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#### How do brain chemicals influence who wins a fight?

Featured scientists: Andrew Bubak and John Swallow from the University of Colorado at Denver, and Kenneth Renner from the University of South Dakota

### Research Background:

In nature, animals compete for resources. These resources include space, food, and mates. Animals use aggression as a way to capture or defend these resources, which can improve their chances of survival and mating. **Aggression** is a forceful behavior meant to overpower opponents that are competing for the same resource. The outcome (victory or defeat) depends on several factors. In insects, the bigger individuals often win. However, if two opponents are the same size, other factors can influence outcomes. For example, an individual with more experience may defeat an individual with less experience. Also individuals that are fighting to gain something necessary for their survival have a strong drive, or motivation, to defeat other individuals.

Researchers Andrew, Ken, and John study what role an animal's brain plays in regulating behavior when motivation is present. They wanted to know if specific chemicals in the brain influenced the outcome of a physically aggressive competition. Andrew, Ken, and John read a lot papers written by other scientists and learned that there is a brain chemical that plays an important role in regulating aggressive behavior. This chemical is called **serotonin** and is found in the brains of all animals, including humans. Even a small amount of this chemical can make a big impact on aggressive behavior, and perhaps the outcome of competition.



Picture 1. Two stalk-eyed flies rearing/extending forearms in battle. Photo credit: Sam Cotton.

The researchers decided to do an experiment to test what happens to aggression during competition as serotonin levels in the brain increase. They used stalk-eyed flies in their experiment. Stalk-eyed flies have eyes on the ends of stalks that stick out from the sides of their heads (Pictures 1 & 2). They reasoned that brain serotonin levels in stalk-eyed flies influence their aggressive behaviors in battle and therefore impact the outcome of competition. If their hypothesis is true, they predicted that increasing the brain serotonin in a stalk-eyed fly would make it more likely to use aggressive behaviors, and flies that used more aggressive behaviors would be more likely to win. Battling flies use high-intensity aggressive attacks like jumping on or striking an opponent. They also use less aggressive

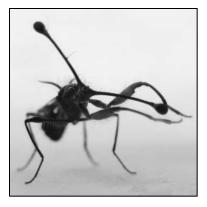


Picture 2. A male stalk-eyed fly compared to the size of a dime. Photo credit: Andrew Bubak, June 2016.

behaviors like flexing their front legs or rearing up on their hind legs.

To test their hypothesis, the researchers set up a fair test. A **fair test** is a way to control an experiment by only changing one piece of the experiment at a time. By changing only one variable, scientists can determine if that change caused the differences they see. Since larger flies tend to win fights, the flies were all matched up with another fly that was the same size. This acted as an experimental control for size, and made it possible to look at only the impact of serotonin levels on aggression. The scientists also controlled for the age of the flies and made sure they had a similar environment since the time they were born. The experiment had 20 trials with a different pair of flies in each. In each trial, one fly received corn mixed with a dose of serotonin, while another fly received plain corn as a control. That way, both flies received corn to eat, but only one received serotonin.

Each pair of flies was placed in a fighting arena and starved for 12 hours to increase their motivation to fight over food. Next, food was placed in the center of the arena, but only enough for one fly! The researchers observed the flies, recording three types of behaviors for each opponent. High intensity behaviors were when the fighting flies touched one another. Low-intensity behaviors were when the flies did not touch each other, for example jump attacks, swipes, and lunges. The last behavior type was retreating from the fight. Flies that retreated fewer times than their opponent were declared the winners. After the battles, the researchers collected the brains of the flies and measured the concentration of serotonin in each fly's brain.



<u>Scientific Question:</u> How does serotonin level affect aggressive behavior and, therefore, the probability of winning against an opponent of similar size?

<u>What is the hypothesis?</u> Find the hypothesis in the Research Background and underline it. A hypothesis is a proposed explanation for an observation, which can then be tested with experimentation or other types of studies.

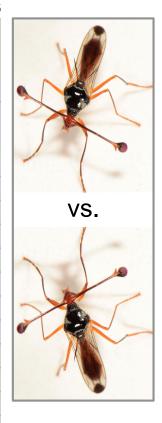
#### Scientific Data:

## Use the data in the following two tables to answer the scientific question:

Table 1. Serotonin Levels vs. Outcomes of Stalk-Eyed Flies

Serotonin Levels (concentration measured in picograms of serotonin per microgram of brain matter [pg/ $\mu$ g])

Loser (pg/ $\mu$ g) Loser (pg/ $\mu$ g)
20 15
62 45
90 38
34 113
57 24
99 59
23 32
39 21
67 16
30 26
21 26
15
49 22
19 16
69 29
75 24
39 21
46 38
97 36
51 24
21 106



The units used by the researchers are picograms (pg) and micrograms ( $\mu$ g). A picogram is one-trillionth (1/10<sup>12</sup>) of a gram and a microgram is one-millionth (1/10<sup>6</sup>) of a gram. The level of serotonin found in the brain is given using the ratio of serotonin measured in picograms to brain matter in micrograms.

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Table 2. Stalk-Eyed Fly Behaviors vs. Outcomes In Battle

Observed Behaviors In Battle	How many winners did this?	How many losers did this?
High-Intensity	16	5
Swipe/lunge	11	4
Jump Attack	11	2
Retreats	2	20

High-intensity behaviors include any behavior where the flies came in contact with each other. Low-intensity behaviors included swipe/lunge and jump attacks.

Data for serotonin levels of the winners and losers are listed in Table 1. As mentioned before, the researchers fed one of the two stalk-eyed flies serotonin-rich food before each trial. They did this to make sure the difference in serotonin between the two flies was high enough to be measured and have an effect on behavior. However, there were times where the natural level of serotonin in the control fly was higher than that of the treated fly. Therefore, the data in Table 1 compares serotonin levels for winners and losers, but does NOT identify whether a fly was treated or not. Table 2 shows frequencies of behaviors compared to outcome.

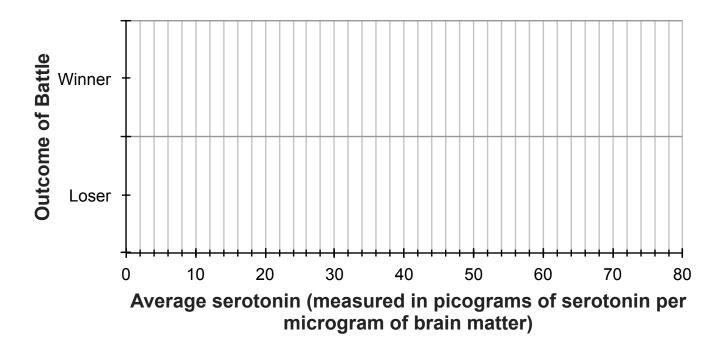
What data will you graph to answer the question?

Table	1:
	Independent variable:
	Dependent variable:
Table	2:
	Independent variable:
	Dependent variable:

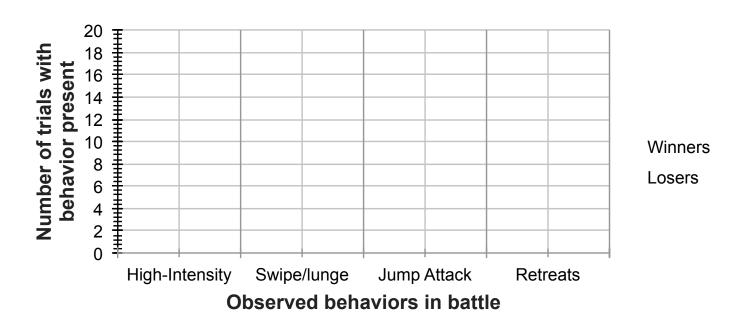
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<u>Draw your graphs below</u>: Identify any changes, trends, or differences you see in your graphs. Draw arrows pointing out what you see, and write one sentence describing what you see next to each arrow.

# Graph 1



Graph 2



Name	-
Interpret the data:  Make a claim that answers the scientific question.	
What evidence was used to write your claim? Reference specific parts of the table graphs.	∍s or
Explain your reasoning and why the evidence supports your claim. Connect the databack to what you learned about how brain chemicals influence animal behavior.	ata

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Did the data support Andrew, Ken, and John's hypothesis? Use evidence to explain why or why not. If you feel the data were inconclusive, explain why.
Your next steps as a scientist: Science is an ongoing process. What new question(s)
should be investigated to build on Andrew, Ken, and John's research? What future data should be collected to answer your question(s)?