

The Effect of Epinephrine on Clam Heart Rate and the Effect Ethanol on Clam Heart Rate in Relation to the NMDA receptor

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[Introduction](#)

[Materials and Methods](#)

[Results](#)

[Discussion and Conclusions](#)

[Bibliography](#)

Introduction

Ethanol (C_2H_6O) is a molecule which is soluble in water. It can be fermented to make fuel but it is more commonly known as a drug found in many alcoholic beverages to induce intoxication (Wikimedia 2005). It is a drug which has various serious physiological effects. Ethanol acts as a central nervous system depressant and increases levels of norepinephrine and dopamine in the brain. It also decreases the amount of serotonin in the brain. Over prolonged use, alcohol can cause serious damage to the brain, particularly the frontal lobes. Ethanol has been used throughout history and is the most commonly used drug in society today. For a drug that is so powerful, there is a significantly incomplete understanding. At low concentrations, ethanol can impair concentration, reduce tension, and slow reflexes. At higher concentrations, it can alter emotions, induce vomiting, and result in unconsciousness or even a coma (Chudler 2000). Ethanol is the cause of these physiological effects but why? The manner in which ethanol effects the brain, behavior, and cells is currently still a incompletely understood.

Epinephrine, otherwise known as adrenaline, acts to produce energy by signaling glycogen to break down into glucose-1-phosphate which is important in muscular function and activity (Cooper 2004) as well as play a central role in short-term stress reaction in the sympathetic nervous system. In extreme conditions (exciting or threatening situations) epinephrine will produce a fight-or-flight response in an organism (Wikimedia 2005). Unlike ethanol, which has been known to decrease heart rate in organisms such as *Daphnia* (crustaceans commonly known as water fleas (Carlson 1999), epinephrine typically elevates heart rate (Wikimedia 2005).

Cell receptors such as the N-methyl D-aspartate (NMDA) receptor, a receptor highly expressed in brain neurons, are used to receive cell signals in order to initiate a series of signaling pathways that regulate cell behavior. The substance received by the NMDA receptor is a neurotransmitter, glutamate, which is released by synaptic terminals to transmit information from a nerve cell to another nerve cell which contains the NMDA receptor (Paves, 1997). The NMDA receptor is hypothesized to have a correlation with the way in which ethanol interacts with cells, resulting in a person's intoxication. Once in contact with a cell, ethanol causes the NMDA receptor to be blocked. However, the biochemical mechanism in which ethanol causes the inhibition of the NMDA receptor is not yet known. The NMDA receptor's role is to allow the molecules Ca^{2+} , Na^{+} , and K^{+} into the cell when glutamate, a salt or ester of glutamic acid, binds to it (Kandel, 2000). At high concentrations, Mg^{2+} ions can block the NMDA receptor. The NMDA receptor is composed of the subunits R1, R2A, R2B, R2C, and R2D which are all regulated by phosphorylation and kinases. These receptors

are currently being tested and researched by various researchers to form conclusions about the exact way in which they are effected by ethanol.

The clam, a member of the phylum Mollusca, was used in this experiment to view how ethanol affects heart rate. The clam was used in this experiment because it is readily available and could be dissected and observed with ease. In this experiment, I compared the resting heart rate of a clam to the heart rate of a clam under the influence of ethanol and then under the effect of epinephrine. From the data recorded, the heart rates could be compared and and conclusions could be drawn about the effect that these drugs have on the NMDA receptor. The primary focus of this paper is how ethanol interacts with the NMDA receptor. I hypothesized that the heart rate of the clam would decrease under the influence of ethanol.

[Introduction](#) [Materials and Methods](#) [Results](#) [Discussion and Conclusions](#) [Bibliography](#)

Materials and Methods

Dissection of Clams

All clams were dissected by cutting the adductor muscles starting at the umbo of the clam and cutting along the inside of the shell, avoiding any other muscles. The umbo was pointed away from the body and only the left adductor muscles were cut. Once the adductor muscles were cut and the shell naturally opened, the shell free of the clam was pulled back and off. The dissected clam was immediately placed in 200 mL filtered sea water.

Control: Finding Heart and Heart Rate of Clam

A clam was placed underneath the dissecting microscope. Tissue covering the gills and heart were cut away and pulled back using a probe and scalpel. An additional 50 mL of filtered sea water was warmed for 30 seconds in a microwave. 20 mL of the warmed filtered sea water was added to the dissecting dish with the clam in order to increase the heart rate to ease observation. A movie of the heart rate was recorded over an interval of one minute. This heart rate was recorded. This procedure was repeated with two more clams.

Epinephrine Preparation

To obtain a 50 μM which is equal to 9.2 mg/10 L, epinephrine solution, add 13mg epinephrine to 14 mL distilled H_2O (Beech 2005).

Finding Clam Heart Rate With Clam Under Influence of Ethanol

A clam was dissected following the procedure of *Dissection of Clam*. The heart of the clam was found by using a probe and scalpel to cut away and pullback tissue covering the gills and heart. A video of the heart rate was taken over an interval of one minute. This heart rate was recorded. Next, 4 mL of ethanol was added to the filtered seawater with the clam to create a 2% v/v solution of ethanol. After one minute, another video was taken of heart rate over an interval of one minute. This heart rate was counted and recorded. Three minutes after the ethanol was added to the filtered sea water with the clam, a third video of the heart rate was recorded over an interval of one minute. This heart rate was counted and recorded. This procedure was repeated with two more clams.

Finding Clam Heart Rate With Clam Under Effect of Epinephrine

A clam was dissected following the procedure of *Dissection of Clam*. The heart of the clam was found by using a probe and scalpel to cut away and pullback tissue covering the gills and heart. A video of the heart rate was taken over an interval of one minute. This heart rate was recorded. To expose the clam to a 5×10^{-5} epinephrine solution, 100 μM was added to the dissecting dish containing 200 mL filtered sea water and the clam. After 1 minute, a video of the heart rate was recorded over an interval of one minute. This heart rate was counted and recorded. Three minutes after the epinephrine was added to the filtered sea water solution, another video of the clam heart rate was recorded over the interval of one minute. This heart rate was counted and recorded. This procedure was repeated with two more clams.

Comparing Resting Heart Rate of Clam to Heart Rate of Clam Under Effect of Epinephrine

A clam was dissected following the procedure of *Dissection of Clam*. The heart of the clam was found by using a probe and scalpel to cut away and pullback tissue covering the gills and heart. A video of the heart rate was taken over an interval of one minute. This heart rate was recorded. A video of the heart rate was taken again after two minutes and five minutes, both over the interval of one minute.

A clam was dissected following the procedure of *Dissection of Clam*. The heart of the clam was found by using a probe and scalpel to cut away and pullback tissue covering the gills and heart. 300 μ M epinephrine was added to the dissecting dish containing 200 mL filtered sea water and the clam. A video of the heart rate was taken over an interval of one minute. This heart rate was recorded. A video of the heart rate was taken again after two minutes and five minutes, both over the interval of one minute.

[Introduction](#) [Materials and Methods](#) [Results](#) [Discussion and Conclusions](#) [Bibliography](#)

Results



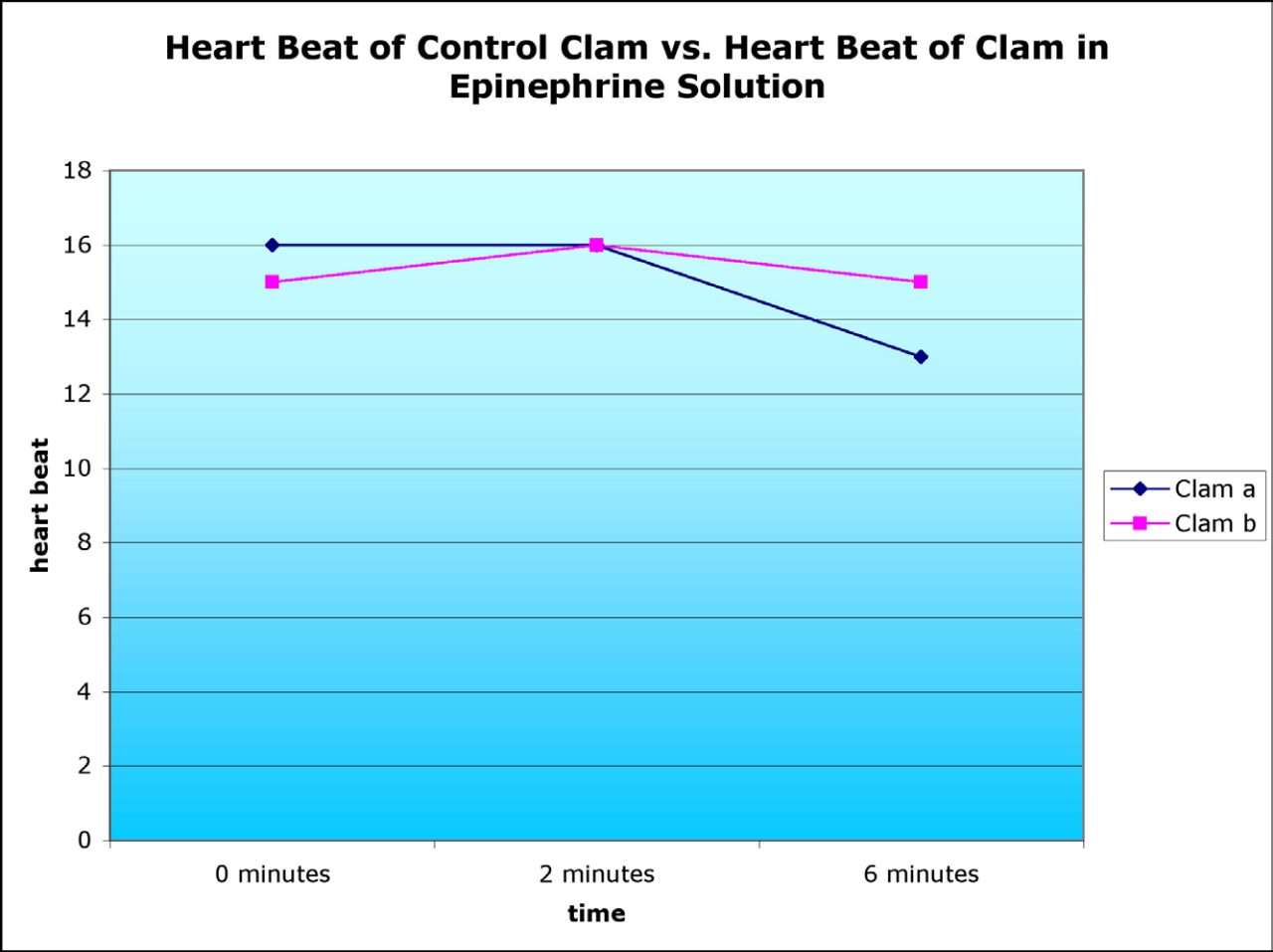
Fig. 1: The clam heart at resting heart rate. The clam heart is located at the center of this picture. It was just underneath a layer of tissue and is located above the gills which resemble white feathers.

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The average heart rate of the three clams in 2% v/v ethanol solution was 15 beats per minute before the ethanol was

added, 15 beats per minute 1 minute after the ethanol was added, and 12 beats per minute 6 minutes after the ethanol was added. On average, underneath the influence of ethanol, the clam heart rate decreased after 1 minute. Clam 1 showed slight deviation in heart rate after 1 minute compared to the rest of the data. In comparison, the average heart rate for 3 different clams in 5×10^{-5} M epinephrine solution was 11 beats per minute before the epinephrine was added, 10 beats per minute 1 minute after the epinephrine was added, and 9 beats per minute 6 minutes after the epinephrine was added. On average, the heart rate of the clams under the effect of epinephrine decreased at a steady but slow rate. Individually, the heart rate of each clam either decreased slightly or remained consistent.

Graph: Clam Control Compared to Clam in Solution with 300 μ M epinephrine



The heart rate of the clam control decreased at a faster rate after 2 minutes than the heart rate of the clam in solution with 300 μ M epinephrine which remained consistent.

<>The average heart rate of a clam after being under the influence of ethanol for 6 minutes is lower than both the clam control and the clam with 300 μ M after 5 minutes. Though there is a slight difference in time, it is possible that the heart rate of a clam under the influence of ethanol is either the same or slightly lower than the heart rate of the control clam which is not exposed to either ethanol or epinephrine.

Discussion and Conclusions

I hypothesized that the heart rate of the clam would decrease under the influence of ethanol. The results from this experiment show that the heart rate of a clam under the influence of ethanol decreases steadily. The clam heart rate of a clam in solution with epinephrine decreased at a slower rate than a clam under the influence of ethanol and at some points remained constant. The data from the clam control as opposed to the clam in solution with 300 μM in 200 mL filtered sea water further supports this conclusion.

The results concerning the clams under the influence of ethanol could support the conclusion that ethanol slows heart rate. However, there is no data for clam heart rate under the influence of ethanol observed from the time it is dissected and through the duration of 5 minutes to compare with the clam control and clam with 300 μM epinephrine as shown in Table 3. The experiment would have been stronger with this data.

This could suggest that the heart rate of a human decreases for a period of time with alcohol consumption. This is probably more likely with higher concentration or intake of alcohol considering the effects of alcohol are mild at low concentrations. One effect which ethanol has either on the NMDA receptor is the increase of protein kinase C (PKC) and protein kinase A (PKA) phosphorylation on the NR1 subunit (Morrow 2004). PKC cleaves PIP2 to form IP3 and diacylglycerol. IP3 then binds to a receptor on the membrane of the endoplasmic reticulum and triggers the efflux of Ca^{2+} into the cytosol of the cell (Cooper, Hausman 2004). I speculate that the way in which the subunits of the NMDA receptor react to ethanol in some way not only causes the physically visible signs of intoxication such as slurred speech and loss of inhibitions but other physiological effects like the involuntary response of slowing of heart rate.

Additional research on the NMDA will make the molecular explanation for intoxication less ambiguous. Further experimentation could include studying the NMDA receptor more directly by applying a channel blocker such as MK-801 and ifenprodil, both potential blockers for different subunits on the NMDA receptor and studying the subsequent cellular effects. This method could be applied using mice as the experimental organism while making sure to follow humane methods and in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. (Boyce-Rustay 2004.)

[Introduction](#)

[Materials and Methods](#)

[Results](#)

[Discussion and Conclusions](#)

[Bibliography](#)

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