Supplementary Text S1

Effectiveness of preamplifier attachment to butterfly and reference electrode location for successful monopolar recording of butterfly flight muscle.

We first conducted differential recording with 800-mm long bipolar electrodes (50 µm in diameter) during the free forward flight of the butterflies, without the onboard preamplifier. The bipolar electrodes were inserted into the left DLM, and a reference electrode (800-mm long, 80 µm in diameter) was inserted into the abdomen. The output signals showed a sizable drift from the baseline, presumably due to the mechanical noise during the flight (see Supplementary Figure S1A, upper trace). It was difficult to distinguish the periodic EMG burst of each stroke cycle, even after the signal was filtered with a bandpass of 100–3000 Hz (see Supplementary Figure S1A, lower trace).

Next, we attached the preamplifier on the dorsal mesonotum, and inserted two electrodes into the left DLM and a reference electrode into the abdomen. As the preamplifier allowed shortening of the signal electrodes (10–20 mm), the signal drift, observed when the long electrodes were used, was not seen in this setting (see Supplementary Figure S1B). Each of the preamplifier output signals showed EMG bursts that consisted of relatively large spikes around the dorsal stroke reversal timing. However, it was still difficult to distinguish the burst corresponding to each stroke cycle because of the continuous noise generated over the stroke cycle. These noises were diminished by calculating the difference between the electrodes, making the inter-burst intervals visible (see Supplementary Figure S1B, red arrows in the bottom trace). These results indicated that the noises on both electrodes contained common-mode signals. Differential recordings with bipolar electrodes could produce a reasonable outcome. However, because the butterfly thorax cuticle is soft, bipolar electrode insertion into each muscle would cause more mechanical damage to the cuticle than a single monopolar electrode as done for the wireless EMG measurement in free-flying hawkmoths (Ando et al., 2002).

The interference of the nearby DVM activity, operating in an opposite phase to the DLM, could be one of the common-mode noise causes (Ando and Kanzaki, 2004). We conducted EMG recordings from the bilateral DLM, using monopolar electrodes, to investigate this possibility. If the common-mode noise arises from the DVM, the DVM signal on each side will interfere with the DLM signal on the same side. We expected the DVM signals to remain even after calculating the difference between the two recordings because of the difference between the left and right activities. However, the difference between the bilateral DLM indicated that the common-mode signal had been reduced (see Supplementary Figure S1C, red brackets with arrowheads), and only the

DLM-related signals were observed (see Supplementary Figure S1C, black parentheses), as in the case when two electrodes were inserted into the same DLM (see Supplementary Figure S1B). The common-mode noise was observed regardless of the position of the recording electrodes and was also observed in non-flying butterflies by fixing the wings (see Supplementary Figure S3). From these results, we concluded that the cause was the position of the reference electrode in the abdomen. Because the butterfly moves the abdomen intensively during flapping flight (see Supplementary Movie S1; Sunada et al., 1993; Senda et al., 2012a], abdominal muscle activity or mechanical vibrations are thought to be the cause of this common-mode noise.

Supplementary References

Ando N, Kanzaki R (2004). Changing motor patterns of the 3rd axillary muscle activities associated with longitudinal control in freely flying hawkmoths. Zool Sci 21: 123–130

Ando N, Shimoyama I, Kanzaki R. (2002). A dual-channel FM transmitter for acquisition of flight muscle activities from the freely flying hawkmoth, *Agrius convolvuli*. J Neurosci Methods 115: 181–187

Senda K, Obara T, Kitamura M, Nishikata T, Hirai N, Iima M, et al. (2012). Modeling and emergence of flapping flight of butterfly based on experimental measurements. Robotics Auton Syst 60: 670–678

Sunada S, Kawachi K, Watanabe I, Azuma A (1993). Performance of a butterfly in take-off flight. J Exp Biol 183: 249–277