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Abstract of Doctoral Dissertation

Degree requested: Doctor of Life Science Applicant's name: RAHMAN SADNIMAN

Title of Doctoral Dissertation

Relative value learning in *Drosophila melanogaster* larvae

（キイロショウジョウバエ幼虫における相対的価値学習）

The ability to learn from past experiences to inform future decision-making is crucial for humans and animals alike. One question with important implications for adaptive decision-making is whether we learn by comparing the values of different options and associate the outcome of this comparison with stimuli (called relative value learning) or whether we learn about values irrespective of their alternatives (called absolute value learning). Many studies in humans and vertebrates demonstrated relative value learning even when it leads to suboptimal decisions. While some studies in insects have demonstrated that insects do discriminate values of different rewards or punishments and can learn accordingly, none of them clearly showed whether the value associated with one option changes because another option with a different (better or worse) value had been present. Thus, it has remained unclear whether organisms with simple nervous systems (such as insects) learn by the comparison of different values and thus whether the principles of value-based learning are shared across evolutionarily distant species.

To answer this question, I investigate whether and how insects learn by comparing the values of rewards and punishments during classical conditioning using *Drosophila melanogaster* larvae. I carefully designed associative odour-taste learning experiments to distinguish absolute and relative value learning. At first, larvae were trained using two odours, n-amylacetate (AM) and 1-octanol (OCT), each paired alternately with reward or punishment substrates of two different strengths. Neutral training conditions were provided using tasteless agar. Larvae underwent three alternating training cycles in which each odour was paired with its assigned substrate. Only AM was given during the test which allowed a direct evaluation of the associative value attributed to that odour alone. The numbers of animals selecting each side of the test dish were analyzed using quasibinomial generalized linear models (GLMs), followed by Wald tests to evaluate the significance of predictors, and the Quasi-Akaike's Information Criterion (QAIC) was used to compare model fits. In four independent experiments, I observed that the larvae's behaviour was mostly determined by the comparison of the reward or punishment strengths during the training, which corresponds to the definition of relative value learning as it is used in human research.

To test whether learning by comparing reward values requires the presence of two odours during training, I performed one-odour training experiments where larvae experienced the to-be-tested odour paired with either the reward or the neutral substrate, while the alternative training phase included no odour. The results of three experiments, each using another odour, suggested that learning by comparing reward values does not require the presence of two odours during the

training. However, the results differed depending on the odours being used. For AM, I found evidence suggesting a potential dominance of the reward value paired with the odour whereas when using OCT and Benzaldehyde (BA), the larvae's behaviour was best explained by a simple subtraction of the two reward values.

In addition, to know whether relative value learning depends on the sequence of training experiences, I performed one-trial training experiments in which larvae were trained with one training cycle with OCT or AM being presented first in separate experiments. I found that while the training sequence had a significant effect, in both sequences the larvae were able to learn by comparing the two reward values.

In summary, this work using *D. melanogaster* larvae strongly suggests that insects can learn by comparing reward or punishment values during training, equivalent to what is called relative learning in humans. These findings reveal that relative value learning is not a high-level cognitive feature restricted to complex brains but an evolutionarily adaptive process which can be observed even in a nervous system with only ~10,000 neurons. Given the great potential of *D. melanogaster* as a model organism for in-depth neurobiological analyses, this study opens up the opportunity to reveal the mechanism underlying relative value learning in unprecedented detail. In my thesis, I discuss one simple and plausible possibility for such a neuronal mechanism, based on the rich existing literature about the mushroom body, the memory centre of insects.