Drosophila acquires seconds-scale rhythmic behavior

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（ショウジョウバエにおける秒単位の周期的な行動の獲得）

神経行動分野

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Introduction

Time-based information is crucial to optimize physiology and behavior of organisms from amoebae to humans. Insects, for example, change their behavior according to a wide range of time scales, such as in the generation of courtship songs, and in circadian rhythm. Perception of interval timing has scarcely been investigated, especially in invertebrates, despite its relevance to immediate behavioral adaptations. Here I examined if the fruit fly, *Drosophila melanogaster*, can acquire rhythmic behavior in the range of seconds.

Design & Methods

Temporal conditioning is a direct experimental procedure which can behaviorally demonstrate the perception of interval timing. In temporal conditioning, animals receive repeated stimuli that induce behavioral responses (unconditioned stimulus [US]) at a regular interval. After a given number of US presentations, the subject is tested to see whether it keeps behavior responses at the traine interval. In this study, electric shock stimuli were repetitivel presented to flies at regular (experimental group) or random interval (control group: Fig. 1).

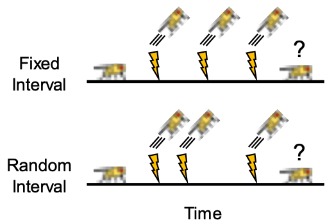

Figure 1. Experimental Design.

Figure 2. Behavioral responses to electric shocks.

To measure whether flies acquired a trained interval, electric shocks were given and behavioral responses like jumping walking, and small movement were detected (Fig. 2). A time-frequency analysis was applied to behavioral time series (Fig. 3). The difference in periodic behavior of the experimental and control groups following training was defined as the conditioned response (CR: i.e., rhythmic behavior). I tested following parameters: repetition, inter-stimulus-interval (ISI), and age.

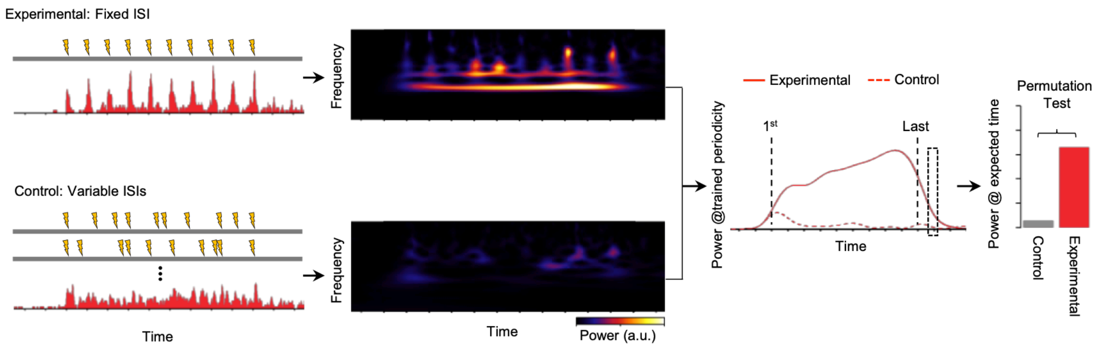

Figure 3. Time-frequency analysis reveals the acquisition of rhythmic behavior in temporal conditioning. Power spectral density of jumping behavior is calculated by wavelet transformation. Power at the trained frequency is extracted from the experimental and control groups, and these values especially at the first expected shock after conditioning (dashed rectangle) are compared by permutation test.

Results

1. Behavioral rhythm lasts after temporal conditioning

I subjected flies to a repetition of 10 electric shocks at a 2 s interval and analyzed the periodicity of different behavioral components. The regularity of jumps was notable during the whole of the training phase, and lasted even after termination of the shock, but not for control groups. These results indicate that flies acquire 2 s rhythmic behavior following the presentation of repeated electric shocks.

2. Critical factors influencing acquired rhythmic behavior

First, I examined the effect of shock repetition on CR in my assay. A statistically significant difference of periodic jumping was only detected for the 10 and 20 repetitious shock groups. The difference of regularity between the experimental and control groups was at a maximum in the 10 shock group and decreased with repetition (Fig. 4). This suggests that increasing over the optimal number of repetitions does not improve, and may even worsen, the acquisition of CR.

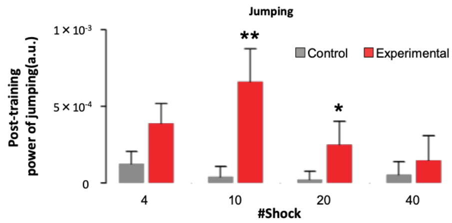

Figure 4. Repetition effect on CR.

To characterize the time range which flies can acquire behavioral rhythms, I trained them with four different ISIs. CR was observed in jumping or small movement after 2 or 1.4 sec interval respectively, but not for 3 and 5 s. These results suggest that flies can acquire behavioral rhythms up to 2 s and they represented the rhythm in different types of behavior in different intervals.

Past studies in mammals have suggested an association between age and the accuracy of interval timing. To address this in the fly, I examined whether aged flies can acquire the 2 second rhythmic behavior. I found that shock-induced jump bouts declined with aging. Post-conditioning periodic jumping in 1- and 2-week-old flies persisted strongly, but was dramatically decreased in 3- and 4-week-old flies (Fig. 5). Interestingly, the period of jumping right after conditioning became shorter than the trained frequency in the older flies (Fig. 5), suggesting that their interval perception is changed with aging.

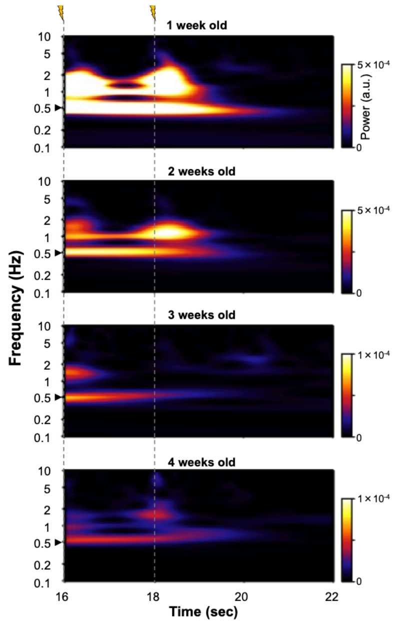

Figure 5. Spectrograms of jumping in the last phase of and after the temporal conditioning. Arrowheads indicate the trained frequency. The age-associated decline in periodic jumping is noticeable.

Discussion

It has been found that invertebrates, like the bumble bee, can distinguish between “longer” and “shorter” intervals(36 s and 6 s, for example). However, the interval range that they can accurately reproduce has not been investigated to date. In this study, I demonstrated that *Drosophila* can reproduce time intervals of at least up to 2 seconds. The range of time that invertebrates can reproduce is more narrow than what they can distinguish, suggesting interval-reproduction and interval-differentiation may rely on different mechanism.

Flies acquired time intervals with much less repetition than mice. Many studies with mice used appetitive stimulus as US, thus, this valence difference might lead to different acquisition speed. On the other hand, I found that the interval perception of flies shared a key feature with humans; a life-course decline of temporal coordination. These findings suggest that invertebrate and mammals may have some commonality in the mechanism of interval perception. Thus, temporal conditioning in *Drosophila* will provide a useful experimental platform to tackle the neuronal mechanism of seconds-range time perception.