



MSc in Human Biology

Making a UCPH thesis template

A case study for all faculties at University of Copenhagen

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Supervised by John Doe and Jane Doe

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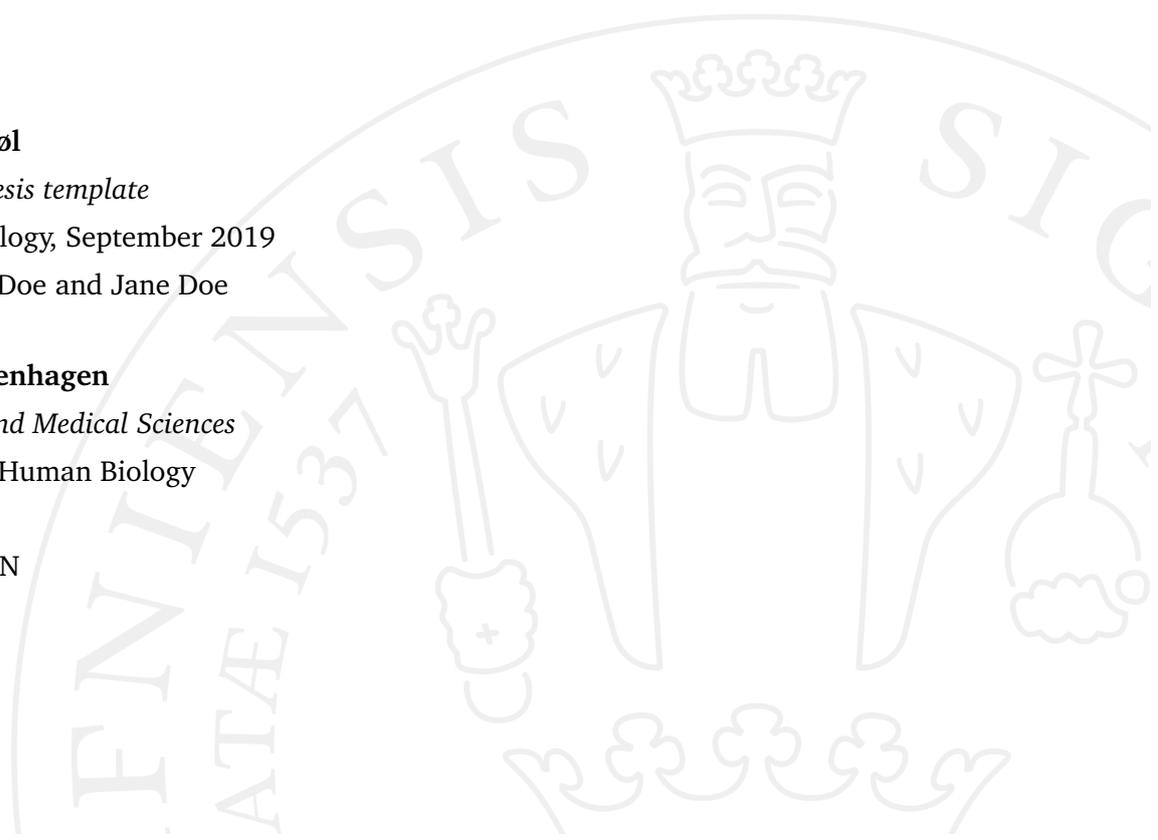
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"I want to say a word for the study of comparative physiology also for its own sake. You will find in the lower animals mechanisms and adaptations of exquisite beauty and the most surprising character, and I think nothing can be more fascinating than the senses and instincts of insects as revealed by the modern investigations."

August Krogh, 1929

"It is certain that there may be extraordinary mental activity with an extremely small absolute mass of nervous matter: thus the wonderfully diversified instincts, mental powers, and affections of ants are notorious, yet their cerebral ganglia are not so large as the quarter of a small pin's head. Under this point of view, the brain of an ant is one of the most marvelous atoms of matter in the world, perhaps more so than the brain of a man."

Charles Darwin, 1872

Preface

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Acknowledgements

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Abstract

Optic flow, the measure of the speed at which visual input moves past the eye, is a general feature of biological vision. As the performance of the visual system is thought to affect behaviour, we set out to investigate whether self-induced optic flow affects walking behaviour of the wood ant (*Formica rufa*). Previous investigations of the use of optic flow in walking insects have been performed using freely moving animals, which does not permit full control of the visual experience. To overcome this we developed a novel virtual reality setup, which we validated by testing whether wood ants interact with a black beacon in virtual reality. We found that wood ants preferentially faced the beacon and exhibited aggression towards it. We take this to provide convincing evidence that wood ants interact with and utilise visual information within a virtual reality setup. To study if and how wood ants use self-generated optic flow to control walking behaviour they were placed in a completely white environment with a black pattern on the floor. We show that wood ants respond to decreased gain (x0.3) of self-induced translational optic flow by increasing their walking speed by 29-98% and increasing their translational walking duration by 34-118%, whereas neither normal or increased (3x) gain has any effects. We further show that the increased walking duration results both from increasing duration of walking bouts and decreasing duration of pauses. This is the first evidence that wood ants use self-induced optic flow, and the first direct evidence that the sensory experience affects locomotion duration and intermittency in any animal.

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Rationale

Locomotion is thought to be a tightly controlled behaviour, ensuring good sensory performance, especially visual, and reducing energy expenditure (Bénichou *et al.*, 2011; Kramer and McLaughlin, 2001). Whether this is the case in the wood ant *Formica rufa* remains unknown, despite many aspects of ant locomotion having been well studied (Lipp *et al.*, 2005; Wahl *et al.*, 2015). The wood ant is an eusocial insect capable of using vision for navigation (Harris *et al.*, 2007) and associative learning (Fernandes *et al.*, 2017). Furthermore, other ant species have been found able to use optic flow for path integration (*Cataglyphis*, Ronacher and Wehner 1995; Pfeffer and Wittlinger 2016). It could thus be speculated that vision actively controls general walking features such as speed, duration and pausing in the wood ant, *Formica rufa* (like bees control speed, Schöne, 1996; Linander *et al.*, 2015). We ask the following question: Does control of walking speed and pausing depend on the presence and speed of optic flow?

A promising approach for performing behavioural experiments in a laboratory setting is the use of virtual reality. Insects have recently been shown to exhibit naturalistic behaviour in closed-loop within a virtual environment (Takalo *et al.*, 2012; Buatois *et al.*, 2017; Seelig *et al.*, 2010). Such setups allow data sampling at precise temporal and spatial resolution whilst animals walk unrestricted for long periods of time. They further enable the recording of brain activity directly, either by electrophysiological recordings or calcium imaging (Seelig *et al.*, 2010). However, despite continued efforts from multiple laboratories it has not yet been possible to make a setup in which ants will readily behave within closed-loop. Nevertheless, we set out to develop a virtual reality paradigm for wood ants (*Formica rufa*). We propose a paradigm based on a modified closed-loop version of the trackball setup by Dahmen *et al.* (2017) combined with the virtual reality software developed by Aronov and Tank (2014).

Research Questions

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Introduction

Locomotion is the means by which animals move around the world. However, movement comes at a price. Sensory performance is decreased as motion blur hampers visual discrimination (Kramer and McLaughlin, 2001; Land, 1999) and extraction of relevant visual information may prove more difficult (Land, 1999). This is particularly important in unpredictable environments. Furthermore, it is dependent upon muscular work and it is thus inherently energetically costly. An example is the 15-50 fold increase in oxygen consumption in flying locusts compared to rest-state (Krogh and Weis-fogh, 1949). It is thus reasonable to believe that metrics of walking are evolutionarily optimised. That would entail animals choosing walking speeds that allows extraction of visual information, such as distance cues, whilst also being energetically efficient.

In this chapter, I will review the:

- Evidence for visual control of locomotion
- Evidence for energetic control of locomotion
- The use of virtual reality in animal behaviour research

3.1 Control of locomotion

3.1.1 Optic flow: Definition and features

Optic flow is the movement of structured light across the retina (Raudies, 2013). The practical meaning of this is that optic flow does not estimate the physical speed of things but rather the speed at which they move past the eye (in degrees/s). Optic flow can be divided roughly into two parts, rotational and translational both of which can occur around all axes. Both contain information about the speed and direction of movement. Translational optic flow from forward movement is generated around a central focal point with vectors pointing in opposite directions for the two eyes. During forward motion, the flow points away from the focal point and is said to be expansive. During backwards motion, on the other hand, it points towards the focal point and is retracting. Translational optic flow provides information regarding the translational distance of movement, and also allows for inference of depth due to the relative movement of components across the visual field, called motion parallax (e.g. when an animal moves forward, the translational optic flow generated by objects nearby will be larger than that generated by objects far away). Insects like locusts and mantids use motion parallax by translating sideways (peering) without rotating, to infer distance (e.g. Sobel, 1990). In rotational optic flow, vectors are pointing in the same direction for both eyes, providing directional information. However, it does not allow for depth perception as retinal displacements are independent of the distance to the retina. As movement is often a mixture of simultaneous translation and rotation rather than pure translation, this makes efficient extraction of distance information more difficult. In this thesis, I only discuss translational optic flow unless otherwise stated.

Optic flow was first proposed in a biological setting by Gibson in 1958 (Gibson, 1958) and has since been confirmed to be behaviourally relevant in a wealth of species, including humans (Warren and Hannon, 1988), rats (Kautzky and Thurley, 2016), zebra fish (Wang *et al.*, 2019), crabs (Horseman *et al.*, 2011), bees (Linander *et al.*, 2015), blowflies (Longden and Krapp, 2009) and desert ants (Pfeffer and Wittlinger, 2016). Furthermore, it has been shown to influence a multitude of behaviours such as collision avoidance, mate following,

predator avoidance, feature detection and path integration (e.g. Srinivasan *et al.*, 1996).

Within insects, David (1982) was the first to investigate the use of optic flow in the control of locomotion speed in a series of experiments on *Drosophila*. It had previously been shown that some flying insects can maintain their flight speeds constant relative to the ground (David, 1979). David (1982) made flies fly inside a "barber's pole" wind tunnel, enabling him to manipulate the visual motions around the flies. He was able to make the flies adopt a stable position within the tunnel by imposing a headwind in the tunnel. Flies kept a steady ground speed in head winds ranging from 0.2-1.0m/s. He then manipulated the diameter of the tunnel, the wavelength of the revolving pattern and the pitch of the revolving lines. Thus, he could infer which aspects enabled to fly to maintain a preferred speed and concluded that it was due to the optic flow.

These findings were extended to another flying species, the honey bee, by Srinivasan *et al.* (1996). They had previously shown that honey bees use optic flow in their centering response, which enables them to keep objects on either side at equal distances (Srinivasan *et al.*, 1991). This response is a way of keeping them from colliding with nearby objects. They now set out to investigate if optic flow was also responsible of the control of their flight speed. By showing that bees slow down when flying through a narrowing gap with vertical lines on the walls, they showed that bees do indeed use optic flow. To control that it was not confounded by the fact that closer lines appear larger, they had bees fly through a tunnel in which the lines had a short wavelength and the other had a greater wavelength. Bees showed no change of flight speed, confirming that optic flow was indeed the metric used by the bees. The findings have since also been confirmed in bumblebees (Linander *et al.*, 2015).

Methods and Materials

4.1 Animals and Animal Preparation

Experiments were performed using wood ants (*Formica rufa* L.). Colonies were collected during summer 2018 from Ashdown Forest, Sussex, UK (N 51 4.680, E 0 1.800). They were kept in large plastic containers with Fluon coated walls at 26 degrees celsius with a 12h light: 12h darkness light cycle regime. They were fed *ad libitum* with 33.3% sucrose solution.

For experiments, ants were chosen based on their size and level of activity (large, active ants were preferred). Subsequently, these ants were painted and placed in small groups for 30 minutes to reduce ant-ant aggression due to the paint odour before being transferred to a small portable nest-like box with unpainted nest mates. To prepare an ant for the experiment, they were first put on ice for 5-10 min to cool them reducing movement before an insect pin (Austerlitz Insect Pin) was attached to the ants back with ultraviolet-light-sensitive glue (5 Second Fix) under a microscope (Olympus Corporation, Tokyo, JP). The pin was bent to a 135° angle about 2/3 up the pin. The ant was then transferred to a separate box where it was kept until the first trial. After the first trial the ant was transferred to nest-like box and kept for 2-4h before the second trial. Ants were picked up with tweezers by the pin and harnessed to the trackball system (described in detail later). After finishing the experiments, animals were discarded in ethanol ($\geq 99.8\%$, Sigma-Aldrich Ltd, Dorset, UK).

Results

5.1 Validation of virtual reality setup

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6.1 Summary of findings

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Conclusion

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The development of a virtual reality setup brings about exciting possibilities. Hopefully, we will soon see ants express innate behaviours such as searching, navigation, associative learning, etc. without moving in physical space. Coupled with the potential of exploring the neural basis of these behaviours is very promising indeed. To enable this, I believe work should be targeted in two directions:

Implement new virtual reality software. The current software (ViRMEn) has allowed us to probe the use of virtual reality with wood ants, however, it imposes certain limitations in the long term. It is written in Matlab, and customising it has remained a challenge throughout. Furthermore, one is limited to simple virtual environments created within ViRMEn. There are other potential software solutions openly available, e.g. MouseoVeR/FlyOver from Janelia Research Campus (Cohen *et al.*, 2017). This software is written in the open source programming language Python, and uses environments created in the open source 3D software, Blender. Not only will adopting this solution improve our ability to customise experiments to our needs, it will also improve the reproducibility by being based solely on open source software.

Develop reward system. Associative learning experiments entails establishing an association between a cue and a reward (e.g. Fernandes *et al.*, 2017), as does traditional navigation experiments with central place foragers (e.g. Buehlmann *et al.*, 2018). To allow such behaviour, the setup needs to allow distribution of reward. This could potentially be accomplished by introducing a syringe with a sucrose solution, however, this approach will first have to be developed and validated before beginning any learning experiments.

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