

Mutual Information Analysis Reveals Millisecond Level Precision Across Flight Muscles in *Manduca sexta*

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Abstract

Motor control has long been thought to be primarily encoded in spike rate, however recent work has highlighted the importance of spike timing. Despite possessing a good understanding of spike timing precision in sensory systems, the same understanding has not been established for their motor counterparts. We utilize EMG and motor output recordings from the flight system of the hawk moth *Manduca sexta* to characterize the level of spike timing precision in the motor system. We show through two complementary information theoretic methods, including a novel noise corruption method, that the scale of temporal precision in all muscle activations is on the millisecond-scale, comparable to many sensory systems. Additionally, we establish that the novel method is capable of resolving precise precision values in systems that traditional methods struggle to characterize. This indicates that this precision must arise within the circuit.

Context

Understanding how motor information is encoded is vital to our understanding of how the nervous system handles data and transforms it into motor output. Traditionally, motor information was thought to be encoded in the spiking rate of neurons [1]. While this is true in many cases, recent studies have demonstrated that a large amount of motor information is encoded in the precise spike timing, or precision, of various neurons [2]. Action potentials, or spikes, are defined as large, near-digital changes in voltage across the neuron’s membrane caused by the movement of ions through said membrane. This movement propagates along a portion of the neuron, where it is eventually transmitted to muscle cells. Spike timing in this context refers to the specific intervals between individual action potentials. For example, if there are three spikes in a 6-millisecond time window, the average spike rate is 500 spikes per second. However, this spike train can have the same spike rate, where the exact interval between the spikes, to be investigated in this study, may be varied. In that scenario, different spike timing encodes different information, which is known to occur in the mechanosensory system [3]. This project aims to uncover the level of intramuscle spike timing in the flight system of *M. sexta*. The precision level of intramuscular timing has not yet been established. Characterizing muscle timing would lead to a better understanding of how precise spike timing is used by the nervous system to modulate motor power output.

Introduction¹

Neurons in both sensory and motor systems convey information through the precise timing of spikes. Though temporal encoding in sensory systems has been acknowledged for several decades now [4, 5], it is only recently that the role of temporal encoding in motor systems has been appreciated. While a gross correlation between spike rate and muscle force production exists, the precise timing of spikes in motor neurons matters due to nonlinearities in muscle force production and mechanical interactions of musculoskeletal systems within themselves and with the environment [6]. Motor circuits in cortex [7], cerebellum [8], descending interneurons [9], and the periphery [2, 10, 11] carry information in neural spike timings. This information can be encoded in the difference between timings in two spike trains [2], the inter-spike interval [7], or temporal patterning [10], providing motor control systems the potential for a rich, multiplexed code that utilizes the high potential bandwidth inherent to timing codes. However, we know little about what time scale precision information is encoded in motor systems, and how all this potential bandwidth is utilized. It has been shown previously that small changes in bilateral spike timing can vastly change power output [2].

In some sensory systems, the precision of temporal encoding has been explored. Neurons are capable of generating spikes with incredible precision, due to their biophysical properties that reduce the effect of noise on spike timing. Extreme cases in auditory systems like bat echolocation and

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owl interaural hearing are capable of encoding timing differences on the order of microseconds and demonstrate low noise phase-locking across behaviorally relevant frequency bands [12]. However, the precision of firing of single neurons in sensory systems has been estimated to occur on the millisecond or sub-millisecond scale, a much longer timescale [5]. In cortical motor circuits, spike timing precision has also been estimated to the millisecond-scale in a songbird vocalization area [7]. Comparatively little work has been done to establish temporal precision in peripheral motor circuits due to the difficulty of obtaining spike resolution and limitations of current methods to determine precision. Most studies of peripheral motor systems either measure only the absolute activation of muscles through electromyography (EMG) or calcium imaging. EMG in vertebrates sample from too many motor units to generate discriminable spikes in recordings, and the calcium dynamics in imaging techniques occur over greater time-scales than the width of neural spikes, making it difficult to resolve single spike precision. Estimating the spike timing precision in a comprehensive peripheral motor circuit would be a starting comparison point between cortical and sensory circuits. It also would give us a more integrated view of how precision arises and is perpetuated through neural circuits from sensation to action.

The hawk moth, *Manduca sexta*, is a great organism to investigate spike timing precision in peripheral motor circuits. These moths use a set of only 10 muscles as the primary actuators of their flight and each of these muscles is innervated by one or very few motor neurons, enabling simultaneous recording of a nearly complete, or comprehensive, spike-resolved motor program. Additionally, we know that spike timing encodes information about motor output and coordination between pairs of muscles across the hawk moth motor system [11]. Insects provide a compelling test-bed for questions of precision due to their fast, complex, and dynamic motor behaviors that challenge the inherent biophysical speed of neural firing. The earliest examples of precise temporal encoding in motor circuits were found in invertebrates like crabs, flies and locusts [13–15]. The hawk moth motor circuit, due to its few muscles and spike resolution, enables us not only to estimate timing precision, but discover whether this precision changes depending on muscle type, actuation, or functional properties across a nearly complete motor program. How muscles are controlled in a limited data set may help us understand both how the brain controls movement and also how we can mimic this control architecture in flying robots.

Previous methods for estimating precision include variability on stimulus-response curves and information theoretic methods using discrete representations of motor output and response [5, 7]. However, by discretizing the motor response, these methods may miss out on important features of the motor output that require spike timing precision. Here, we use two methods to estimate spike timing precision in each of the 5 muscle types in the hawk moth motor program: an established discrete information theoretic method previously used to estimate spike timing precision [7, 16] and a new method of adding noise on spike timings and using the continuous Kraskov k-nearest neighbors Mutual Information estimation method, an extension of methods previously introduced [10, 11, 17]. Our new method makes spike timing precision estimates in the continuous space to give us a more accurate and precise picture of the time scales necessary for encoding motor commands.

Materials and methods

Comprehensive, Spike-Resolved Motor Program Data Set

The previously published data set used in this analysis recorded the comprehensive, spike-resolved motor program of the hawk moth, *Manduca sexta* ($N = 7$), and its motor output in a tethered flight preparation (Data deposited in the Dryad repository: <https://doi.org/10.5061/dryad.r4xgxd280>) [11, 18]. EMG signals from the 10 primary muscles actuating each moth’s wings were recorded with spike-level resolution using implanted silver wire electrodes. These muscles are treated as single motor units, since each is innervated by one or very few motor neurons. Therefore, the muscle action potentials (MAPs) captured correspond directly to neural spiking activity.

The moths were tethered using super glue to a 3D-printed ABS plastic rod attached to a custom six-axis force/torque (F/T) transducer (ATI Nano17Ti, FT20157; calibrated ranges: F_x , F_y =

± 1.00 N; $F_z = \pm 1.80$ N; $\tau_x, \tau_y, \tau_z = \pm 6250$ mN-mm). After tethering, the moths were given thirty minutes to recover from the surgery and adapt to dark conditions, since these are crepuscular moths that typically fly at dusk and dawn. The moths were then presented with a 3D-printed plastic flower oscillating horizontally in a 1 Hz sinusoidal trajectory; these flowers have been used to elicit flight variation previously [19]. The EMG recordings were sampled at 10000 Hz, amplified using a 16 channel amplifier (AM Systems Inc., Model 3500), and acquired using a data acquisition board (National Instruments USB-6529 DAQ) and custom MATLAB software. The same model of DAQ board was used to acquire the strain gauge voltages from the ATI F/T transducer, also sampling at 10000 Hz.

The structure of the data set relates spike counts and spike timings in segmented wing strokes to the scores of the first 2 principal components (PCs) of the within-wing stroke yaw torque (τ_z) produced in each individual moth (see [11] for details).

We adapted two different information theoretic methods, one discrete and the other continuous, to estimate the spike timing precision in each of the 10 muscles of the comprehensive, spike-resolved flight motor program.

Method 1: MI Estimation Using the NSB Method with Discrete Bins

We first estimated spike timing precision by adapting a previously described Nemenman, Shafee and Bialek entropy estimation (NSB) method [7, 16, 20]. We directly estimated the mutual information between spike timings and the scores of the first 2 PCs of the yaw torque by discretizing the probability distributions of both the neural input and the motor output. The neural input was discretized using spike "words" created by binning the spikes in each wing stroke using different numbers of bins, n_s . The number of spikes that occur in each bin sets the value of that bin, and each unique sequence of bin values across all wing strokes is a spike "word". The prevalence of each spike word provides a discrete probability distribution that describes the neural input in each individual muscle. The number of bins n_s used to create this discrete distribution was varied; the size of each of these bins set the resolution of spike timing r as:

$$r = \frac{t_{max} - t_{min}}{n_s}, \quad (1)$$

where t_{max} is the highest spike time recorded in that muscle and t_{min} is the lowest. This is the maximum range within a wing stroke where spikes occur. When this range is split into n_s bins the size of each bin is r in milliseconds.

To create a discrete probability distribution for the motor output, we binned the 2 PC scores with a number of bins n_m on each axis. At each value of n_m , we generated a series of torque "words" from the two binned PC scores, similar to the spike words described above. The prevalence of each torque word provides a discrete probability distribution that describes the neural output in each individual muscle, n_m was varied between 1 and 3, with no unexpected results. As such, $n_m = 2$ was chosen, which generates four discrete motor states.

Using these two discrete PDFs, we estimated the mutual information (MI) between the spike "words" (the neural input) and the PC score states (the motor output) using the direct method:

$$I(S, M) = H(S) - H(S|M), \quad (2)$$

where the mutual information $I(S, M)$ between the neural input S and the motor output M is equal to the total entropy of the neural input $H(S)$ minus the entropy of the neural input given knowledge of the motor output state $H(S|M)$. Since all direct estimates with finite data have bias, we used the NSB entropy estimation for both entropies in this equation [7, 16, 20], which can estimate these entropies with minimal bias even when severely under-sampled.

Method 2: MI Estimation Using the Kraskov k-Nearest Neighbors Method with Added Noise

Our second method of estimating the spike timing precision used a continuous Kraskov k-nearest neighbors MI estimation [17]. We generated a random uniform distribution of noise U between values of 0 and r , where r is the upper limit of the distribution. This adds a window of noise equivalent to a value r , in milliseconds, shifting spike timing values S . We then estimate the MI between the noise corrupted spike timing values and M across values of r :

$$I(S + U(0, r), M) = \sum_{i=1}^{C_{max}} p(C = i) I(S + U(0, r); M | C = i) \quad (3)$$

where C is the number of spikes in a wing stroke and C_{max} is the maximum count of spikes in a single wing stroke for each muscle. This formulation for the information between spike timings and the motor output allows for a variation in the number of spikes in each wing stroke to be correctly taken into account. The mutual information between the spike timings with added noise and the scores of the first 2 PCs in each wing stroke was estimated 150 times for each value of r tested, reducing the effect any individual estimation run has on the final mutual information value.

We define the precision as the point where the MI estimate fell below the lower bound of the estimated standard deviation of the MI at $r = 0$ (i.e. the original data) based on variance in data fractions [21]. The spike timing precision value p was determined for each muscle in each moth in the data set. To test for statistically significant differences between the spike timing precision p of different muscles, we used one factor ANOVA and Kruskal-Wallis tests across individual moths.

Results and Discussion

Continuous MI method gave an exact estimate of millisecond-scale spike timing precision

The continuous noise addition method resulted in stable estimates, with the expected decrease in mutual information that corresponded to the amount of noise added (Fig. 1A). Method 1 fails to establish this lower bound on precision due to instabilities in the mutual information estimates that are not seen in the second method (Fig. 1B).

Method 1, a traditional information theoretic method, failed at high precision due to instabilities in the entropy estimate at low bin sizes. At high bin numbers, the large number of possible torque and spike words lead to some word combinations that occur only a small number of times in a given trial, making it impossible to capture the full spectrum of word combinations, and therefore impossible to accurately calculate the actual word distribution. Method 2, due to continuously adding noise to the system, is able to capture more of the possible motor space and therefore does not suffer the same instabilities seen in method 1. It is important to note, however, that the continuous method is not immune from data limitations. In addition to an increase in stability, due to the continuous nature of the noise added, the precision added in method 2 can be incremented in very small amounts, unlike a discrete method, which will by nature be restricted by discrete bin sizes. This extended continuous noise method allows for the analysis of systems that so far have resisted characterization. Applying method 2 across all muscles demonstrates consistent millisecond level precision. Method 1 supports this conclusion, with MI increasing at small bin sizes. While this investigation sets an upper bound on the level of precision present in the system, further work would be necessary to determine whether the timing differences between muscles truly are statistically significant, and therefore whether the control strategy used by the nervous system is related to the purpose of a muscle within the flight system.

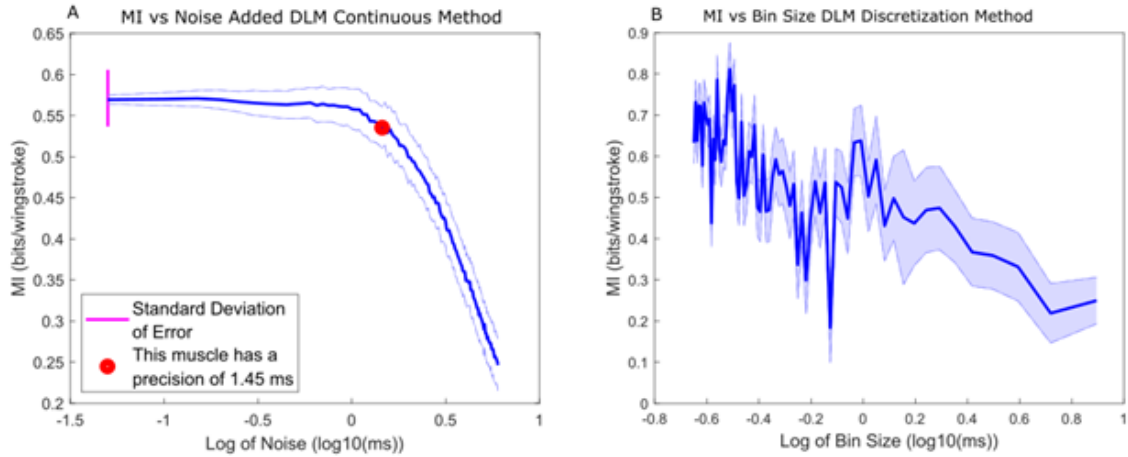


Fig 1. A. MI at values of noise added, plotted on a log scale (red circle - estimated precision, blue line - mean MI \pm STD, purple line- error estimate of MI at zero noise added). B. NSB method estimation of a sample DLM muscle (MI estimate \pm STD). The estimation increases at small bin sizes but never forms a plateau.

Millisecond-scale spike timing precision spans every muscle in the system

Method 2 demonstrates millisecond-scale precision across all ten muscles in the motor system. While there were marginally statistically significant differences across the muscles (One-factor ANOVA = 0.0147, Kruskal-Wallis, $p = 0.0655$), the central conclusion is that mean precision of all the muscles across individuals was within the range of 0.6 to 1.65 ms (Fig. 2). The lowest mean precision values were the subalar (SA) and upstroke power muscles (DVM), with an average precision value of 0.75 and 0.8 milliseconds, respectively. The 3rd axillary (3AX) muscles have the largest mean precision value at 1.6ms. These results are consistent with previous work, Sponberg et al. demonstrated sub-millisecond level precision between bilateral pairs of DLMs [2]. We demonstrate that the precision for the individual muscles in the complete set of flight muscles in *Manduca sexta* have millisecond-scale precision. This scale is on the same order of magnitude as the spike timing precision present in many sensory systems [12]. There does not appear to be a link between the level of spike timing precision and amount of information encapsulated by spike timing in a muscle.

Spike timing must be essential for sensorimotor integration

The existence of millisecond-level precision across the entire muscle set, which is consistent with the scale of many sensory systems, indicates that precise timing must either be preserved throughout the nervous system or otherwise arises within the system. The origin of this precision should be investigated, as it is not clear whether ventral nerve chord has enough precision to preserve these precise spike timings. An alternative possibility is that a CPG present in the thoracic ganglion provides precision based on wing mechanosensory input. The answer to these questions would inform us on the purpose of temporal encoding to control motor output.

Implications

The utilization of highly precise spike timing at the motor output could be a representation of sensory input that is preserved for use in motor control, or alternatively timing may be a useful method to incorporate feedback to modulate the power output of the flight system. As the precision is consistently on the millisecond scale over a mostly-complete motor set, the presence of highly temporal precise coding is not a special case reserved for certain functionalities, but rather a

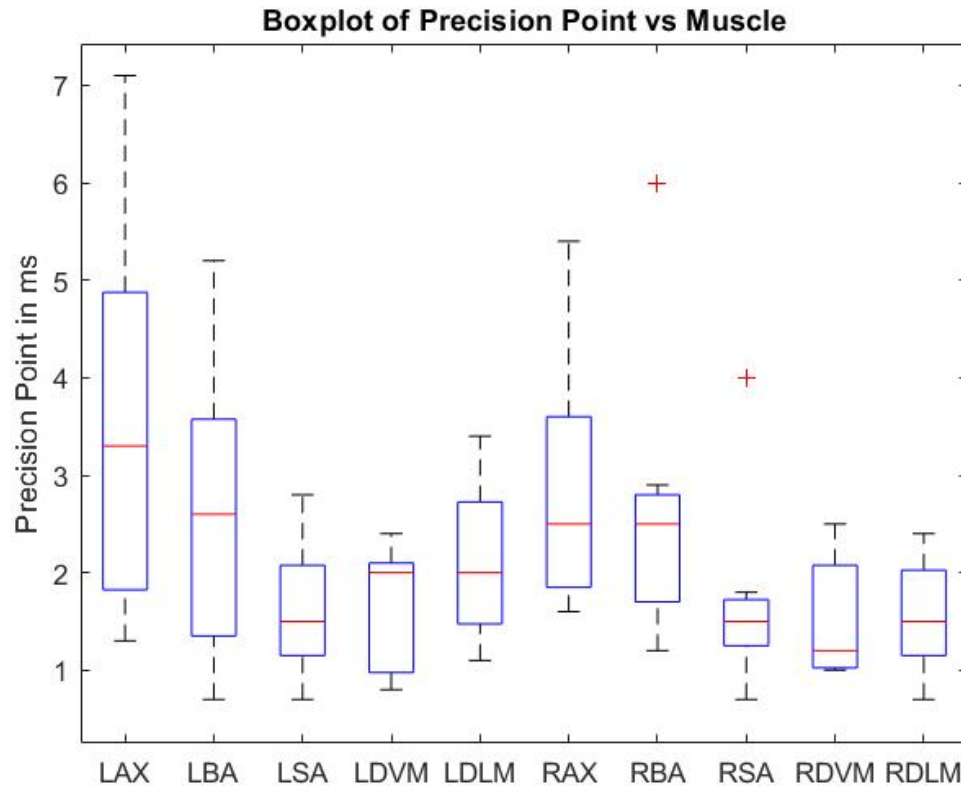


Fig 2. Spike timing precision values for all moths reported as boxplots (red line - median, blue box - 25th and 75th percentiles, whiskers - all data except outliers, red crosses -outlier points) as estimated by method 2.

consistent encoding strategy used by the nervous system.

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