Muscular Excitability Under the Influence of Alcohol and Caffeine

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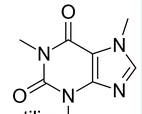
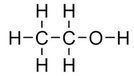
Introduction



- Caffeine, a legal stimulant, is a component that can be derived from various seeds, nuts, and plant leaves.
- Many people worldwide frequently utilize caffeinated products for their stimulatory and psychoactive effects.
- Alcohol (ethanol) is produced by fermentative processes in which yeast is broken down glucose into carbon dioxide and alcohol.
- Alcoholic drinks like beer, wine and liquor etc. are used worldwide for their euphoric effects. Ethanol is a psychoactive drug that can act as either a stimulant or depressant, depending on the quantity consumed



Background

- **Caffeine**
  - CNS stimulant.
  - antagonist of adenosine (A1 and A2).
  - indirectly alters the release of norepinephrine, dopamine, acetylcholine, serotonin, glutamate, and GABA; it's stimulatory effect on these compounds has been found to have inhibitory effects on neuronal function.
  - regulates the activity of Ryanodine receptors and thus it has a direct effect on the release of intracellular calcium.
  - Studies on zebrafish embryos have noted motor neuron defects, such as reduced tactile sensitivity and muscle fiber misalignment,
- **Alcohol**
  - CNS depressant.
  - modifies the inhibitory and excitatory synaptic transmission within the CNS
  - modulates ion channels involved with mediating excitability.
  - studies on the giant squid axon, evidence leads us to believe that ethyl alcohol depresses neural excitability.
  - Similar effects indicating decreased excitability was first noted in frog neurons and frog skeletal muscle.
  - Regarding neuromuscular junctions, alcohol inhibits the conductivity of Na+ ions, prevents muscle fatigue, potentiates the neuromuscular transmission, and reduces the amplitude of excitatory responses.

Methods

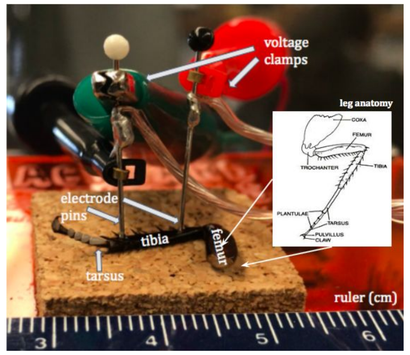


Figure 1 . Experimental Setup and Leg Anatomy of the Cockroach

- Model organism of choice: Madagascar Hissing Cockroach (Gromphadorhina portentosa)
- Experimental group conditions:
  - no drug (control) n=5
  - saline (control) n=6
  - caffeine (drug) n=6
  - ethanol alcohol (drug) n=4
- To account for the variable responsive ranges demonstrated by each individual organism, we ran a no-drug control trial before subjecting the leg to each of the prior listed conditions. This allowed us to monitor and isolate the effects of time and fluid displacement apart from pitch sensitivity.
- To anesthetize, we immersed each cockroach body into a traditional ice bath. Once the insects movement and sensory responsivity ceased, we removed one hind limb (either from the left or right side) using a pull method.
- Following the dissection, we mounted the leg onto the cork of the Spiker box device. We placed one electrode recording pin into the tibial region proximal to the tarsus and the other in the tibial region proximal to the femur-tibia joint.
- Voltage clamps were attached to the recording pins; their placement of which allowed for the transmission of a range of pitches.
- We used an online tone generating device to generate the various pitch frequencies; in response, we watched for signs of muscular excitation.
- We noted the minimal and maximal frequencies that produced a muscular response and then denoted this as our responsive range.

Questions and Hypothesis:

★ We hypothesize that the the responsive pitch range will decrease when under the influence of ethanol, and decrease upon the administration of caffeine.

Results: Administration of Drugs does not affect low pitch responsiveness

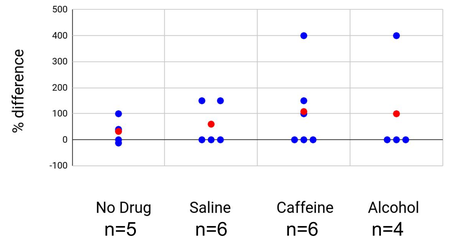


Figure 2. Low pitch responsiveness was the same amongst all trials. The graph above shows the percent increase or decrease in low extreme response values from control to experimental conditions. All calculated percent difference data is in blue and red dots depict group means. One way ANOVA revealed a insignificant effect of treatment on low extreme responsivity, $F(3, 16)= 1.0259$, $p = 0.4074$.

Results: Administration of Drugs does not affect high pitch responsiveness but shows trends

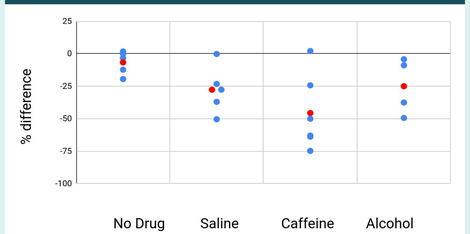


Figure 3. High pitch responsiveness was statistically the same amongst all trials. The graph above shows the percent increase or decrease in high extreme response values from control to experimental conditions. All calculated percent difference data is in blue and red dots depict group means. One way ANOVA revealed a insignificant effect of treatment on high extreme responsivity, $F(3, 16)= 3.0131$, $p = 0.0608$.

Results: Administration of Caffeine Decreases Muscular Responsivity

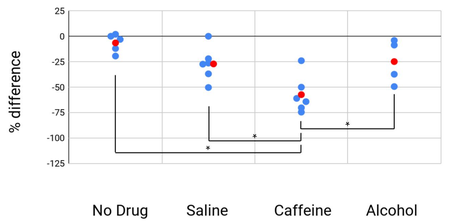


Figure 4. Administration of drugs affects overall muscular responsivity. One Way ANOVA Analysis of percent decreases revealed a significant effect of treatment on responsivity, $F(3, 16)= 8.7576$, $p = 0.0009$. All calculated percent difference data is in blue and red dots depict group means.

Results: Image J analysis

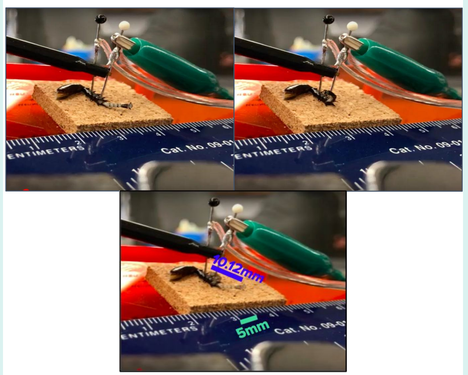


Figure 5. Image J analysis reveals Muscular response varies between legs. Using image J analysis we set out to measure the exact distance of contraction when under influence of experimental drug and electrical stimulus. Legs at baseline varied in the distance they contracted; some just twitched, others contracted fully. Percent differences could not be determined due to the set-up variability Ensuring that the leg is in the same orientation and position in relation to ruler and camera is essential for consistent measurements. Pin placement could also influence the varying leg contraction distance.

Conclusions

- Caffeine decreases muscle responsivity compared to No Drug, Saline, and Alcohol trials
- Alcohol had no significant effect on muscle responsivity compared to controls
- The volume injected may play a role in muscle responsivity

Future Directions

- Consistently utilize the same removal technique (either snipping or pulling) for all recorded trials
- Obtain official camera gear to document each muscular response from multiple perspectives (aerial, front, and back views etc.)
- Set markers/borders to specify the setup arrangement of the camera documenting device, measurement tool, and leg angle positioning
- Use imageJ software to quantify the contractive responses observed in each trial
- Test an alternative model organism, like crickets
- Investigate muscular excitability in response to the dual administration of both caffeine and alcohol.

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