An investigation into cockroach extensor muscle control over $0°$, $45°$, and $90°$ terrains

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Abstract

After reading how cockroach neuromechanics did not differ when running on flat versus rough terrain, we were inspired to investigate how the neuromechanics differed in running versus climbing. We investigated cockroach locomotion on flat, 45°, and 90° terrain, examining specifically the extensor muscle. When climbing, the EMG recordings showed a transition in the number of spikes (i.e., more 3 and 4 spikes, less 1 and 2 spikes) than when running.

BACKGROUND

Increasingly, cockroaches are being used as model organisms for several research fields [1]. One growing field is that of neurobiology . Because the cockroach nervous system is rather simple [9] when compared to mammalian nervous systems, it is rather easy to conduct experiments on a single muscle and the one or two nerves that enervate the muscle. However, due to the homology found in animal nervous systems [6], the results found in cockroach studies can generally be extrapolated and applied to mammalian biology [1].

Additionally, cockroach neuromechanics has had exciting applications in the field of robotics. The first noteworthy paper discussing the application of cockroach mechanics to robotics was published in 1999 [4]. The researchers constructed an algorithm based on cockroach mechanics to modify and increase the efficiency of the robot's posture and locomotion. Recently, Sanchez et al. [5] designed a robot implementing not just the surface locomotion patterns of the robot, but also the neuromechanical control systems. Furthermore, it is possible to learn about the biology of the cockroaches from robotic design as well. One uses the information gained from animal studies to design the robot, but in designing the robot, one could also discover facets or parameters previously not considered in the animal model. This is one of the more interesting facets of interdisciplinary studies.

Despite the vast amount of insight one can acquire by investigating cockroach neuromechanics, it is still a relatively unexplored field. Some of the more recent publications look into how cockroaches transition from walking straight to turning [8], differences in neuromechanics while running on flat versus rough terrain [7], visual analyses of transitions from running to climbing [3], and running in confined spaces [2].

The motivation for this investigation comes from Dr. Sponberg and Dr. Full's paper [7]) investigating rough versus flat terrains. To do so, they generated a terrain consisting of heights chosen from a gaussian distribution of null to $4 - 6$ the cockroaches' hip height, Figure 1. Using EMG analyses and high speed camera recordings, Sponberg and Full concluded there was no significant different in the neuromechanics involved in running on flat versus rough terrain. Given Sponberg and Full's results, we hoped to expand that research into the cases of vertical and angled terrain. We were also inspired by Dr. Goldman's previous research on the kinematics of cockroach climbing vs. running.

It is worth a paragraph to note, the advancement of cellular technology allows us to accomplish this experiment with a minimal amount of advanced and expensive technology. Traditional EMG machinery can be replaced with a simple electronic breadboard circuit and a phone app called Backyard Brains. Our experiments were recorded with a high speed camera, which again was simply a smartphone camera with a "high speed" mode. Once costly and complicated experiments are now accomplishable with only a smartphone.
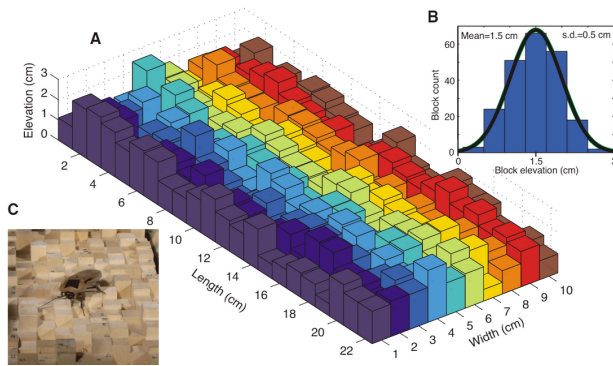
Figure 1: *Figure reproduced from [7]. Cockroaches (B. discoidalis) were run over a rough terrain (A) with a Gaussian distribution of surface heights (B) up to three times the hip height of the animal (C). The cockroach experienced repeated random perturbation while negotiating the terrain. Offsetting the terrain from the entry and exit tracks resulted in very large (4 − 6 times hip height) perturbations to the first and last steps on the terrain. The level surface of all blocks was 1cm ∗ 1cm.*

Animal Preparation

Adult (either male or female) specimens of *Blaberus discoidalis* were selected after ensuring they possessed a full set of appendages. The specimens were then placed in a cooling unit until they were unconscious. In Figure 2, we see the next steps. The specimens are pinned to a flat surface and one of two muscles, either the extensor or the flexor muscle, are chosen for each experiment. To achieve a potential signal gap in a muscle, two wires were pinned roughly 2-3mm apart. In our first implantations, we used 0.008" gauge wire and then transitioned to 0.005" gauge wire. The wires were secured using a minimal amount of gel superglue and cured with baking soda. Experiments began once the specimen regained full mobile ability.

Arenas

The arena consisted of a simple textured bottom with plexiglass walls of suitable height to prevent escape attempts. To achieve $90°$, the arena was placed on its short side such that the long axis was vertical. To achieve $45°$, the Protractor phone app was used to determine the angle to within $0.3°$ degrees, and the arena was propped using various weights and blocks at said angle.
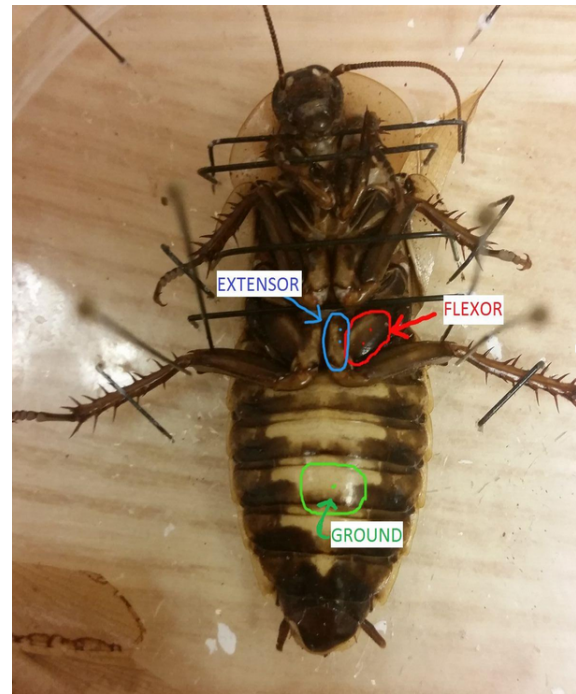

Figure 2: *An image depicting the anatomy and partial methodology of wiring a cockroach. A specimen is pinned to a flat surface and one muscle is selected for study: either the flexor muscle (red) or the extensor muscle (blue). Two wires are implanted in the chosen muscle and a third wire is implanted into the abdominal region and serves as ground.*

Experimental Setup

After placing the specimen in the arena, extension wires were attached to the wires trailing from the cockroach using alligator clips. The wires were then connected to the electronic circuit pictured in Figure 3, where the signal was run through an RC circuit to help reduce noise. An audio jack then transmitted the signal from the circuit to the cellular phone, where Backyard Brains (BYB) displayed the signal in time versus mV graphs. The scales on the axes are adjustable. For our experiments, the time scale was 440ns and the voltage scale was 0.35mV.

Figure 4 shows an informal picture of the experimental setup.

A camera filming at high speed was connected to a tripod and used to film each run (where 'run' is defined as the time in which the Backyard Brains app is recording and the specimen begins running). Once both phones were recording, specimens were positioned at an end of the arena for horizontal runs, and the bottom of the arena

for titled and vertical runs, and prodded to movement. We stopped the recordings when the specimens ceased movement.
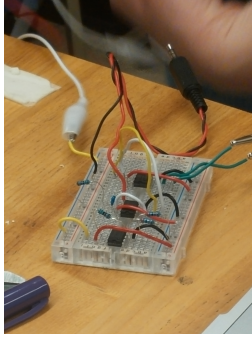

Figure 3: *The electronic circuit implemented in the experimental setup. Wires connected to the cockroach are plugged into the circuit (t ground to ground, the two electrodes into an RC circuit to reduce noise. The signal is then transmitted into a phone via an audio jack, where the Backyard Brains app would allow us to view the signal in mV versus time.*

Figure 4: *The experimental setup. The complete experimental setup included the arena (pictured on the left), an electronic circuit connecting electrode signals to a cellular phone, the cellular phone running Backyard Brains app, and a tripod positioned above the arena so that the experiments may be filmed.*

CALCULATIONS

Two methods of analysis were used on the data we recorded. After being run through data quality checks to ensure the analysis was being done on physical signals, five runs each were used from motion at 0°, 45° and 90°. To ensure only accurate data was used, any EMG recording showing significant amount of noise was not considered during analysis. Additionally, the 0.008" gauge wire was found to significantly alter the specimen's mobility. To ensure this "hindered" data did not skew results, the EMG recordings were not included during analysis.

Once the data quality checks were run and inadequate data were thrown out, a comparison to the raw data collected by Dr. Sponberg was performed. Qualitatively, our data resembled the raw data he collected. By itself, this does not necessarily mean that our data is good data, but the similarities, combined with the fact that analysis of our data revealed similar results to Dr. Sponberg's (as seen in the "Results" section) was enough to convince us that our data was physical.

Each run was examined individually. By eye, the number of spikes present in each step was counted and recorded. Second, a histogram was made of the number of spikes in a burst, combining all of the horizontal runs, all of the vertical runs and all of the 45° runs.

RESULTS

To begin, the raw data collected by the Backyard Brains app is shown below (Fig. 5). Backyard Brains saves the signals as .wav files, and MATLAB is used to visually represent these files as waveforms. Shown are three waveforms: one from a horizontal run, one from a vertical run and one from a run at 45°. After several runs were done at each angle by the same creature, analysis was performed on the number of spikes per step of the cockroach's leg (each "burst" of spikes in the waveform is one step.)

The tables below (Tables 1, 2, and 3) show the results from analyzing every run individually. It is important to note that the data presented here was collected from the same cockroach. This is done to introduce the fewest number of variables possible. Three of the horizontal runs follow the pattern given by Dr. Sponberg's paper [7] - a peak at 2-spike bursts, with more 3-spike bursts than 1-spike bursts. The other two horizontal runs show more 1-spike bursts than 3-spike bursts. It is unclear at this time if this discrepancy is an issue in sample size, where more runs would bear out the pattern given by Dr. Sponberg, or if it is some peculiarity in the animal itself. Very few 4-spike bursts are seen in horizontal running.

Analysis of the vertical runs shows a difference in the

signal output between running and climbing. Generally, vertical climbing shows a somewhat higher concentration of 3-spike bursts; however, the true difference occurs in the count of 4-spike bursts. Whereas only 2 of the 5 horizontal runs contained any 4-spike bursts, and each of those two only contained 1 such burst, every run of vertical climbing contained a 4-spike burst, and some contained multiple. Finally, the 45° runs show a 4-spike burst count roughly between the horizontal and vertical, but interestingly shows more 3-spike bursts than either.

The histogram (Fig. 6)bears out the pattern seen in the analysis of individual runs. Because step count was not equal across runs, each run does not carry equal weight to the histogram. For this reason, the 2 horizontal runs, which had more 1-spike bursts than 3-spike bursts, actually weigh more than the 3 which had more 3-spike bursts.
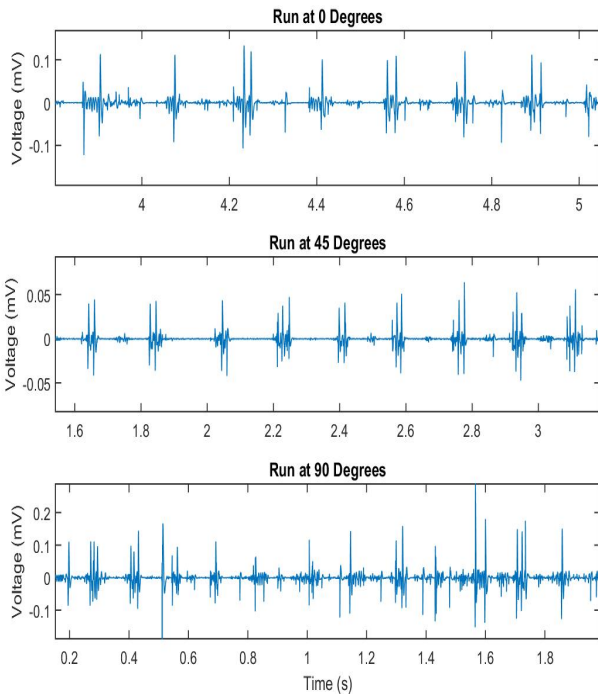

Figure 5: *Waveforms collected by the Backyard Brains app. These recordings are made as the cockroaches move across the arena. Each collection of spikes represents a step the cockroach made with the wired leg. To generate the data presented in this paper, the number of spikes seen at each "step" are counted and tallied.*

Table 1: *Proportion of steps with each number of spikes in horizontal motion. Very few 4-spike bursts are seen; mostly 2- and 3-spike bursts, with a smaller number of 1-spike bursts.*

		Proportion of Spikes			
		1	2	3	4
Run	1	.38	.47	.14	0
	2	.11	.55	.33	0
	3	.11	.44	.33	.11
	4	.33	.33	.25	.08
	5	.1	.5	.4	0

Table 2: *Proportion of steps with each number of spikes in vertical motion. A larger number of 4-spike bursts are seen in vertical climbing, with very few 1-spike bursts.*

		Proportion of Spikes			
		1	2	3	4
Run	1	.18	.43	.31	.06
	2	.2	.5	.2	.1
	3	.25	.375	.25	.125
	4	.125	.4375	.3125	.125
	5	.2	.4	.27	.133

Table 3: *Proportion of steps with each number of spikes in motion at 45 degrees. An intermediate number of 4-spike bursts are seen, but the 45° run shows more 2- and 3-spike bursts than either horizontal or vertical.*

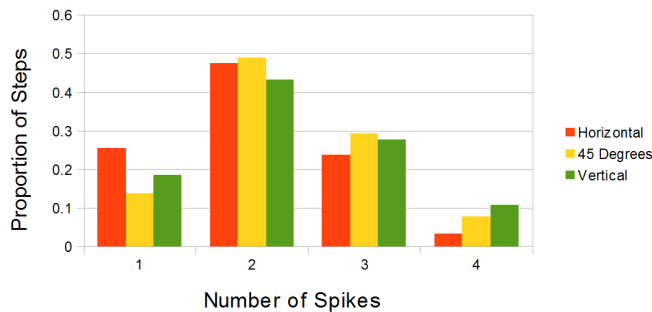|		Proportion of Spikes			
		1	2	3	4
Run	1	.11	.55	.33	0
	2	.22	.44	.33	0
	3	.2	.4	.33	.067
	4	0	.55	.22	.22
	5	.11	.55	.22	.11

Figure 6: *A histogram combining every run at a given angle. The horizontal run shows the most 1-spike bursts and a proportion of 2-spike bursts almost equal to that of $45°$, higher than that of $90°$. The vertical run shows a larger proportion of 3- and 4-spike bursts than horizontal. The $45°$ shows the highest proportion of 2- and 3-spike bursts.*

DISCUSSION

The data collected seems to suggest some difference in the neuromechanics of running vs. climbing. These results are very preliminary, but should they hold, it would suggest that a cockroach climbing is not simply running up a wall, but is behaving differently when it climbs. At this point, the data seems to corroborate the results from Dr. Goldman's lab; the force applied by cockroaches to the surface is different when running versus climbing. Thus, one would expect to see some difference in the neuromechanics as well.

While the experiment produced some promising results, room for improvement does exist. First, the electronic experiment used is prone to random noise, which influences both the amount and quality of the data. For some periods of time, the circuit would not cooperate well with cellular devices, and valuable experimentation time was lost trying to troubleshoot the circuit, which would spontaneously begin working again. Additionally, the goal of using the camera to capture both the cockroach and the phone screen while recording a run was to be able to match a run to the specific BYB recording and determine exactly where in the recording the cockroach began running. However, this matching process became mostly guesswork. To ensure a more exact reading, we would like to implement an LED component into the circuit. By running the electronic signal coming from the cockroach through an amplifier and using it to power the LED, we should be able to determine exactly when the cockroach

is taking a step.

Additionally, the methodology can become more standardized. For example, cockroaches were simply cooled to the point of unconsciousness, which was checked periodically but not at specific intervals. To ensure each cockroach is treated uniformly in the future, we should endeavor to determine exactly how much time it takes to sufficiently cool a cockroach and then use that time within one standard deviation on all cockroaches.

Future Steps

To continue this project, there are many facets we are willing to investigate. First, as we only analyzed the extensor muscle in this project, the flexor muscle's control signals remain undetermined in both our and in Dr. Sponberg's and Dr. Full's paper [7].

We should examine intermediate angles (e.g., $30°$, $60°$) to investigate if the transition we see in 6 from horizontal to vertical is linear in nature or not.

To investigate the muscular control systems in a more well-rounded manner, we would like to expand the arenas to include downward motion, granular terrains, quarter- (and perhaps half-) pipe, and perhaps even obstacles courses. Sponberg and Full [7] investigated upward and downward steps of a reasonably size in their paper. We would like to expand this to investigate how the signals will change as the steps increase in height from non-significant to just shy of sustained climbing.

REFERENCES

[1] *Cockroaches as Models for Neurobiology Applications in Biomedical Research, Volume I*, chapter Introduction. CRC Press, 1990.

[2] K. Jayaram, D. Goldman, and R. J. Full. Effect of friction on cockroaches running in confined spaces. *INTEGRATIVE AND COMPARATIVE BIOLOGY*, 54(1):E101, 2014. Annual Meeting of the Society-for-Integrative-and-Comparative-Biology, Austin, TX, JAN 03-07, 2014.

[3] K. Jayaram, J. M. Mongeau, B. Mcrae, and R.J. Full. High-Speed Horizontal to Vertical Transitions in Running Cockroaches Reveals a Principle of Robustness. *INTEGRATIVE AND COMPARATIVE BIOLOGY*, 50(1):E83, JUL 2010. Annual Meeting of the Society-

for-Integrative-and-Comparative-Biology, Seattle, WA, JAN 03-07, 2010.

[4] GM Nelson and RD Quinn. Posture control of a cockroach-like robot. *IEEE CONTROL SYSTEMS MAGAZINE*, 19(2):9–14, APR 1999.

[5] Chiu C.W. Zhou Y. Gonzalez J.M. Vinson S.B. Sanchez, C.J. and H. Liang. Locomotion control of hybrid cockroach robotos. *Interface*, 12(105):, APR 2015.

[6] D. Sandeman. Homology and convergence in vertebrate and invertebrate nervous systems. *Naturwissenschaften*, 1999.

[7] S. Sponberg and R.J. Full. Neuromechanical response of musculoskeletal structures in cockroaches during rapid running on rough terrain. *J. Exp. Bio*, 2007.

[8] NicholasS. Szczecinski, AmyE. Brown, JohnA. Bender, RogerD. Quinn, and RoyE. Ritzmann. A neuromechanical simulation of insect walking and transition to turning of the cockroach blaberus discoidalis. *Biological Cybernetics*, 108(1):1–21, 2014.

[9] Majeed Z.R. Hartman H.B. Burns E. Titlow, J.S. and R.L. Cooper. Neural circuit recording from an intact cockroach nervous system. *J. Vis. Exp.*, 2013.