing and lead to death. Mu-opioid receptors are also found in a brainstem region called the pre-Botzinger complex. This region of the brain controls breathing. Come up with a model to why an opioid overdose can lead to death?
2. Would you use Naloxone as a drug to treat people that have overdosed on opioids (use your answer in question 9 to help your answer)?
3. Ethics - The NEJM published a non-reviewed letter that concluded that despide opioid use, the “development of addiction is rare.” This led to numerous citations, including those by Purdue Pharma, maker of Oxycontin. Discuss the impact of the following NEJM letter on the opioid epidemic.