Behavioural responses to olfactory input may be reflected in the *Schistocerca gregaria* flight central pattern generator

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Abstract

The locust flight central pattern generator (CPG) is a model circuit frequently used to study rhythmic pattern formation. Sensory information is integrated into the locust CPG by way of the tegula stretch receptors. Additionally, olfaction is an important sensory modality in many organisms, especially insects. In the following experiment, the role of olfactory top-down sensory input will be explored in the *Schistocerca gregaria* flight pattern CPG, by introducing female locusts to attractants and repellants and analyzing their steering. Tendencies towards changes in direction and steering occurred for attractive and aversive odors.

Introduction

Many organisms rely heavily on olfaction for survival. It is used to localize prey, food, and to detect pheromones (Schneider, 1969). Locusts have an olfactory system similar to most insects, in which the odor is transduced to an electrical impulse in the antennae by olfactory receptor neurons (ORNs), which is sent to the antennal lobe (AL), where the ORNs separate into glomeruli, depending on their receptor type (Wilson et al, 2006), and finally to the mushroom body and lateral protocerebrum, which are responsible for higher order processes such as associative odor learning (Schneider, 1969, Saha et al, 2013, de Belle et al, 1994). Olfaction and other sensory modalities are instrumental in guiding the behaviour of locusts and help them to find food, avoid prey or toxic substances, and



between antennae and the nature (attractant or repellent) of the

odorant.

behaviours can be adjusted by presenting aversive or attractive stimuli to the animal (Haskell et al, 1962). It is the relative difference of odorant concentration between the two antennae that accounts for an insect's ability to localize the source and steer towards or away from it (Hansson, 1999). For

example, an attractive odorant on the insect's right hand side will diffuse away from its source, and be at a higher concentration near the right antenna than the left. The insect will detect this difference, and steer towards it. The same model applies to repellants,

and the insect will steer away from high concentrations (Fig. 1).

Although observations of an animal's behaviour can lead to qualitative conclusions about attraction and repulsion, one must also find ways to quantify changes in behaviour using its underlying physiological mechanisms. The presence of a well-studied and stereotypical central pattern generator for flight in locusts makes them an ideal model for electrophysiological recordings that will be used to this end.

Central pattern generators are circuits in the central nervous system that need no sensory input or descending control to allow muscles to contract in a certain rhythmic pattern of fixed timing (Marder et al, 2001). In the case of locusts, this pattern relates to flight and involves both elevators and depressors of forewing and hind-wing. Donald

localize an appropriate mate (Anton et al, 2006). In an experimental context, these

Wilson discovered this CPG in 1961, when he observed "fictive flight" in locusts, recording full motor flight patterns when the only remaining parts of the locust were its head and thoracic ganglia; sensory input was completely absent (Wilson, 1961). Since their discovery, CPGs have been found for many motor patterns such as walking, breathing and swimming in many different animals, including humans (Marder et al, 2001). The locust flight circuit, however, is more complex than a simple central pattern generator. It also involves initiative input from the sensory tegula organ, which activates upon being compressed by wing depression and affects the speed of the rhythm (Wolf et al, 1988). Because of this known role of input for the locust CPG, study of other sensory modalities and their effects on the wing-beat pattern are necessary.

Locust flight musculature is partially direct and partially indirect (Josephson, 2006). Direct flight muscles attach to each wing, one lateral and one medial to the wing's focal rotational point. The lateral muscle (depressor) pulls the wing down, while the medial muscle (elevator) pulls it up. Conversely, indirect flight muscles do not attach to the wings. Instead, they function by deforming the thorax. Contractions of dorso-ventral muscles pull the dorsal thorax (tergum) down, causing wing elevation, while contractions

of the longitudinal muscles shorten the thorax and depress the wings (Josephson, 2006). Locusts change orientation while flying by skipping wing beats for muscles ipsilateral to the direction of the turn (Wilson, 1968). Furthermore, in studies on phonotaxis, forewing depressor timing increases in frequency for the muscle on the inside of the



Figure 2: The M97 forewing elevator muscle is highlighted in red. It is located laterally in the anterior aspect of the thorax.

turn while steering to avoid or approach a sound (Dawson et al, 1997). Together, these findings indicate the forewing depressor (M97) as a candidate for electrophysiological recordings regarding steering in response to sensory cues (Fig. 2).

While many CPGs have been found, one must not take for granted that they generate their patterns in perfect accordance with the definition of a central pattern generator. Often, sensory input is required for some aspect of the pattern. In locusts, stretch receptors are essential in maintaining the correct frequency of flight rhythm (Wolf et al, 1988). Further studying other sensory inputs and their effects on the locust flight CPG is an interesting and relevant step in understanding neural circuits.

Methods

We used seven female *Schistocerca gregaria* because of their large size as compared to males, and for their tendency to fly more consistently. We selected locusts that had two intact antennae to ensure normal olfaction. Before recording, we removed all of the locusts' legs and mounted them by the pronotum to the head of a large nail hammered into a block of wood, using wax. We then implanted both M97 forewing depressor muscles wire electrodes, whose insulation was intact so as to record from a small area. These electrodes were waxed to the locusts' ventral thorax to stop the wires from becoming dislodged during the experiment. We used the recordings from these muscles as a proxy for flight pattern and steering as outlined above. We inserted a reference electrode with wire insulation removed so as to record from a large area, into the abdomen of each locust and served as a comparison point for the changes in potential in the forewing M97 muscles. All three wires were secured to a second nail in the block of

wood using modeling clay to further avoid displacement during flight, and were fed to an A-M Systems Inc. Model 3000 AC/DC differential amplifier. The amplifier's leads were sent to a computer for recording and analysis of traces using the Axoscope 9.0 software.

During recording sessions, we placed the locust, attached to its block of wood, in the middle of a large cardboard box with the top cut out (Fig. 3A). The box served to block out visual sensory input that may have affected the results, which were to be based solely on olfaction. We coated the locust's left antenna in Vaseline, which served to numb the antenna and enhance the locust's ability to detect a difference in concentration between left and right antenna, since the left antenna would detect little concentration at



all (Kennedy et al, 2011). In the middle of each side of the box, we cut a hole and inserted a

Figure 3: Box apparatus view from A) the top with the locust inside on a block of wood, and toilet paper tube B) the side with toilet paper tubes inserted.

into it (Fig. 3B). The outer end of the tube was covered by a perforated piece of tissue to facilitate airflow. At the beginning of each trial, we placed an odorant in the inside, open end of the right hand tube. Odorants were a bruised lettuce attractant, 6x300mg valerian root capsules, each containing the valeric acid equivalent (0.8%) of 2.4g of valerian root as a repellant, no odorant as a control, or ethanol, which is known to elicit no response as an odorant and was used as a second control (Haskell et al, 1962). We placed a very weak fan on the tissue end of the odorant tube to help air flow into the box apparatus, and

once a minute had passed to ensure the odorant's proper diffusion into the locust's olfactory range, we blew air at the locust's head to cause flight. Between each trial, we waited for a five-minute rest period to allow complete dissipation of the previous odorant before a tube containing the new odorant was inserted into the box. Each odorant had its own toilet paper tube to avoid mixing of odors.

After we had run trials for each odorant, we dissected the locust to ensure we had

been recording from the M97 depressor muscle. First, we removed the head, then the abdomen and gut. Then we cut along the dorsal and ventral midlines and separated the right and left sides of the locust. We removed connective tissue and the longitudinal muscles on each side, and specify which one contained the base of the



Figure 4: Inside dissected locust, no muscles removed. Posterior sides facing inward, anterior facing outward, ventral at bottom, electrode is inserted into M97 muscle.

We analyzed traces in Microsoft Excel 2008.

the longitudinal muscles on each side, and continued to remove muscles until we could specify which one contained the base of the recording electrode (Fig. 4)



First, we counted the number of muscle contractions and wing beat skips from the Axoscope 9.0 files for each odor on each wing and chose a threshold for skips equal to the voltage value of the largest contraction divided by two (Fig. 5). To find the wing beat rate and skip rate, we divided these values by the

Figure 5: Example of two Axoscope 9.0 traces with one skip in the top right hand corner.

amount of time over which they were counted. We

calculated the ratio of rates of wing beats and of spikes between the left and right wings

for each odorant because of varying time stretches of usable data. Standard deviations from the mean were calculated for each parameter (N=7), except for skip rate ratio, where we divided the locusts into two groups per odorant per wing in order to avoid division by zero (N=2). For beat and skip rates, we did two-tailed, paired t-tests between left and right wing values within odorants, and between all combinations of odorants for each wing. For ratios, we did two-tailed, paired t-tests for all combinations of odorants (See Appendix). In two locusts, recordings appeared to be from both elevator and depressor muscles, and so we divided the counted wing beats by two.

Results

To determine whether olfactory information is integrated into the *Schistocerca gregaria* flight rhythm CPG, we recorded from the forewing depressor M97 muscles because of their known relation to steering (Dawson et al, 1997). The odorant parameter was varied using an attractant, a repellent, and two controls, all presented on the right hand side. We expected the locust to steer towards the attractant, a movement that would be accompanied by an increase of beat skipping on the right hand wing and an increase in the right wing frequency, reflected in the beat rate. Conversely, when presented on the right with a repellent, we expected the locust to fly away from it by increasing the left wing skips and increasing the beat rate on the same wing. The ratio of left to right skips or rate reflects how much more the locust skipped beats or increased frequency on the left wing, and therefore we expected it to be higher for the repellant and lower for the attractant. None of the results were significant (p > 0.05), likely due to the small number of locusts used, and large standard deviation, but some results suggested a possible trend in the data towards what we expected.

For the lettuce attractant, the right wing (0.471 ± 0.446) skipped more often on average than the left (0.228 ± 0.335) (Fig. 6A) and had a very slightly higher rate



Figure 6: Differences in average skip rates and average beat rates between left and right wings for each odorant. See appendix for standard deviation values.

(15.282±2.376) as compared to the left wing (14.839±2.452) (Fig. 6B). There was also



Figure 7: Average skips per second between odors.

an increase in average right wing skips for lettuce (0.471 ± 0.446) as compared to some other odorants (no odor: 0.4 ± 0.554 , valerian: 0.405 ± 0.455) 7B). (Fig. Finally, there was an overall decrease of the left to right ratio of wing beats for lettuce (0.972±0.059 ethanol: VS. 0.984±0.040, no odor: 1.003 ± 0.036 valerian 1.001±0.031) (Fig. 9B), which indicates that the rate of the right wing was higher than that of the left and that the locusts tended to steer towards the

right hand side. All of these results suggest that with a larger sample size, there may be a significant increase of skips and a higher right wing beat rate as compared to the left. These changes in the flight rhythm CPG would indicate that the locust was flying to the right, towards the attractive odor and verify that there is sensory olfactory input from the brain, which affects the locust central pattern generator.

Similarly, some of the data







Figure 8: Averaage wing beat rate of A) right wing and B) left wing over all odorants.

for the repellent valerian root extract suggested a trend towards avoidance. The left average wing beat rate was higher in valerian extract (16.119 ± 1.945) than in any other odorant (ethanol: 15.590 ± 2.127 , no odor: 15.586 ± 1.423 , lettuce: 14.839 ± 2.452), indicating that animals were flying to the left, away from the odorant (Fig. 8B). Furthermore, the ratio of skips was highest for valerian extract (0.869 ± 0.699) out of all

В



Figure 9: Left to right ratio of A) skips and B) wing beat rates over odorants.

odorants (ethanol: 0.414 ± 0.020 , no odor: 0.443 ± 0222 , lettuce: 0.550 ± 0.071), and the ratio of wing beat rates for valerian extract was higher than lettuce and the ethanol control (but not the odorless control) (see above) (Fig. 9). This means that the left wing skipped more and beat faster for valerian extract than other odorants (except the odorless control, in which it beat faster). If these trends continue with a larger sample size, they would suggest that sensory olfactory avoidance information is also involved in the *Schistocerca gregaria* flight central pattern generator.

The rates and skips between wings and between odors for the ethanol and odorless controls remained similar throughout the experiment, which suggests that between the two, they do not elicit differing behaviour from the locust and are both suitable controls.

Discussion

Animals must adapt their behaviour to meet their own needs and to overcome challenges in their environments. However, using a group of seven female locusts, no significant impact of olfactory sensory input was found on the *Schistocerca gregaria* central pattern generator. In spite of this, we speculate that given the tendencies of the data to coincide slightly with previous studies, a larger sample size and a smaller standard deviation would likely prove that there is information being integrated from the brain that impacts the pattern of flight in locusts, although it is largely regulated by a circuit that needs very little sensory input to function. Our results match those described in the literature. Experiments in phonotaxis have recorded locusts' response to bat-like sounds and have seen significant steering both towards and away from this stimulus, which suggests that ultrasound information is processed by flight circuits (Dawson et al, 1997). Furthermore, locusts exposed to an artificial visual horizon would rotate and steer to match the horizon's angle (Taylor, 1981), and upon having a light shined lateral to their flight path, locusts steer towards the illumination (Baker, 1978). Finally, locusts exposed to different orientations of wind tunnels will vary their flight patterns and wing beat amplitude according to the direction and speed of the air (Baker, 1978). In addition to a wealth of experiments exploring how olfaction affects flight, all of this data suggests that many other sensory modalities impact the flight generation circuit. The use of other sensations compounded with the similarities between our data and previous olfactory studies (in spite of our insignificant results) strongly indicates that olfactory information processing is integral in the locust flight circuit.

Because of this need for behavioural adaptation, central pattern generators are not static circuits. Investigations into the method of CPG modulation in several model species, such as mice and lobsters, have been done. It is accepted that Gprotein coupled neuromodulatory transmitters such as dopamine and serotonin act on many different cells in CPG circuits, up or down-regulating the activity of each neuron involved and thereby allowing for many combinations of action, each one appropriate for its stimulus (Harris-Warrick, 2011). We propose a mechanism in which the upper levels of the olfactory system such as the mushroom body and the lateral protocerebrum integrate olfactory information in the brain, associating different odors with different environmental stimuli. These brain centers will then send descending axons down the nerve cord, which synapse on one or more CPG neurons and modulate the motor rhythm output it will generate. Depending on the attractive or aversive nature of the stimulus, descending neurons must stimulate and repress the correct muscles ipsilateral to the inside of the turn. Together, the CPG and descending sensory input create a stereotyped, but malleable behavioural system.

Certain improvements could be made on our experiment. Odors could have been presented from both sides separately to each locust, ensuring that one antenna was not more functional than the other and reading false concentration differences in odorant molecules. The experiment could also have been done in a quiet room to minimize the effect of auditory stimuli in the lab, which may have added accessory sensory input to the system and confounded the results. Finally, to eliminate all visual stimulation, a cover could be placed over top of the box opening.

There is much room for exploration in the field of central pattern generators and olfactory input in locusts. Firstly, this same experiment should be carried out with more locusts to ensure the role of olfaction in the CPG output. Other pairs of muscles could also be recorded from to test the model of olfactory input in the flight circuit. Additionally, similar experiments could be done on male locusts to look for differences in the nature of odorants. Finally, the study of pheromones and their effect on flight patterns is an interesting subject, especially because their effects differ temporally, causing different amounts of attraction in locusts as they mature, and between sexes (Anton, 2006).

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<u>Appendix</u>

SAMPLE AVERAGE CALCULATION:

(average skip rate left, ethanol)

$$x_{bar} = \frac{\sum x_i}{N}$$
 (from i = 1 to i)

$$x_{\text{bar}} = \frac{0.2 + 1 + 0.1 + 0 + 0 + 0.1 + 0.2}{7}$$

$$x_{bar} = \frac{1.6}{7} = 0.23$$

SAMPLE STANDARD DEVIATION CALCULATION:

(standard deviation skip rate left, ethanol)

SD =
$$\sqrt{\frac{\sum(x-\bar{x})^2}{N-1}^2}$$

$$SD = \sqrt{\frac{\Sigma(0.2 - 0.23)^{2} + (1 - 0.23)^{2} + (0.1 - 0.23)^{2} + (0 - 0.23)^{2} + (0 - 0.23)^{2} + (0.1 - 0.23)^{2} + (0.2 - 0.23)^{2}}{7 - 1}}$$

$$SD = \sqrt{\frac{0.7343}{6}}$$

SD = 0.1224

SAMPLE T-TEST CALCULATION:

(t-test average skip rate left, ethanol vs. control)

**N = 7
ethanol = X
$$(X - avgX)^2$$
 control = Y $(Y - avgY)^2$
0.2 0.000816245 0.4 0.077602042
1 0.595104245 0 0.014744898
0.1 0.016530245 0 0.014744898
0 0.052244245 0 0.014744898
0 0.052244245 0 0.014744898
0.1 0.016530245 0.2 0.014744898
0.1 0.016530245 0.2 0.00617347
0.2 0.000816245 0.25 0.016530613
avgX = 0.228571429
sumX = sum((X - avgX)^2) = 0.121428571
sumY = sum((Y - avgY)^2) = 0.159285714
s²_x = sum((X - avgX)²/N - 1 s²_y = sum((Y - avgY)²/N - 1

 $s_y^2 = sum((Y - avgY)^2)/N - 1$ 0.026547619

t = <u>avgX – avgY</u>									
$\sqrt{s^2 + s^2}$	Two Tailed Significance								
$\sqrt{\frac{s_x}{N} + \frac{s_y}{N}}$	Degrees of freedom (n-1)	α = 0.20	0.10	0.05	0.02	0.01	0.002		
V IN IN	1	3.078	6.314	12.706	31.821	63.657	318.300		
	2	1.886	2.920	4.303	6.965	9.925	22.327		
t = 0.229 - 0.121	3	1.638	2.353	3.182	4.541	5.841	10.214		
	4	1.533	2.132	2.776	3.747	4.604	7.173		
0.122 0.027	5	1.476	2.015	2.571	3.305	4.032	5.893		
$1/\frac{1}{1}+\frac{1}{1}$	s <u>113</u>	a such as	1.000			all all all a	and the second s		
$V 7 \cdot 7$	6	1.440	1.943	2.447	3.143	3.707	5.208		
,	7	1.415	1.895	2.365	2.998	3.499	4.785		
	8	1.397	1.860	2.306	2.896	3.355	4.501		
	9	1.383	1.833	2.262	2.821	3.250	4.297		
t = 0.108	10	1.372	1.812	2.228	2.764	3.169	4.144		
/	S S								
$\sqrt{0.0213}$	11	1.363	1.796	2.201	2.718	3.106	4.025		
V 0.0210	12	1.356	1.782	2.179	2.681	3.055	3.930		
	13	1.350	1.771	2.160	2.650	3.012	3.852		
	14	1.345	1.761	2.145	2.624	2.977	3.787		
t = 0.740	15	1.341	1.753	2.131	2.602	2.947	3.733		

t < 2.447 therefore, not significant p > 0.05

RAW DATA:

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1 alcohol left 150 15 0.987 2 10 100	locust	trial	lead	spikes	time (s)	(spikes/s)	ratio	#skips	time (s)	(skips/s)	first 4	/last 3
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6 alcoholright1701017010007 alcoholright1691016.94100.401 controlleft1591015.90.9884100.402 controlleft77515.41.041050003 controlleft1811018.11.04601000004 controlleft76515.20.93805030.65 controlleft76515.21155030.66 controlleft76515.21155030.67 controlleft128816128001400.4142 controlright74514.8851.600 <td>5</td> <td>alcohol</td> <td>right</td> <td>84</td> <td>5</td> <td>16.8</td> <td></td> <td>3</td> <td>5</td> <td>0.6</td> <td>7</td> <td></td>	5	alcohol	right	84	5	16.8		3	5	0.6	7	
7 alcohol right 169 10 16.9 4 10 0.4 1 control left 159 10 15.9 0.988 4 10 10.4 4 0.2857143 2 control left 77 5 15.4 1.041 0 5 0 - 3 control left 181 10 18.1 1.046 0 10 0 - 4 control left 76 5 15.2 0.33 0 5 0 - - 5 control left 128 8 16 1 2 8 0 0 0 .04 14 2 control left 128 8 16 1 2 8 5 1.6 - <td>6</td> <td>alcohol</td> <td>right</td> <td>170</td> <td>10</td> <td>17</td> <td></td> <td>0</td> <td>10</td> <td>0</td> <td></td> <td></td>	6	alcohol	right	170	10	17		0	10	0		
1 control left 159 10 15.9 0.988 4 10 0.4 0.2857143 2 control left 77 5 15.4 1.041 0 5 0 0 3 control left 181 10 18.1 1.046 0 10 0 3 0 4 control left 76 5 15.2 0.938 0 10 0 3 0.66 5 control left 76 5 15.2 1 1 5 0.22 0 3 0.66 6 control left 76 5 15.2 1 1 5 0.22 0 3 0.66 7 control right 161 10 16.1 4 10 0.4 14 0 0.4 14 0 0.4 14 0 0.4 15 0.2 0 15 0.0 15 0.0 0 0 0 0 0 0 <td>7</td> <td>alcohol</td> <td>right</td> <td>169</td> <td>10</td> <td>16.9</td> <td></td> <td>4</td> <td>10</td> <td>0.4</td> <td></td> <td></td>	7	alcohol	right	169	10	16.9		4	10	0.4		
2 control left 77 5 15.4 1.041 0 5 5 6 3 control left 181 10 18.1 1.046 0 10 0 0 0 5 control left 133 10 13.3 1.008 0 10 0 3 0.6 6 control left 7 5 15.2 1 1 5 0.2 0	1	control	left	159	10	15.9	0.988	4	10	0.4	4	0.2857143
3controlleft1811018.11.0460101010104controlleft76515.20.938050030.65controlleft1331013.31.00801010030.66controlleft76515.2115 0.22 030.67controlleft1288161280.25001controlright74514.8851.6000 <t< td=""><td>2</td><td>control</td><td>left</td><td>77</td><td>5</td><td>15.4</td><td>1.041</td><td>0</td><td>5</td><td>0</td><td></td><td></td></t<>	2	control	left	77	5	15.4	1.041	0	5	0		
4 control left 76 5 15.2 0.938 0 5 0 3 0.6 5 control left 133 10 13.3 1.008 0 10 0 3 0.6 6 control left 76 5 15.2 1 1 5 0.2 1 7 control left 78 8 16 1 2 8 0.25 1 1 control right 161 10 16.1 4 10 0.4 14 2 control right 74 5 14.8 8 5 1.6 2 3 control right 173 10 17.3 2 10 0.2 2 10 0.2 10 4 control right 132 10 13.2 0 15.3 10.0 15.3 10.0 10 0.4 5 10 5 control right 128 16.1 10.0	3	control	left	181	10	18.1	1.046	0	10	0		
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	4	control	left	76	5	15.2	0.938	0	5	0		
6 control left 76 5 15.2 1 1 5 0.2 7 control left 128 8 16 1 2 8 0.25 1 control right 161 10 16.1 4 10 0.4 14 2 control right 74 5 14.8 8 5 1.6 3 control right 173 10 17.3 2 10 0.2 4 control right 81 5 16.2 0 5 0 5 control right 132 10 13.2 4 10 0.4 5 6 control right 76 5 15.2 1 5 0.2 1 7 control right 128 8 16 0 8 0 0 1 1 lettuce left 195 0.995 2 <td< td=""><td>5</td><td>control</td><td>left</td><td>133</td><td>10</td><td>13.3</td><td>1.008</td><td>0</td><td>10</td><td>0</td><td>3</td><td>0.6</td></td<>	5	control	left	133	10	13.3	1.008	0	10	0	3	0.6
7controlleft128816128 1123 123 1123 1controlright1611016.14100.4142controlright74514.8851.6143controlright1731017.32100.2164controlright81516.2050165controlright1321013.24100.456controlright128816080167controlright128816080161lettuceleft1531015.31.0078100.8100.52lettuceleft1951019.50.9952100.2163lettuceleft1951019.50.9952100.2164lettuceleft127815.875108060.66lettuceleft1231012.30.969010016161lettuceright1521012.50.9776100.616161lettuceright15211101.12016 <td>6</td> <td>control</td> <td>left</td> <td>76</td> <td>5</td> <td>15.2</td> <td>1</td> <td>1</td> <td>5</td> <td>0.2</td> <td></td> <td></td>	6	control	left	76	5	15.2	1	1	5	0.2		
1 control right 161 10 16.1 4 10 0.4 14 2 control right 74 5 14.8 8 5 1.6 3 control right 173 10 17.3 2 10 0.2 10 4 control right 81 5 16.2 0 5 0 10 5 control right 132 10 13.2 4 10 0.4 5 6 control right 76 5 15.2 1 5 0.2 10 0.8 0 10 0.4 10 0.4 10 0.4 10 0.4 10 10 0.4 10 0.4 10 0.4 10 0.4 10 0.4 10 0.4 10 10 0.4 10 0.4 10 0.5 10 10.5 10 10.5 10 10.5 10 0.5 10 0.5 0 10 0.5 10 <	7	control	left	128	8	16	1	2	8	0.25		
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	1	control	right	161	10	16.1		4	10	0.4	14	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	2	control	right	74	5	14.8		8	5	1.6		
4 control right 81 5 16.2 0 5 0 1 5 control right 132 10 13.2 4 10 0.4 5 6 control right 76 5 15.2 1 5 0.2 1 7 control right 128 8 16 0 8 0 1 1 lettuce left 153 10 15.3 1.007 8 10 0.8 10 0.5 2 lettuce left 146 10 14.6 1.013 0 10 0 0 10 0.5 3 lettuce left 195 10 19.5 0.995 2 10 0.2 10 0.2 10 0 10 0 10 0 10 0.5 0 10 10 1.5 10 12.5 0.7 6 10 0.6 10 16 10 1.6 10 1.6 10 1.6<	3	control	right	173	10	17.3		2	10	0.2		
5 control right 132 10 13.2 4 10 0.4 5 6 control right 76 5 15.2 1 5 0.2 1 7 control right 128 8 16 0 8 0 1 1 lettuce left 153 10 15.3 1.007 8 10 0.8 10 0.5 2 lettuce left 146 10 14.6 1.013 0 10 0	4	control	riaht	81	5	16.2		0	5	0		
6 control right 76 5 15.2 1 5 0.2 7 control right 128 8 16 0 8 0 0 1 lettuce left 153 10 15.3 1.007 8 10 0.8 10 0.5 2 lettuce left 146 10 14.6 1.013 0 10 0	5	control	riaht	132	10	13.2		4	10	0.4	5	
7 control right 128 8 16 0 8 0 1 lettuce left 153 10 15.3 1.007 8 10 0.8 10 0.5 2 lettuce left 146 10 14.6 1.013 0 10 0	6	control	right	76	5	15.2		1	5	0.2		
1 left 153 10 153 1.007 8 10 0.8 10 0.5 2 lettuce left 146 10 14.6 1.013 0 10 0 0 3 lettuce left 195 10 19.5 0.995 2 10 0.2 0	7	' control	riaht	128	8	16		0	8	0		
2 lettuce left 146 10 14.6 1.013 0 10 0 3 lettuce left 195 10 19.5 0.995 2 10 0.2 4 lettuce left 69 5 13.8 0.842 0 5 0 5 lettuce left 127 8 15.875 1 0 8 0 6 0.6 6 lettuce left 125 10 12.5 0.977 6 10 0.6 0 7 lettuce left 123 10 12.3 0.969 0 10 0 0 1 lettuce right 152 10 15.2 11 10 1.1 20 1 lettuce right 152 10 15.2 11 10 1.1 20 2 lettuce right 144 10 14.4 6 10 0.6 15 3 lettuce right 196<	1	lettuce	left	153	10	15.3	1.007	8	10	0.8	10	0.5
3 lettuce left 195 10 19.5 0.995 2 10 0.2 4 lettuce left 69 5 13.8 0.842 0 5 0 5 lettuce left 127 8 15.875 1 0 8 0 6 0.6 6 lettuce left 125 10 12.5 0.977 6 10 0.6 0 7 lettuce left 123 10 12.3 0.969 0 10 0 0 1 lettuce right 152 10 12.3 0.969 0 10 0.6 0 1 lettuce right 152 10 15.2 11 10 1.1 20 2 lettuce right 144 10 14.4 6 10 0.6 0 3 lettuce right 196 10 19.6 0 10 0 0 4 lettuce right 82 5 16.4 3 5 0.6 10 </td <td>2</td> <td>lettuce</td> <td>left</td> <td>146</td> <td>10</td> <td>14.6</td> <td>1.013</td> <td>0</td> <td>10</td> <td>0</td> <td></td> <td></td>	2	lettuce	left	146	10	14.6	1.013	0	10	0		
4 lettuce left 69 5 13.8 0.842 0 5 0 5 lettuce left 127 8 15.875 1 0 8 0 6 0.6 6 lettuce left 125 10 12.5 0.977 6 10 0.6 7 lettuce left 123 10 12.3 0.969 0 10 0 1 lettuce right 152 10 15.2 11 10 1.1 20 2 lettuce right 144 10 14.4 6 10 0.6 3 lettuce right 196 10 19.6 0 10 0 11 4 lettuce right 196 10 19.6 0 10 0 11 4 lettuce right 82 5 16.4 3 5 0.6 12	3	lettuce	left	195	10	19.5	0.995	2	10	0.2		
5 left 127 8 15.875 1 0 8 0 6 0.6 6 lettuce left 125 10 12.5 0.977 6 10 0.6 7 lettuce left 123 10 12.3 0.9977 6 10 0.6 7 lettuce left 123 10 12.3 0.969 0 10 0.6 1 lettuce right 152 10 15.2 11 10 1.1 20 2 lettuce right 144 10 14.4 6 10 0.6 3 lettuce right 196 10 19.6 0 10 0 4 lettuce right 82 5 16.4 3 5 0.6	4	lettuce	left	69	5	13.8	0.842	0	5	0		
6 left 125 10 12.5 0.977 6 10 0.6 7 leftuce left 123 10 12.3 0.977 6 10 0.6 7 leftuce left 123 10 12.3 0.969 0 10 0 1 leftuce right 152 10 15.2 11 10 1.1 20 2 leftuce right 144 10 14.4 6 10 0.6 3 leftuce right 196 10 19.6 0 10 0 4 leftuce right 82 5 16.4 3 5 0.6	5	lettuce	left	127	8	15.875	1	0	8	0	6	0.6
7 left 123 10 12.3 0.969 0 10 0 1 lettuce right 152 10 15.2 11 10 1.1 20 2 lettuce right 144 10 14.4 6 10 0.6 3 lettuce right 196 10 19.6 0 10 0 4 lettuce right 82 5 16.4 3 5 0.6	6	lettuce	left	125	10	12.5	0.977	6	10	0.6		
1 lettuce right 152 10 152 11 10 1.1 20 2 lettuce right 144 10 14.4 6 10 0.6 3 lettuce right 196 10 19.6 0 10 0 4 lettuce right 82 5 16.4 3 5 0.6	7	lettuce	left	123	10	12.3	0.969	0	10	0		
2 lettuce right 144 10 14.4 6 10 0.6 3 lettuce right 196 10 19.6 0 10 0 4 lettuce right 82 5 16.4 3 5 0.6	1	lettuce	right	152	10	15.2	0.505	11	10	1.1	20	
3 lettuce right 196 10 19.6 0 10 0 4 lettuce right 82 5 16.4 3 5 0.6	2	lettuce	right	144	10	14.4		6	10	0.6	20	
4 lettuce right 82 5 16.4 3 5 0.6	3	lettuce	right	196	10	19.6		0	10	0		
	4	lettuce	right	82	5	16.4		3	5	0.6		
ער אר	5		right	127	א א	15 875		0	ר <u>ק</u>	0.0	10	
6 lettuce right 128 10 12.8 9 10 0.9	6	lettuce	right	127	10	12.8		0 0	10	0 9	10	
Zlettuce right 127 10 12.7 1 10 0.1	7	'llettuce	right	120	10	12.0		1	10	0.1		

	1	valerian	left	164	10	16.4	1	0	10	0	3	0.375
	2	valerian	left	136	10	13.6	0.978	1	10	0.1		
	3	valerian	left	171	10	17.1	1.006	0	10	0		
	4	valerian	left	58	3	19.3333333	1.055	2	3	0.666666667		
	5	valerian	left	170	10	17	1.024	0	10	0	15	1.3636364
	6	valerian	left	144	10	14.4	0.966	6	10	0.6		
	7	valerian	left	150	10	15	0.98	9	10	0.9		
	1	valerian	right	164	10	16.4		0	10	0	8	
	2	valerian	right	139	10	13.9		4	10	0.4		
	3	valerian	right	170	10	17		0	10	0		
	4	valerian	right	55	3	18.3333333		4	3	1.3333333333		
	5	valerian	right	166	10	16.6		2	10	0.2	11	
	6	valerian	right	149	10	14.9		4	10	0.4		
	7	valerian	right	153	10	15.3		5	10	0.5		
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RATIO SKIPS

	average	SD	t test	t	sig
ethanol	0.41428571	0.0202	etoh vs ctrl	0.874334	ns
no odor	0.44285714	0.22223	etoh vs lett	0.163817	ns
lettuce	0.55	0.07071	etoh vs val	0.517017	ns
valerian	0.86931818	0.69907	ctrl vs lett	0.5	ns
			ctrl vs val	0.425902	ns
			lett vs val	0.603293	ns

RATIO BEAT RATE

	average	SD	t test	t	sig
ethanol	0.98357143	0.040319	etoh vs ctrl	0.3816035	ns
no odor	1.003	0.036023	etoh vs lett	0.7089859	ns
lettuce	0.97185714	0.059376	etoh vs val	0.4500615	ns
valerian	1.00128571	0.030728	ctrl vs lett	0.0614321	ns
			ctrl vs val	0.9416499	ns
			lett vs val	0.3854083	ns

AVG SKIPS L

	average left	left SD	t test	t	sig
ethanol	0.22857143	0.34983	etoh vs ctrl	0.509766	ns
control	0.12142857	0.16293	etoh vs lett	1	ns
lettuce	0.22857143	0.33523	etoh vs val	0.676352	ns
valerian	0.32380952	0.38524	ctrl vs lett	0.280055	ns
			ctrl vs val	0.218347	ns
			lett vs val	0.668488	ns

AVG SKIPS R

average right	right SD	t test	t	sig
0.96190476	1.41309	etoh vs ctrl	0.180226	ns
0.4	0.55377	etoh vs lett	0.412656	ns
0.47142857	0.44615	etoh vs val	0.323606	ns
0.4047619	0.45478	ctrl vs lett	0.780166	ns
		ctrl vs val	0.987798	ns
		lett vs val	0.78096	ns
	average right 0.96190476 0.4 0.47142857 0.4047619	average right right SD 0.96190476 1.41309 0.4 0.55377 0.47142857 0.44615 0.4047619 0.45478	average right right SD t test 0.96190476 1.41309 etoh vs ctrl 0.4 0.55377 etoh vs lett 0.47142857 0.44615 etoh vs val 0.4047619 0.45478 ctrl vs lett ctrl vs val lett vs val	average right right SD t test t 0.96190476 1.41309 etoh vs ctrl 0.180226 0.4 0.55377 etoh vs lett 0.412656 0.47142857 0.44615 etoh vs val 0.323606 0.4047619 0.45478 ctrl vs lett 0.780166 ctrl vs val 0.987798 lett vs val 0.78096

AVE BEAT RATE L

	average left	left SD	t test	t	sig
ethanol	15.5904762	2.127485	etoh vs ctrl	0.99437	47 ns
control	15.5857143	1.422941	etoh vs lett	0.47819	89 ns
lettuce	14.8392857	2.451694	etoh vs val	0.65747	18 ns
valerian	16.1190476	1.945161	ctrl vs lett	0.40080	29 ns
			ctrl vs val	0.579	89 ns
			lett vs val	0.23396	22 ns

AVE BEAT RATE R

	average right r	right SD	t test	t	sig
ethanol	15.8619048	2.117688	etoh vs ctrl	0.7223416	ns
control	15.5428571	1.303658	etoh vs lett	0.5987875	ns
lettuce	15.2821429	2.375514	etoh vs val	0.8387862	ns
valerian	16.0619048	1.474654	ctrl vs lett	0.7667546	ns
			ctrl vs val	0.4297951	ns
			lett vs val	0.2974854	ns

DIFF WINGS SKIPS

DIFF WINGS BEAT RATE

	average left	average r	SD left	SD right	t (all ns)	average left	average rigl	SD left	SD right	t (all ns)
ethanol	0.22857143	0.9619	0.3498299	1.413091	0.13449501 ethanol	15.5904762	15.8619	2.127484542	2.1176882	0.3315798
control	0.12142857	0.4	0.1629344	0.553775	0.27688174 control	15.5857143	15.54286	1.422941286	1.3036579	0.8521299
lettuce	0.22857143	0.47143	0.3352327	0.446148	0.07536723 lettuce	14.8392857	15.28214	2.451694021	2.3755137	0.2744923
valerian	0.32380952	0.40476	0.3852437	0.454781	0.56136396 valerian	16.1190476	16.0619	1.945160866	1.4746536	0.7780198