



Supplementary Figure S1. EMG measurements during free-flight at different amplifier positions and reference electrode positions. **(A)** Conventional EMG measurement with long bipolar electrodes (800 mm, $\phi 50 \mu\text{m}$). The bipolar electrodes and reference electrodes were inserted into the left DLM and the abdomen, respectively (schema). The top trace indicates an original signal output from a

differential amplifier located outside of a butterfly, and the bottom trace indicates the signal after filtering through a bandpass of 100–3000 Hz. Cyan vertical bars indicate the timing of wing dorsal stroke reversal (onset of the downstroke). The vertical scale represents amplified voltages. **(B)** EMGs from the DLM with monopolar electrodes. The signals from two monopolar electrodes inserted into the left DLM (top and middle traces) and their difference (electrode 1 – electrode 2, bottom trace) are shown. The bursts (black square parentheses) and inter-burst intervals (red parentheses with arrowheads) appeared in the difference between the two signals whereas the original signals were noisy throughout the recording. **(C)** EMGs of bilateral DLM with monopolar electrodes. The signal from the DLM on each side (top and middle traces) and the difference (left DLM – right DLM, bottom trace) are shown. As in **(B)**, remarkable inter-burst intervals (red parentheses with arrowheads) appeared in the difference between the two signals. **(D)** EMGs of the bilateral DLMs measured with monopolar electrodes (the same data as shown in Fig. 4A). Compared to **(C)**, the cyclic activities of spike bursts (black square parentheses) paused by inter-burst intervals (red parentheses with arrowheads) were observed without differential recording. Signals shown in **(B–D)** were filtered with a bandpass of 100–3000 Hz. For detailed descriptions, see Supplementary Text S1.