

Dopamine lesions alter the striatal encoding of single-limb gait

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Reviewed Preprint

Revised by authors after peer review.

About eLife's process

Reviewed preprint version 2

March 14, 2024 (this version)

Reviewed preprint version 1

December 12, 2023

Posted to preprint server

October 9, 2023

Sent for peer review

September 27, 2023

 https://en.wikipedia.org/wiki/Open_access

 Copyright information

Abstract

The striatum an important role in motor control, and neurons in this area encode the body's initiation, cessation, and speed of locomotion. However, it remains unclear whether the same neurons also encode the step-by-step rhythmic motor patterns of individual limbs that characterize gait. By combining high-speed video tracking, electrophysiology, and optogenetic tagging, we found that a sizable population of both D1 and D2 receptor expressing medium spiny projection neurons (MSNs) were phase-locked to the gait cycle of individual limbs in mice. Healthy animals showed balanced limb phase-locking between D1 and D2 MSNs, while dopamine depletion led to stronger phase-locking in D2 MSNs. These findings indicate that striatal neurons represent gait on a single-limb and step basis, and suggest that elevated limb phase-locking of D2 MSNs may underlie some of the gait impairments associated with dopamine loss.

eLife assessment

This **valuable** work extends previous studies showing that the striatum multiplexes various aspects of locomotion, including velocity and movement transitions, by demonstrating that striatal neurons also encode single-limb gait. The authors present **solid** evidence to show that gait deficits induced by severe unilateral dopamine depletion are associated with an imbalance in the gait modulation of striatal firing. Although the source and function of this gait modulation remain unclear, this manuscript uncovers a role of striatal activity in gait, which may have implications for understanding gait disturbances in Parkinson's Disease.

Introduction

Walking is an essential mode of locomotion which relies on neural systems for carrying out coordinated rhythmic limb kinematics (i.e., gait), regulating speed, as well as starting and stopping movement^{1,2,3,4}. While spinal cord microcircuits are ultimately responsible for producing limb movements, the voluntary control of walking is thought to rely on sensorimotor signals from multiple cortical and subcortical areas, including the basal ganglia^{2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100}. A large body of work

has examined the role of the direct and indirect pathways of the basal ganglia in locomotion, as altered signaling in these circuits is implicated in the motor symptoms of movement disorders such as Parkinson's disease^{5–9}. These pathways, originating in D1 and D2 MSNs in the striatum, have been shown to represent both discrete aspects of locomotion such as the initiation and cessation of movement, as well as continuous aspects such as body speed^{10–16}. Yet, with few exceptions^{17, 18}, most of these studies have relied on relatively low spatial resolution measures of motion – whole-body movements – to link striatal activity specifically to locomotor function (work has examined other types of limb movements, such as lever pressing, but this is a behaviorally distinct process from gait^{19, 20}). While body speed is a product of gait, measuring body speed alone does not adequately capture the kinematics of individual limbs in walking animals. Thus, despite significant conceptual advances, there has been little effort to link D1 and D2 MSN activity to gait with single-limb and step resolution. Dopamine degeneration in Parkinson's disease is associated with impaired gait, but the neural mechanisms underlying many of these behavioral changes are unclear²¹. We therefore hypothesized that D1 and D2 MSNs display a neurophysiological signature of gait on a step-by-step basis, and that this signature is altered in animals with impaired gait performance. We recorded high-speed video of freely behaving mice in an open field and extracted individual limb movements using machine learning-based pose tracking tools. This approach allowed us to obtain the gait characteristics of each limb during bouts of self-initiated walking. In parallel, we recorded single-unit spiking activity of dorsal striatal neurons, and subsequently identified them as D1 or D2 MSNs via optogenetic tagging²². We then examined the relationship between neural activity and locomotion at the level of single-limb kinematics, alongside more common whole-body measures of motion such as the initiation, cessation, and speed of walking. We found that the spike timing of an appreciable subset of striatal neurons was entrained to specific phases of the gait cycle. D1 and D2 MSNs normally showed a balanced encoding of limb phase, whereas dopamine-lesioned animals displayed an imbalance between D1 and D2 MSN limb phase coding properties, with stronger gait cycle coupling in the D2 MSN population. Collectively these results reveal a previously underappreciated property of striatal neurons to encode the phase of individual limbs, which may serve to support the production of rhythmic limb movements during walking, and whose altered activity may underlie some of the gait impairments associated with dopamine loss.

Results

Single-limb gait measurements in freely behaving mice

We employed high-speed (80 fps), high-resolution (0.3 mm/pixel) video recordings and the open-source pose estimation tool SLEAP²³ to track limb movements of freely behaving mice in an open arena (**Figure 1A**). Video was captured from a bottom-up view. Our primary analysis focused on bouts of self-initiated walking that were identified based on body speed and the presence of rhythmic limb motion that is characteristic of the gait cycle. The gait cycle of an individual limb consists of two phases – the stance phase in which the limb contacts the ground, followed by the swing phase in which the limb loses ground contact (**Figure 1B**). A stride is comprised of one full stance/swing cycle. The stance and swing onset times were determined by identifying the trough and peak of the position of each limb projected onto the nose-tail axis. During each 30 minute recording session mice exhibited multiple walking bouts in the arena, typically resulting in hundreds of strides for each limb under a variety of speeds (**Figure 1C,D**). Mice walked with a lateral sequence gait pattern (LR $\diamond$ LF $\diamond$ RR $\diamond$ RF), with the limbs on the same side of the body moving first (**Figure 1E**). Furthermore, the front and rear limbs on each side of the body moved with an approximately anti-phase relationship (180° phase offset, **Figure 1F**)²⁴. We next characterized the properties of individual limb strides, and confirmed that faster strides are associated with higher length and frequency (**Figure 1G,H**)²⁴, and that whole-body speed is strongly correlated with stride speed (**Figure 1I**). Stride parameters in healthy mice appeared normally distributed and were similar across the four limbs (**Figure 1J-L**).

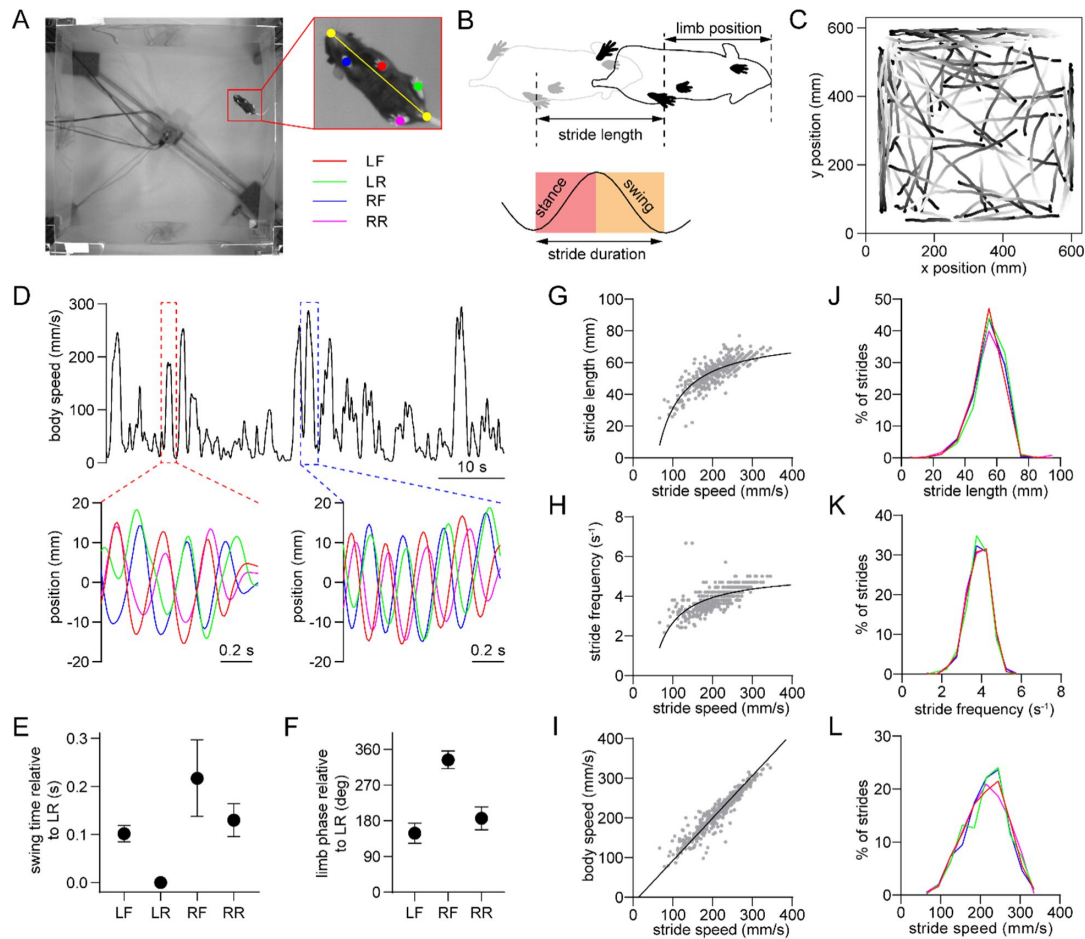


Figure 1.

Single-limb gait measurements in freely behaving mice.

(A) Video frame showing the bottom-up view of a mouse walking in the 60 cm x 60 cm open field. The inset shows the six tracked body parts (four limbs plus the nose and base of the tail). The limbs are abbreviated as LF: left front, LR: left rear, RF: right front, RR: right rear. The yellow line represents the nose-tail axis.

(B) Illustration of the gait cycle comprised of the stance and swing phase. Stride duration corresponds to the time needed to complete one stance/swing cycle, stride length is the distance spanned by the limb during this period, and stride speed is the ratio between these quantities.

(C) Walking bout body trajectories from a recording session in a healthy mouse. Light color represents the start of movement.

(D) Time course of body speed showing a low speed and high speed walking bout (red and blue dashed lines), and the motion of each limb during these walking bouts. Limbs are color-coded according to A.

(E) Mean swing start time of each limb relative to LR, reflecting the lateral sequence gait pattern (LR $\rightarrow$ LF $\rightarrow$ RR $\rightarrow$ RF). Data represent mean $\pm$ SD of all strides from one recording session.

(F) Mean limb phase angle relative to the LR limb, indicating the approximately anticorrelated phase relationship between front and rear limb movements on each side of the body. Data represent mean $\pm$ SD of all strides from one recording session.

(G) LF limb stride length as a function of stride speed from one recording session. Gray dots represent individual strides. Black line represents the best polynomial fit.

(H) Stride frequency (inverse of duration) as a function of stride speed.

(I) Body speed as a function of stride speed. Black line represents the best linear fit (Pearson $R = 0.95$).

(J) Stride length distribution of the four limbs from one recording session. Limbs are color-coded according to A.

(K) Stride frequency distribution of the four limbs from one recording session.

(L) Stride speed distribution of the four limbs from one recording session. All data in this figure were collected from the same recording session.

Dorsal striatal neurons are phase-locked to the gait cycle

Gait measurements were combined with electrophysiological recordings via an opto-microprobe²⁵, a device containing a silicon-based multielectrode array attached to an optical fiber (for optogenetic tagging), which was implanted in the dorsal striatum of the right hemisphere (**Figure 2A**). Measurements were performed in D1-Cre and A2a-Cre mice after virally expressing ChR2 in the striatum to enable optogenetic identification of specific MSN subtypes (**Figure 2B**), though our initial analysis examined all cell types together including those that were unidentified. The recordings yielded spiking activity from multiple striatal units in each animal (**Figure 2C**), allowing us to investigate the relationship between individual neuron spike timing and limb movements during walking. A number of neurons appeared to show an oscillatory discharge that was time-locked to specific time points in a limb's gait cycle (**Figure 2D**). This firing pattern resembles the rhythmic activity of neurons in motor cortex and other supraspinal areas, which was reported in earlier studies with animals walking on treadmills^{27–29}. The stride-to-stride variability of freely behaving mice appeared to attenuate the average oscillatory firing pattern in the time domain. To gain a more reliable means of quantifying spike-limb coupling, we transitioned the firing rate analysis to the phase domain by converting limb position into phase values, with 0° defined as the start of the stance phase (**Figure 2E**). A subset of neurons preferentially fired action potentials at specific phases of the gait cycle (**Figure 2F**). The strength and preferred direction of this gait phase coding phenomenon were characterized in terms of the mean vector length (a parameter which can theoretically vary from 0 to 1) and angle (**Figure 2G**). In total, around 45% of striatal neurons (total includes all cell types) showed significant phase-locking to at least one limb, which was determined via a spike time jitter test (see Methods; **Figure 2H**). Thus, cells that display this effect represent an appreciable population of neurons in the dorsal striatum, suggesting this may be a functionally important signal. There was no significant difference in mean vector length across the four limbs (**Figure 2I**). Consistent with the relative phase of limb motion, the mean spike-limb phase angle distribution was similar for diagonal limbs, and offset by approximately 180° for off-diagonal limbs (**Figure 2J,K**).

Since motion between different limbs during walking is strongly correlated, we next examined whether neurons are either preferentially phase-locked to individual limbs, or with equal strength, but different phase, to each limb. The majority of phase-locked neurons were entrained to either a single limb or pair of limbs (**Figure 2 – figure supplement 1A**). Among the cells that were coupled to only two limbs, most of the limb pairs were diagonal (**Figure 2 – figure supplement 1B**). The preferred coupling to diagonal limbs is likely related to their in-phase motion, as shown in **Figure 1F**. We also observed a significant bias in the mean vector length when ranked based on the single limb preference, ranging from the highest to the lowest (**Figure 2 – figure supplement 1C**). Together, these results support the conclusion that striatal neuron spiking is preferentially coupled to single limbs. However, we speculate that because of the inherently correlated motion across limbs, a subset of striatal neurons also displays significant phase-locking to multiple limbs, particularly to diagonal pairs. While single-limb phase-locking was widespread, on average the vector length did not show a bias for either ipsi- or contra-lateral limbs (**Figure 2 – figure supplement 1D**). Finally, the vector length was negatively correlated with a neuron's firing rate averaged across the entire recording session, while it did not consistently vary along the depth of the dorsal striatum (**Figure 2 – figure supplement 1E,F**).

Mixed striatal encoding of single-limb phase and whole-body movement initiation, cessation, and speed

The dorsal striatum is known from previous work to encode a variety of whole-body kinematic variables including continuous parameters (e.g., speed) and discrete events (e.g., start and stop of movement)^{11, 13, 15}. This suggests that the striatum may contain a mixed representation of both single-limb and whole-body movements related to walking. Consistent with this assumption, our analysis indicated that a substantial proportion of dorsal striatal neurons exhibited significant modulation to single-limb phase, body speed and/or the initiation and cessation of movement

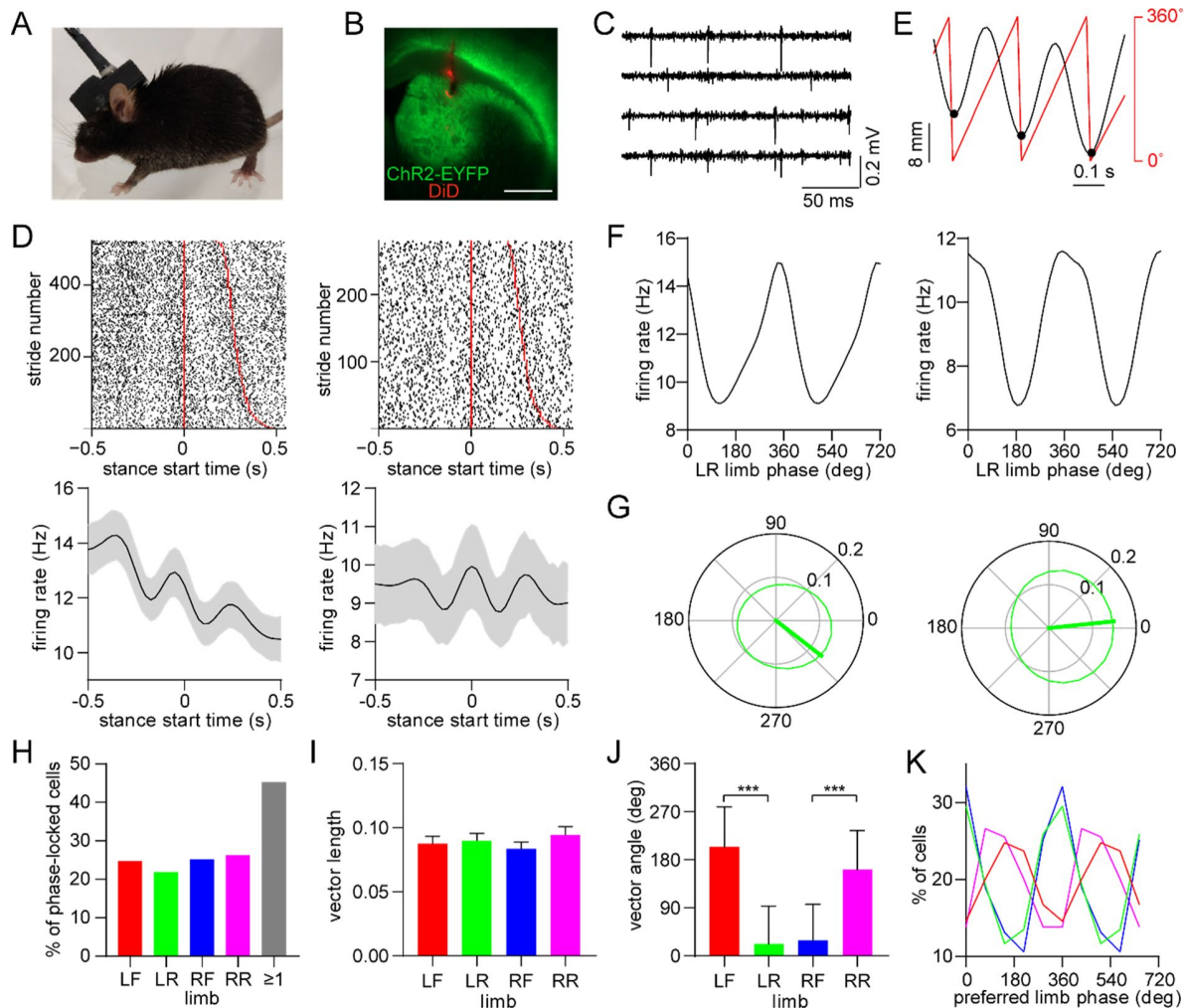


Figure 2.

Dorsal striatal neurons are phase-locked to the gait cycle of individual limbs.

(A) Mouse implanted with an opto-microprobe and head cap housing a miniature electronic head stage.

(B) Opto-microprobe track (red) in the dorsal striatum with virally mediated ChR2-EYFP (green) expression in a D1-Cre mouse. Scale bar: 0.5 mm.

(C) Filtered time traces from four electrodes showing striatal spiking activity.

(D) Top: raster plot of two striatal neurons aligned to the start of the LR limb stance phase. The stride number is sorted by stride duration (represented by the red lines). Bottom: mean $\pm$ SEM firing rate of the same neurons.

(E) Single limb position (black) and the corresponding phase angle (red). Black dots indicate the start of the stance phase (defined as 0°).

(F) Average firing rate as a function of limb phase for the two neurons in D. The limb phase is plotted for two full gait cycles (0-720°) for visual clarity.

(G) Normalized firing rate of the neurons in F in polar coordinates. The thick green line represents the mean vector (left cell: vector length = 0.13 and angle = 322°; right cell: vector length = 0.15 and angle = 6°).

(H) Percentage of limb phase-locked striatal neurons ($n = 274$ total cells pooled from 9 healthy mice).

(I) No significant difference in mean spike-limb phase vector length between the four limbs ($n = 274$ cells, one-way RM ANOVA, $p = 0.07$). Data represent mean $\pm$ SEM.

(J) Mean preferred limb phase angles are significantly different between front and rear limb (angular permutation test adjusted for 6 comparisons, LF-LR: $p < 0.001$; LF-RF: $p < 0.001$; RF-RR: $p < 0.001$; LR-RR: $p < 0.05$). Data represent mean $\pm$ SD.

(K) Distribution of preferred limb phase angles across the four limbs.

(**Figure 3A,B**). Notably, around one third of striatal neurons encoded all three factors (**Figure 3C-G**). These findings suggest that a partially overlapping neural population in the striatum may play a role in regulating multiple aspects of walking (e.g., initiating movement and maintaining a continuous gait at a specific speed).

D1 and D2 MSNs display balanced encoding of single-limb phase but not movement initiation

We next examined the response of specific striatal cell types. D1 and D2 MSNs were identified via an optogenetic tagging protocol performed at the conclusion of each recording session. This involved stimulating ChR2-expressing neurons in the vicinity of the recording electrodes through the optical fiber. We checked for units that were activated with short latency by the laser, and whose spike waveform was similar between the laser stimulation and preceding baseline periods (**Figure 4A-C**).²⁶ In total we identified 39 D1 and 40 D2 MSNs in healthy mice, with a statistically similar average session-wide firing rate (**Figure 4D**). A subset of both cell types displayed rhythmic firing patterns in relation to limb phase (**Figure 4E**). Across all identified cells there was no significant difference between D1 and D2 MSNs in either the mean vector length or angle (**Figure 4F,G**). These results suggest that there is normally a balanced level of D1 and D2 MSN activity coupled to the gait cycle. Furthermore, both populations showed a similar proportion of neurons which encoded limb phase, start of movement, body speed, and the combination of these (**Figure 4H**). Previous work has shown that D1 and D2 MSNs both increase their activity around the time of movement initiation.^{15, 30} In line with these findings, on average we observed an elevated firing rate in both populations during the start of whole-body motion; however, the fractional change in start-related activity was significantly higher in D1 MSNs (**Figure 4I**). Neural responses to movement cessation and speed were similar between D1 and D2 MSNs (**Figure 4J**). Our data indicate that, in healthy animals, D1 and D2 MSNs exhibit similar levels of activity while locomotion is in progress, but that there is an initial bias toward the direct pathway at the start of movement.

Dopamine lesions impair movement initiation and ongoing gait

Since dopamine is hypothesized to regulate motor function by modulating the balance of direct and indirect pathway activity,^{6, 8, 9, 31, 32} we sought to understand whether our findings are altered by dopamine loss. In a separate group of animals, we administered unilateral 6-hydroxydopamine (6OHDA) injections into the medial forebrain bundle, leading to loss of dopamine in the dorsal striatum (**Figure 5 – figure supplement 1A**). We then carried out behavioral and electrophysiological recordings followed by optogenetic tagging at 15 days post-lesion, and compared results to a group of sham-lesioned animals. We first confirmed that dopamine lesions led to impaired locomotion measured at the whole-body level (**Figure 5A-F** and **Figure 5 – figure supplement 1B,C**).^{7, 33} Walking bouts in 6OHDA-injected animals were characterized by frequent ipsiversive turning, lower body speed, shorter overall distance traveled, and a lower rate of initiating locomotion. These deficits in whole-body motion were accompanied by changes in gait performance at the individual limb level. Dopamine-lesioned animals displayed significant changes in stride length and duration, leading to slower strides for each of the four limbs (**Figure 5G-I**).³⁴ We further observed a shorter swing-to-stance duration ratio, indicating that during walking each limb spent more time in contact with the ground (**Figure 5J**). Additional changes in gait included lower coordination between different pairs of limbs, and higher variability in the length and speed of individual strides (**Figure 5 – figure supplement 1D-H**). Overall, it was evident that dopamine lesions impair multiple aspects of locomotion, notably both the ability to initiate movement and, once walking is underway, to perform rapid, sustained, and coordinated limb movements that are typical of a healthy gait pattern.

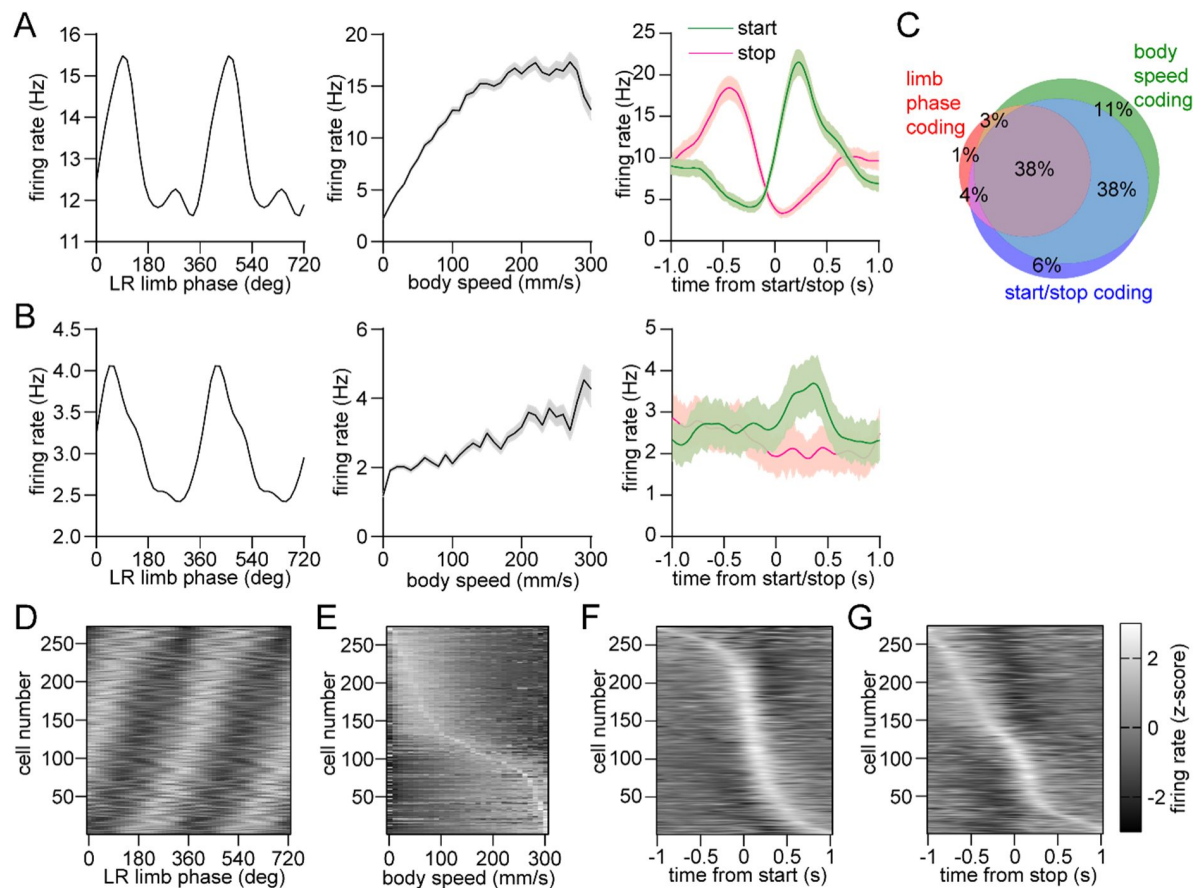


Figure 3.

Mixed striatal encoding of single-limb and whole-body motion.

(A) Response of a neuron which significantly encodes LR limb phase (left), body speed (middle), and start/stop of body movements (right).

(B) Response of a neuron which significantly encodes LR limb phase, body speed and the start of body movements, but not cessation of movements. Data in A and B are represented as mean $\pm$ SEM.

(C) Venn diagram showing the percentage of striatal neurons with significant responses to limb phase (124 out of 274 cells pooled from 9 healthy mice, spike time jitter test, $p < 0.05$ for at least one limb), body speed (245 out of 274 cells, Pearson correlation $p < 0.05$) and start and/or stop of motion (232 out of 274 cells, paired t-test, $p < 0.05$ for either start or stop). See Methods for further details.

(D) Average firing rate (z-scored) as a function of LR limb phase for all striatal neurons (n = 274). The cell number is ordered by the mean vector angle. The limb phase is plotted for two full gait cycles (0-720°) for visual clarity.

(E) Average firing rate (z-scored) as a function of body speed for all striatal neurons. The cell number is ordered by the speed of highest firing.

(F) Average firing rate (z-scored) aligned to the time of movement initiation. The cell number is ordered by the time of maximum firing.

(G) Average firing rate (z-scored) aligned to the time of movement cessation. The cell number is ordered by the time of maximum firing.

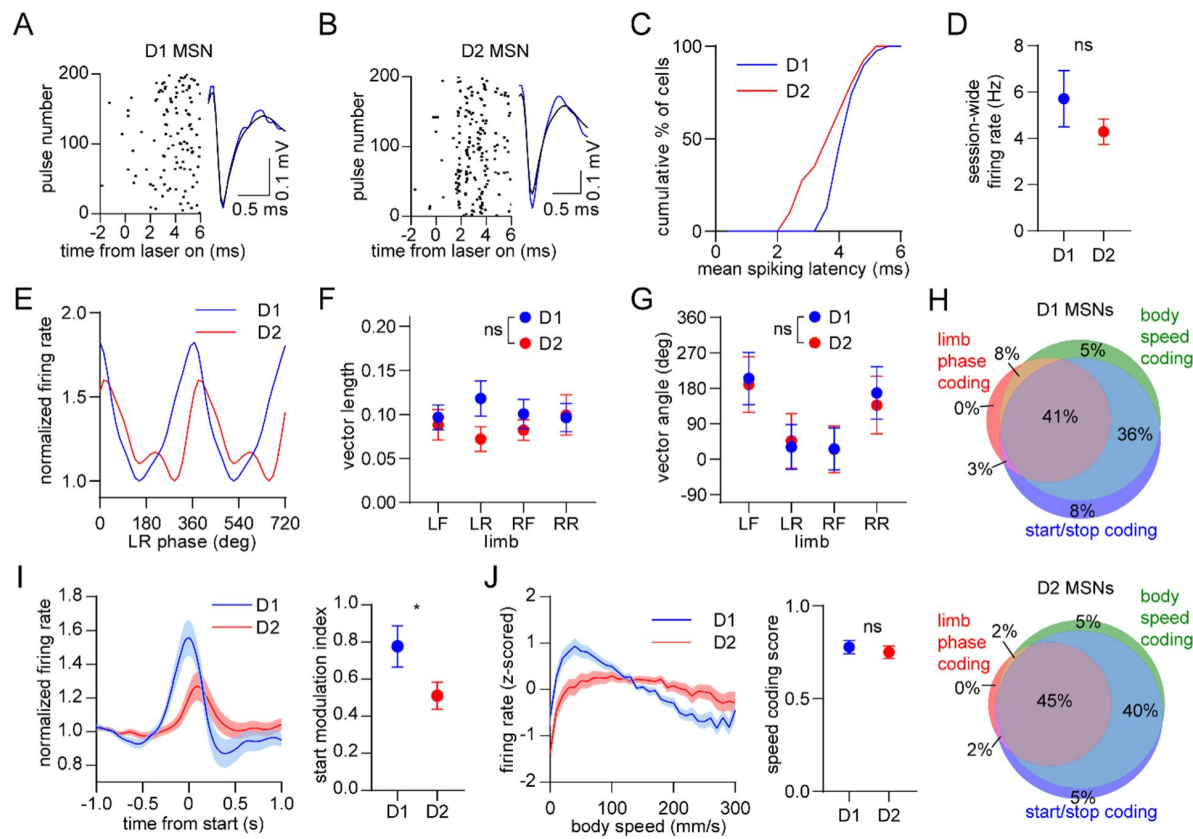


Figure 4.

Balanced D1/D2 MSN activity during continuous walking but not movement initiation.

- (A) Spike raster of an optogenetically identified D1 MSN from a D1-Cre mouse showing a rapid response to optical stimulation, and a similar spike waveform with (blue) and without (black) laser illumination.
- (B) Same as A but for an optogenetically identified D2 MSN from an A2a-Cre mouse.
- (C) Cumulative distribution of the latency to spiking during optical stimulation for all optogenetically tagged cells ($n = 39$ D1 MSNs pooled from 3 mice and 40 D2 MSNs pooled from 6 mice).
- (D) No significant difference in the mean session-wide firing rate between D1 and D2 MSNs ($n = 39$ D1 and 40 D2 MSNs, unpaired t-test, $p = 0.28$).
- (E) Mean normalized firing rate as a function of LR limb phase for a D1 and D2 MSN. The activity of each cell is normalized to the minimum firing rate. The limb phase is plotted for two full gait cycles ($0-720^\circ$) for visual clarity.
- (F) No significant difference in spike-limb phase vector length between D1 and D2 MSNs ($n = 39$ D1 and 40 D2 MSNs, two-way ANOVA, $F_{1,308} = 2.1$, $p = 0.14$).
- (G) No significant difference in mean vector angle between D1 and D2 MSNs (angular permutation test corrected for 4 multiple comparisons, $p > 0.99$).
- (H) Venn diagrams showing the percentage of D1 and D2 MSNs with significant responses to limb phase of at least one limb, body speed, and start and/or stop of motion.
- (I) Left: normalized firing rate relative to the start of movement averaged across all D1 and D2 MSNs. Data are normalized to the mean firing rate in the pre-start baseline period. Right: The start modulation index (fractional change in firing in start period relative to pre-start) of D1 MSNs is significantly higher than D2 MSNs ($n = 39$ D1 and 40 D2 MSNs, unpaired t-test, $p = 0.048$).
- (J) Left: firing rate (z-scored) as a function of body speed averaged across all D1 and D2 MSNs. Right: No significant difference in speed coding score (absolute Pearson r of firing rate with respect to speed) between D1 and D2 MSNs ($n = 39$ D1 and 40 D2 MSNs, unpaired t-test, $p = 0.57$). Angular data are represented as mean $\pm$ SD. All the other data are represented as mean $\pm$ SEM.

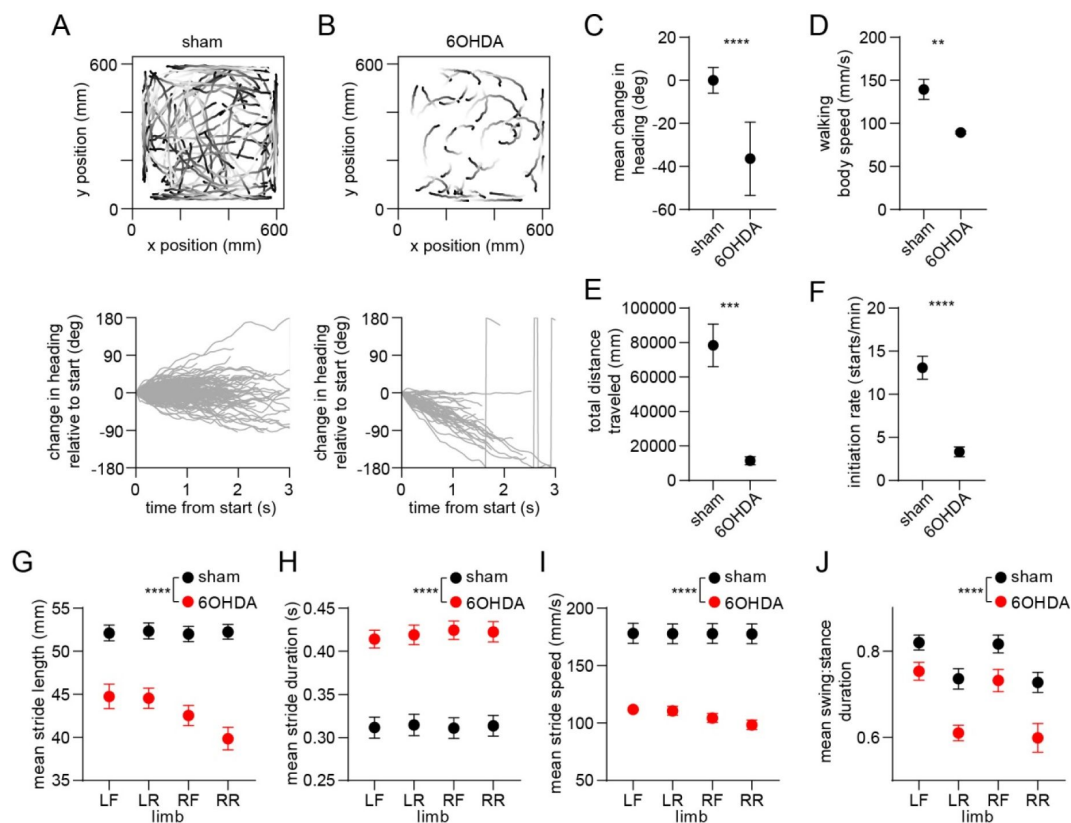


Figure 5.

Whole-body and single-limb motor impairments in dopamine-lesioned mice.

(A) Top: Walking bout body trajectories from a sham-lesioned mouse. Light color represents the start of movement. Bottom: time course of the animal's change in heading during each walking bout. Positive/negative heading indicates contra/ipsi-versive turning.

(B) Same as A but for a 6OHDA-lesioned mouse.

(C) Mean change in movement direction was altered in the 6OHDA group, with negative values indicating ipsiversive turning ($n = 10$ 6OHDA and 14 sham-lesioned mice, angular permutation test, $p < 0.0001$).

(D) Mean body speed was reduced in the 6OHDA group (unpaired t-test, $p = 0.002$).

(E) Total distance covered by each animal in a recording session was reduced in the 6OHDA group (unpaired t-test, $p = 0.0002$).

(F) Rate of initiating movements was reduced in the 6OHDA group (unpaired t-test, $p < 0.0001$).

(G) Mean stride length was reduced in the 6OHDA group ($n = 10$ 6OHDA and 14 sham-lesioned mice, two-way ANOVA, $F_{1,88} = 151.4$, $p < 0.0001$).

(H) Mean stride duration was increased in the 6OHDA group (two-way ANOVA, $F_{1,88} = 157$, $p < 0.0001$).

(I) Mean stride speed was reduced in the 6OHDA group (two-way ANOVA, $F_{1,88} = 174$, $p < 0.0001$).

(J) Mean ratio of the swing:stance phase duration was reduced in the 6OHDA group (two-way ANOVA, $F_{1,88} = 38$, $p < 0.0001$).

Angular data are represented as mean $\pm$ SD. All the other data are represented as mean $\pm$ SEM.

Dopamine lesions alter the relative levels of D1/D2 MSN activity coupled to limb phase and movement initiation

Finally, we investigated the effect of dopamine lesions on the movement-related responses of optogenetically identified D1 and D2 MSNs. We confirmed that, as in healthy mice, sham-lesioned animals displayed similar D1 and D2 MSN limb phase-locking properties measured across the four limbs (**Figure 6A-C**), and a similar mean level of firing across the recording session (**Figure 6D**). However, this originally balanced phase-locking activity was disrupted in 6OHDA-lesioned animals, which exhibited a significantly higher average spike-limb phase vector length among the D2 MSN population (**Figure 6E,F** and **figure 6 – figure supplement 1A**). The average vector angle remained similar between the two MSN subtypes in the 6OHDA group (**Figure 6G**). Thus, dopamine lesions strengthen the coupling of D2 MSNs to the gait cycle, whereas the coupling of D1 MSNs is unaltered. Since the vector length was found to vary in inverse proportion to firing rate (**Figure 2 – figure supplement 1E**), we checked whether dopamine lesions led to appreciable changes in D1 or D2 MSN firing rate. However, the session-wide firing rate between D1 and D2 MSNs remained statistically similar (**Figure 6H**), suggesting that the observed imbalance in vector length cannot be explained by changes in overall firing rate.

We next analyzed the activity of these neural populations in relation to the start of body movements. Since healthy mice exhibited significantly higher D1 MSN activity during movement initiation, we confirmed the same observation in sham-lesioned mice (**Figure 6I**). In contrast, dopamine lesions attenuated this bias, with both MSN subtypes now showing similar levels of start-related activity (**Figure 6J**). Neither the encoding of movement cessation nor body speed showed a significant difference between D1 and D2 MSNs in the sham and 6OHDA groups (**Figure 6 – figure supplement 1B-E**). However, while speed coding remained balanced between D1 and D2 MSNs, there was a substantial reduction in the speed coding score of both cell types after dopamine lesions. Taken together, dopamine lesions appear to primarily alter the relative levels of D1 and D2 MSN activity during two critical stages of locomotion – the initiation of movement and the performance of the gait cycle.

Finally, we sought to clarify whether elevated D2 MSN activity may causally contribute to gait impairments in a manner similar to that observed after dopamine lesions. A subset of healthy Chr2 expressing animals underwent an additional behavioral testing session in which D2 MSNs were optogenetically activated for a 5 minute period (**Figure 6 – figure supplement 2A**). Consistent with previous work^{7,35}, several measures of whole-body motion were reversibly altered (**Figure 6 – figure supplement 2B-E**). Concomitantly, we observed significant changes in single-limb stride duration and speed, as well as more modest changes in stride length and the swing-to-stance duration ratio (**Figure 6 – figure supplement 2F-I**). A limitation of our optical stimulation protocol is that the timing of stimulation was not phase-locked to the gait cycle; nevertheless, the results show that unilaterally raising D2 MSN activity is sufficient to produce both whole-body and single-limb motor impairments with some qualitative similarities to dopamine lesions.

Discussion

The present study investigated the electrophysiological activity of dorsal striatal neurons during self-initiated gait. The single-limb resolution of the behavioral measurements unveiled a sizable fraction (~45%) of striatal neurons which were phase-locked to rhythmic limb movements during the gait cycle. Earlier studies have reported rhythmic firing patterns in the striatum that were attributed to single-limb motion^{13, 35, 36–37}. Here we significantly expanded on prior work, by quantitatively comparing the gait phase coding properties across different limbs, cell types, and dopaminergic states. Our approach of examining neural responses with respect to single-limb gait together with more commonly used whole-body measures of motion led to a number of novel

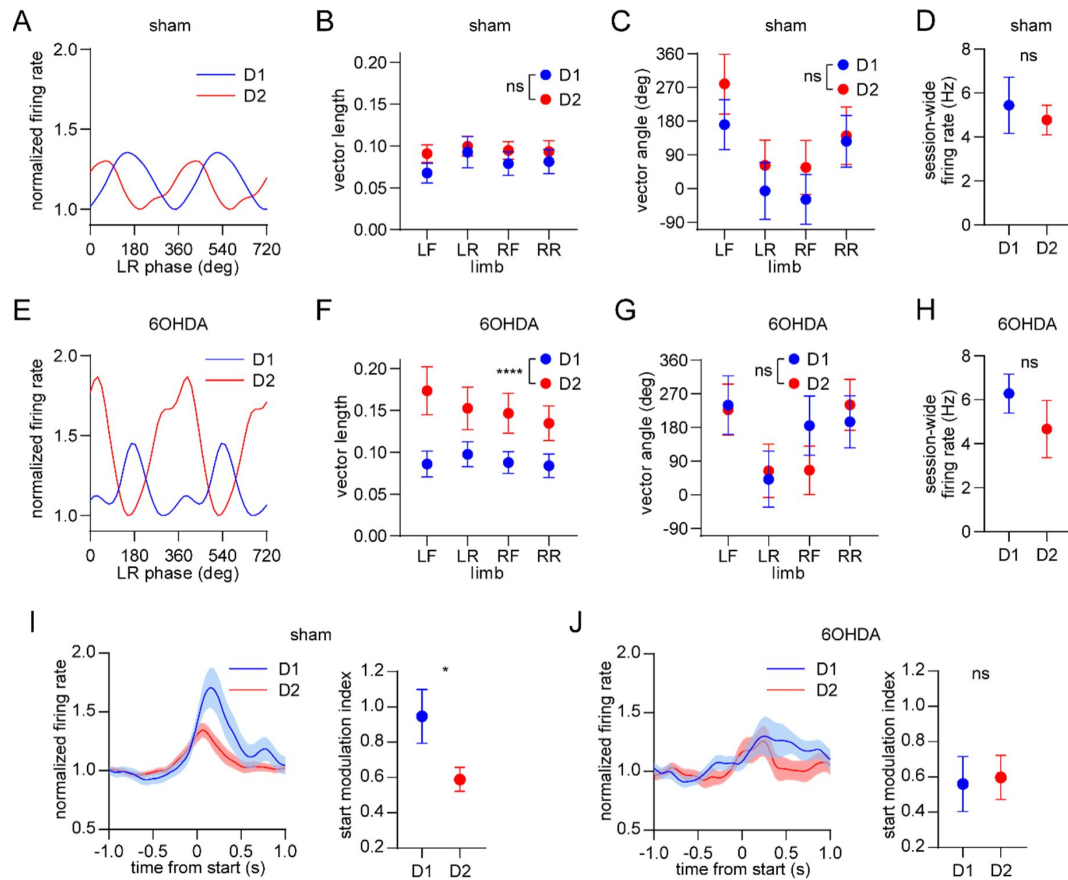


Figure 6.

Dopamine lesions alter the relative level of D1/D2 MSN activity coupled to limb phase and movement initiation.

(A) Mean normalized firing rate as a function of LR limb phase for a D1 and D2 MSN from sham-lesioned mice. The activity of each cell is normalized to the minimum firing rate. The limb phase is plotted for two full gait cycles (0-720°) for visual clarity.

(B) No significant difference in spike-limb phase vector length between D1 and D2 MSNs in the sham group ($n = 22$ D1 MSNs pooled from 5 mice and 57 D2 MSNs pooled from 9 mice, two-way ANOVA, $F_{1,308} = 1.95$, $p = 0.16$).

(C) No significant difference in mean vector angle between D1 and D2 MSNs in the sham group (angular permutation test corrected for 4 multiple comparisons, $p > 0.5$).

(D) No significant difference in the mean session-wide firing rate between D1 and D2 MSNs in the sham group ($n = 22$ D1 and 57 D2 MSNs, unpaired t-test, $p = 0.62$).

(E) Same as A but for cells recorded from 6OHDA-lesioned mice.

(F) Significant difference in spike-limb phase vector length between D1 and D2 MSNs in the 6OHDA group ($n = 31$ D1 MSNs pooled from 5 mice and 28 D2 MSNs pooled from 5 mice, two-way ANOVA, $F_{1,228} = 20$, $p < 0.0001$).

(G) No significant difference in mean vector angle between D1 and D2 MSNs in the sham group (angular permutation test corrected for 4 multiple comparisons, $p > 0.25$).

(H) No significant difference in the mean session-wide firing rate between D1 and D2 MSNs in the 6OHDA group ($n = 31$ D1 and 28 D2 MSNs, unpaired t-test, $p = 0.30$).

(I) Left: normalized firing rate relative to the start of movement averaged across all D1 and D2 MSNs in the sham group. Data are normalized to the mean firing rate in a pre-start baseline period. Right: The start modulation index (fractional change in firing in start period relative to pre-start) of D1 MSNs is significantly higher than D2 MSNs ($n = 22$ D1 and 57 D2 MSNs, unpaired t-test, $p = 0.016$).

(J) Left: normalized firing rate relative to the start of movement averaged across all D1 and D2 MSNs in the 6OHDA group. Data are normalized to the mean firing rate in a pre-start baseline period. Right: No significant difference in the start modulation index between D1 and D2 MSNs ($n = 31$ D1 and 28 D2 MSNs, unpaired t-test, $p = 0.86$). Angular data are represented as mean $\pm$ SD. All the other data are represented as mean $\pm$ SEM.

insights about the diverse role of the striatum in the control of walking. The data suggest that a subset of striatal neurons represents multiple parameters involved in initiating and continuously performing walking, as shown by the finding of mixed coding for single-limb and whole-body motion. A potential interpretation of this mixed code is that the striatum may serve diverse functions required for locomotion, including the initiation of whole-body movements as well as the production or maintenance of ongoing gait at a particular speed.

Movement-related neural activity is widespread in many brain areas, and it is plausible that the striatum receives both motor and sensory signals involved in gait generation. For example, the primary motor cortex, which projects to dorsal striatum, has been shown to exhibit rhythmic spiking activity consistent with gait phase coding²⁷, suggesting a shared mechanism underlying the production of this code. A problem not fully resolved here is whether the observed gait phase coding phenomenon is causally related to gait performance. While the optogenetic manipulations in **Figure 6 – figure supplement 2** indicate that D2 MSNs are capable of influencing gait, those experiments did not directly address whether limb phase-locked spiking activity per se is necessary for this behavior. Furthermore, even if striatal activity is causally linked to the production of gait, it is likely one of many circuits contributing to this motor function³.

Our findings are consistent with a large body of work demonstrating that striatal neurons encode multiple kinematic parameters including initiation, speed, and single-limb motion^{11–13, 15, 38}, as well as a variety of habitual behaviors and action sequences^{14, 17–19, 39}. There is some disagreement about the extent to which striatal neurons represent discrete or continuous actions (e.g., initiation and cessation versus ongoing gait)^{12, 40}. However, the most parsimonious interpretation of our and others' data is that both discrete and continuous actions are represented⁴¹. Behavioral studies provide further evidence that striatal neurons serve a role in both initiating and maintaining locomotion and other actions⁴². For example, activating D1/D2 MSNs increases/decreases the frequency of initiating movement (a discrete event) as well as the speed and duration of each walking bout (measures of continuous motion)^{4, 7}.

We found a different percentage of striatal neurons which encoded limb phase, movement initiation or cessation, and speed (**Figure 3**). Among these three categories, limb phase coding cells represented the smallest population with ~45% of neurons, as opposed to ~90% for start/stop or speed. In addition, nearly all phase coding cells were also significantly responsive to start/stop or speed, whereas a sizable proportion of start/stop or speed coding cells were not entrained to limb phase. It is unclear, however, whether these population size differences reflect a proportionally smaller role for the striatum in regulating single-limb gait as opposed to whole-body movement initiation, cessation or speed.

To delve deeper into the role of different striatal cell types in locomotion, we utilized optogenetic tagging techniques to compare neural activity between D1 and D2 MSNs. We found that healthy animals exhibit a similar strength of neural phase-locking to the gait cycle between these two subpopulations. However, in the same animals, D1 MSNs exhibited higher activity around the time of movement initiation relative to D2 MSNs. These findings have new and important implications for the function of the direct and indirect pathway in locomotion. On one hand, our results provide additional support to several studies demonstrating co-activation of these pathways during movement initiation and ongoing movement^{8, 9, 15, 30, 39, 43}. On the other hand, the data suggest that an imbalance in D1 versus D2 MSN activity may actually be important for the initial, discrete event in the movement execution sequence, but that once locomotion is underway, balanced activity may be optimal for the production of the gait cycle. Taken together, the data suggest the need to distinguish between the relative activity of D1 and D2 MSNs in the “start” and “gait cycle maintenance” stages of locomotion. The significant bias toward higher D1 MSN start activity was not reported in previous studies which relied on measuring calcium dynamics^{8, 9, 15}. We speculate that this discrepancy arose from differences in temporal resolution between single-unit electrophysiology and single-cell calcium imaging. Indeed, both our

data, and a study employing whole-cell membrane potential recordings, suggest that the D1 MSN bias is only apparent in the initial stages of body or whisker movements⁴⁴, a response that may have been missed by slower temporal resolution measurement techniques. We also note that another study employing optogenetic tagging did not find significant D1/D2 MSN differences in start/stop activity²⁶. However, the movement being measured was an instrumental action (reward-guided lever pressing), as opposed to self-initiated motion examined in our work. This suggests either that imbalances between D1 and D2 MSN start activity may be more pronounced under specific behavioral conditions, or that results vary depending on how movement initiation and cessation events are identified.

To further understand the potential behavioral significance of our findings we investigated if changes in normal D1 and D2 MSN activity patterns accompany changes in motor function. Problems with initiating movement (akinesia) and maintaining gait at normal speed and rhythm (bradykinesia) are both major motor symptoms characteristic of Parkinson's disease²¹. Unilateral dopamine lesions via 6OHDA injection recapitulated many of these impairments, including reduced walking bout initiation frequency, and shorter, slower, and more variable limb strides. We identified two prominent effects of dopamine lesions on the relative strength of D1 and D2 MSN activity. First, dopamine loss shifted single-limb phase-locking from a balanced state to an imbalanced state favoring D2 MSNs. Second, these lesions shifted movement start activity from an imbalanced state favoring D1 MSNs to a balanced state with no clear bias for either cell type. Both effects appear consistent with the classical model of basal ganglia function in the sense that loss of dopamine has a net effect of lowering the amount of D1 MSN relative to D2 MSN activity^{5, 8, 32}. However, the data suggest that the specific direction in which D1 and D2 MSN activity is altered, varies across different measures of movement. In terms of the functional consequence of these dopamine-mediated electrophysiological effects, one possibility is that the first effect (increase in D2 MSN limb phase-locking strength) contributes to bradykinetic symptoms, specifically the production and maintenance of a normal gait cycle and rhythm, while the second effect (reduction in D1 MSN start activity) contributes to akinetic symptoms associated with dopamine loss. Alternatively, these effects may reflect a homeostatic mechanism to compensate for altered motor function³¹.

Unilateral 6OHDA lesions produced a strong asymmetry in gait, which was evident both in the significant increase in ipsiversive turning, as well as the greater reduction in the stride length of ipsilateral relative to contralateral limbs (**Figure 5G**). This raises some uncertainty in interpreting the observed imbalance in D1/D2 MSN phase-locking strength, as the imbalance could either reflect a general gait deficit, or a higher incidence of turning. To address this, one approach could be to compare activity during similar turning and straight walking trajectories in healthy and dopamine lesioned animals. However, there were insufficient straight walking bouts in lesioned animals, and turning bouts in healthy animals, to make such an analysis possible with the available data. Nevertheless, some insight into this problem is provided by another study, which employed calcium imaging to compare D1/D2 MSN activity during locomotion⁴⁵. That study reported a small bias toward D1 MSN activity during turning, thus potentially allaying the concern that higher D2 MSN phase-locking merely reflects turning signal.

In addition to characterizing ongoing locomotion via single-limb gait measurements, we employed whole-body speed, which was confirmed to be widely represented by striatal neurons^{9, 12}. However, unlike the measure of phase-locking strength, which displayed significant changes following dopamine lesions, our measure of whole-body speed coding strength remained statistically similar between D1 and D2 MSNs under all experimental conditions, even though dopamine lesions reduced the overall speed coding score for both cell types. These results demonstrate that studying the neural basis of gait at the resolution of individual limb strides reveals insights that are less accessible with lower resolution whole-body speed measurements. In

closing, this study provided an enhanced understanding of striatal dynamics during locomotion, and uncovered a potential neurophysiological mechanism for impaired gait following dopamine loss.

Methods

Animals

All procedures were approved by the University of California, Los Angeles Chancellor's Animal Research Committee. We used transgenic mice of both sexes (D1-Cre, Tg(Drd1-cre)EY262Gsat/Mmucd; and A2a-Cre, Tg(Adora2a-cre)KG139Gsat/Mmucd. Transgenic mice were maintained as hemizygous in a C57BL/6J background (The Jackson Laboratory 000664). Animals were 10-14 weeks old at the time of the initial surgery. Animals were kept on a 12 hour light cycle, and group housed until the first surgery.

Opto-microprobe

Electrophysiological recordings and optogenetic-tagging were performed with an opto-microprobe²⁵, a single-shank 64 electrode silicon microprobe (model 64D-sharp, Masmanidis lab) modified by attachment of an optical fiber. The 0.2 mm diameter fiber terminated 0.1 mm above the most dorsal electrode, allowing delivery of laser illumination to the recording field. To read out electrical signals from the probe, it was wire bonded to a flexible printed circuit board (PCB) containing two electrical connectors compatible with a miniature head stage (White Matter LLC). The silicon microprobe designs and information on the assembly of opto-microprobes are available on a Github file repository (<https://github.com/sotmasman/Silicon-microprobes>).

Surgical procedures

Animals underwent up to three surgical procedures under aseptic conditions and isoflurane anesthesia on a stereotaxic apparatus (Kopf Instruments). The first surgical procedure involved attaching a custom 3D printed head cap on the skull, drilling a craniotomy window, and injecting 0.5 μ l Cre-dependent adeno-associated virus (AAV) expressing ChR2 in the dorsal striatum of the right hemisphere (0.9 mm anterior, 1.5 mm lateral, 2.8 mm ventral relative to bregma). The head cap contained a base plate which was securely fixed to the skull using dental cement, and a cap which was attached to the base plate with a pair of screws. The head cap featured two through-holes, facilitating the connection of an optical fiber for optogenetic tagging, and an electronic cable for electrophysiological data transmission. At the conclusion of the first surgery, the exposed skull area was covered with silicone sealant (Kwik-Cast, WPI). All animals were individually housed after the first surgery and at least 4 weeks elapsed before beginning habituation in preparation for electrophysiological recording. For dopamine lesion experiments, an additional surgical procedure was performed 2 weeks after the first surgery in which 6-hydroxydopamine hydrochloride (6OHDA, 1.2 μ g dissolved in 0.5 μ l) was injected in the right medial forebrain bundle (-1.2 mm anterior, 1.2 mm lateral, 4.75 mm ventral relative to bregma). An intraperitoneal injection of desipramine (10 mg/kg) was administered 30 min before the 6OHDA injection to increase 6OHDA selectivity for dopamine. Sham lesioned animals received medial forebrain bundle saline injections instead of 6OHDA. Animals recovered on a heating pad and body weight was monitored daily. Soft moistened food was placed in a clean area of the home cage to avoid excessive weight loss. A final surgical procedure was performed one day before the scheduled electrophysiological recording session to implant the opto-microprobe in the dorsal striatum (probe tip at 0.7 mm anterior, 1.5 mm lateral, 3.3 ventral relative to bregma). After inserting the microprobe, a layer of Vaseline was applied to cover the exposed microprobe and brain surface, followed by dental cement to fix the microprobe to the skull. A craniotomy was made over the left hemisphere to accommodate a stainless steel ground screw, which was connected to the connectors on the flexible PCB via a stainless steel wire, and then fixed in place with dental

cement. The flexible PCB portion of the opto-microprobe was plugged into a 64 channel miniature head stage (HS-64m, White Matter LLC), and then carefully folded into the head cap assembly. All custom 3D printed part designs can be found on a Github file repository (<https://github.com/LongYang10/Opto-microprobe-implantation> [↗](#)).

Motion tracking

Spontaneous limb movements were monitored in a large open arena (60 cm x 60 cm) containing a transparent floor to allow bottom-up imaging with a high-speed camera (Basler acA2040-90umNIR) under infrared illumination. The spatial resolution was calibrated at 0.3 mm/pixel. Video was captured at 80 fps and streamed via Streampix 8 software (Norpix). To ensure synchronization between the video data and electrophysiological data, the camera was triggered using a shared 80 Hz clock signal generated by a DAQ (NI USB-6356). Prior to the electrophysiological recording session animals underwent a habituation phase, wherein they had the opportunity to freely explore the arena for 15 min per day over three days. Limb motion was tracked offline with SLEAP software²³ [↗](#), which was trained to estimate the 2D coordinates of six body parts (four limbs plus the nose and base of the tail) at each video frame. Whole-body speed was calculated from the derivative of the average position of the six tracked body parts.

Single-limb gait analysis

We performed a semi-automated identification of well-defined bouts of walking to eliminate periods when animal movement did not correspond to locomotion (e.g., grooming). This step involved replaying the tracked body coordinates during candidate walking frames (initially identified automatically via a body speed threshold), and manually accepting only frames in which the limbs displayed a clear rhythmic motion characteristic of gait. Spatial coordinate data were then smoothed with a third order Savitzky-Golay filter. To identify the onset and offset times of individual strides, limb positions were projected onto the nose-tail axis, and bandpass filtered from 0.5-8 Hz. This filtered signal revealed the cyclical motion of limbs during gait (**Fig. 1d** [↗](#)). Each stride begins with the stance phase, whose onset corresponds to the minima in the gait cycle (indicating that the limb is closest to the nose). The stance is followed by the swing phase, whose onset corresponds to the maxima in the gait cycle (indicating that the limb is furthest from the nose). The end of the swing coincides with the start of the next stride's stance. The stride duration is defined as the time interval between the start of the current stride's stance and next stride's stance. The stride length refers to the Euclidean distance spanned by the limb between these two time points. Stride speed is calculated from the ratio between length and duration. The limb phase angle is defined as 0 degrees at the stance start time and is subdivided into equal angular increments up to 360 degrees at the start of the next stride's stance.

Electrophysiology and optogenetic tagging

On the day of electrophysiological recording the head stage in the head cap was connected to a flexible wire tether via an in-line electrical rotary joint (White Matter LLC), which allowed the animal to freely move in the arena. Electrophysiological data were sampled at 25 kHz per channel. Only one 30 min electrophysiological recording session was performed per animal to avoid potentially double-counting cells. The optical fiber remained disconnected during the 30 min recording period to maximize the animal's mobility. At the end of this period, the optical fiber was coupled to a 473 nm laser and an optogenetic tagging protocol was performed, in which 200 pulses of light were delivered (10 ms pulse duration, once pulse every 3 s, with optical intensity calibrated to 5 mW at the opto-microprobe fiber tip). Offline, raw data were bandpass filtered from 600-7000 Hz, spike sorted with Kilosort⁴⁶ [↗](#), and manually curated with Phy. Session-wide firing rate was calculated as the average spike rate over the entire recording session duration, until the time of the first laser pulse. Optogenetic tagging analysis assessed the following three criteria, based on previous work²⁶ [↗](#) and established at the beginning of the study: 1) significant excitatory response within a spike latency of 6 ms from the laser onset time; 2) strong correlation

between the mean optically evoked and mean baseline spike waveforms (Pearson $r > 0.95$); and 3) to address the scale invariance of Pearson correlations, a similar ratio between the voltage minimum of the mean optically evoked spike waveforms and voltage minimum of the mean baseline spike waveforms (ratio < 2). Neurons that satisfied all three criteria were labeled as tagged D1 or D2 MSNs. Optical stimulation produced photoelectric artifacts which sometimes resembled single-unit spikes, but these were confined to a brief (sub-millisecond) period when the laser was turned on. To prevent these artifacts from inflating our estimate of optically responsive neurons, spikes within a 0.6 ms time window from laser onset were removed from the optogenetic tagging analysis. Since the shortest latency for optogenetically evoked neural responses was found to be 2 ms from laser onset, removing spikes within 0.6 ms of laser onset did not adversely affect the tagging analysis.

Optogenetic control of behavior

A subset of healthy Chr2-expressing Adora2a-Cre mice used for optogenetic tagging experiments underwent an additional behavioral testing session on a subsequent day. The 15 minute session consisted of 5 minutes pre-stimulation, 5 minutes laser stimulation (5 mW, 20 Hz frequency and 10 ms pulse duration), and 5 minutes of post-stimulation.

Gait phase coding analysis

Mean firing rate was calculated as a function of single-limb phase angle by dividing the total number of spikes per phase by the number of frames per phase angle, and multiplying by the frame rate. The limb phase angle was calculated as described above for each stride from all the walking bouts identified in a recording session (see “Single-Limb Gait Analysis”). Across all strides we then counted the total number of spikes occurring at each phase angle from 0 to 360 degrees, in bins of 15 degrees. Next, we counted the total number of frames that occurred at each phase angle in bins of 15 degrees. To quantify gait phase coding we adapted an approach used to analyze spike-field coupling⁴⁷, by employing circular analysis methods to calculate the mean vector length and angle of the firing rate versus phase angle distribution. The mean vector length is a unitless quantity from 0 to 1 indicating the strength of neural entrainment to the gait cycle. On average, this parameter was found to have a value of 0.1~0.2. Cells with vector lengths exceeding 0.9 were considered outliers and removed from further analysis, including speed and start/stop coding. Out of a total of 222 optogenetically tagged units in this study, only 5 cells were excluded because of this criterion – further, including these outliers slightly enhanced D1 vs D2 MSN group differences in mean vector length in dopamine lesioned animals, but did not alter the statistical significance of the results. The mean vector angle indicates the preferred limb phase at which spiking occurs. To determine if a cell was significantly entrained to limb phase, we employed a spike time jitter test in which the mean vector length was recalculated after adding a random jitter of up to ± 0.5 s to each spike time. This was repeated for 100 iterations. Cells whose real vector length exceeded more than 95% of the jittered vector lengths with respect to at least one limb's phase were labeled as gait phase coding neurons.

Start/stop coding analysis

To establish the start and stop times of whole-body motion bouts, we initially identified all time points during which body speed exceeded a speed of 50 mm/s, designating them as motion bouts. Subsequently, motion bouts lasting less than 0.3 s were excluded. For each confirmed motion bout, we conducted retrograde and forward scans to determine the frames at which body speed surpassed or declined below 20 mm/s, marking the defined start and stop times, respectively. To identify start/stop coding cells we compared the mean firing rate from -5 to -1 s prior to movement initiation or cessation (baseline window activity), and the mean firing rate within ± 0.5 s (event window activity) using a paired t-test with a significance threshold of $p < 0.05$. The start/stop modulation index was obtained by first normalizing the firing rates by the mean rate in the baseline window, such that baseline rate (FR_{baseline}) was approximately equal to 1. We then

binned the activity in the event window in 20 ms increments and found the maximum rate in this time window (FR_{event}), corresponding to the highest level of start or stop activity. The modulation index was obtained from the expression:

$$modulation\ index = |FR_{event} - FR_{baseline}| / FR_{baseline}$$

This index was a unitless positive quantity with higher values representing greater changes (positive or negative) in start or stop activity with respect to baseline.

Speed coding analysis

Speed coding analysis was performed on whole-body speed data from the entire recording session. Speed data were binned in increments of 10 mm/s. We calculated the average firing rate at each speed bin. The speed coding score represented the absolute value of the Pearson correlation coefficient between firing rate and speed. To determine if neurons were significantly correlated with speed we recalculated the Pearson correlation after shuffling the speed bins. This was repeated for 100 iterations. Neurons whose real correlation coefficient exceeded the shuffled data on more than 95% of iterations were defined as speed coding.

Quantification and statistical analysis

Statistical analysis was performed using custom Matlab and Python code and Prism software. A custom circular statistic, referred to as the angular permutation test, was used to compare angular distributions between two groups. This test involved first computing the mean vector length and angle for two angular distributions using unit vector lengths for each sample. We then calculated the Euclidean distance between the resulting vectors. Subsequently, the samples from each group were randomly shuffled and the Euclidean distance recalculated for a total of 1000 iterations. If the real Euclidean distance exceeded that of 95% of the resampled data, the angular distributions were deemed statistically different. Angular permutation tests were adjusted for multiple comparisons using Bonferroni's correction. The SD of angular distributions was calculated from the angular deviation function in Ref.⁴⁸. Significant differences throughout all the figures are represented by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Data and code availability

Data from this study are available at: <https://doi.org/10.5281/zenodo.10452838>.

Code for analyzing the data in this study is available at: <https://github.com/LongYang10/Gait-Analysis-of-Freely-Walking-Mouse>

Acknowledgements

L.Y. was supported by the Marion Bowen Neurobiology Postdoctoral Grant Program at UCLA. S.C.M. was supported by NIH grant NS125877.

Author contributions

L.Y. designed and carried out experiments, analyzed data, and wrote the manuscript. A.K.W and D.S. carried out experiments and analyzed data. K.A.C. contributed to the interpretation of the results. S.C.M. conceived the project, designed experiments, analyzed data, and wrote the manuscript. All authors discussed and commented on the manuscript.

Declaration of interests

The authors declare no competing interests.

Figure supplements

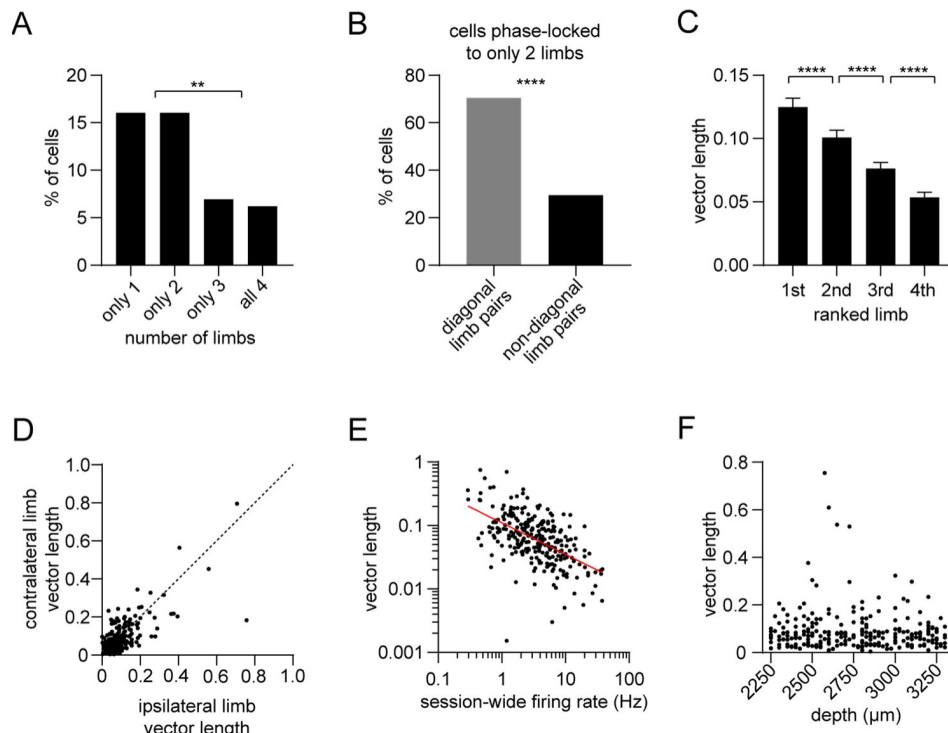


Figure 2 - Figure Supplement 1.

Single-limb phase locking strength in dorsal striatal neurons.

(A) Percentage of striatal neurons with significant phase-locking to only 1, only 2, only 3, or all 4 limbs ($n = 274$ total cells pooled from 9 healthy mice, chi-square test adjusted for 6 comparisons, 1 vs 3 and 2 vs 3: $p = 0.003$; 1 vs 4 and 2 vs 4: $p = 0.0009$).

(B) Among the neurons which were phase-locked to only 2 limbs ($n = 44$ cells), a greater proportion of limb pairs were diagonal (chi-square test, $p < 0.0001$).

(C) Significant difference in spike-limb phase vector length when averaged by limb rank in order of highest to lowest vector length ($n = 274$ cells, one-way RM ANOVA, $p < 0.0001$). Data represent mean $\pm$ SEM.

(D) The mean vector length of striatal neurons shows no preference for the contralateral (LF) or ipsilateral (RF) limb (paired t -test, $p = 0.3$). Each dot represents one neuron.

(E) Significant negative correlation between the vector length and session-wide firing rate per cell. Vector length is calculated from the LF limb ($n = 274$ cells, Pearson $r = -0.32$, $p < 0.0001$). Each dot represents one neuron. Red line represents the best linear fit, plotted on a logarithmic scale.

(F) Mean vector length per cell as a function of the neuron's estimated depth in the dorsal striatum relative to bregma (Pearson $r = -0.08$, $p = 0.22$). Each dot represents one neuron.

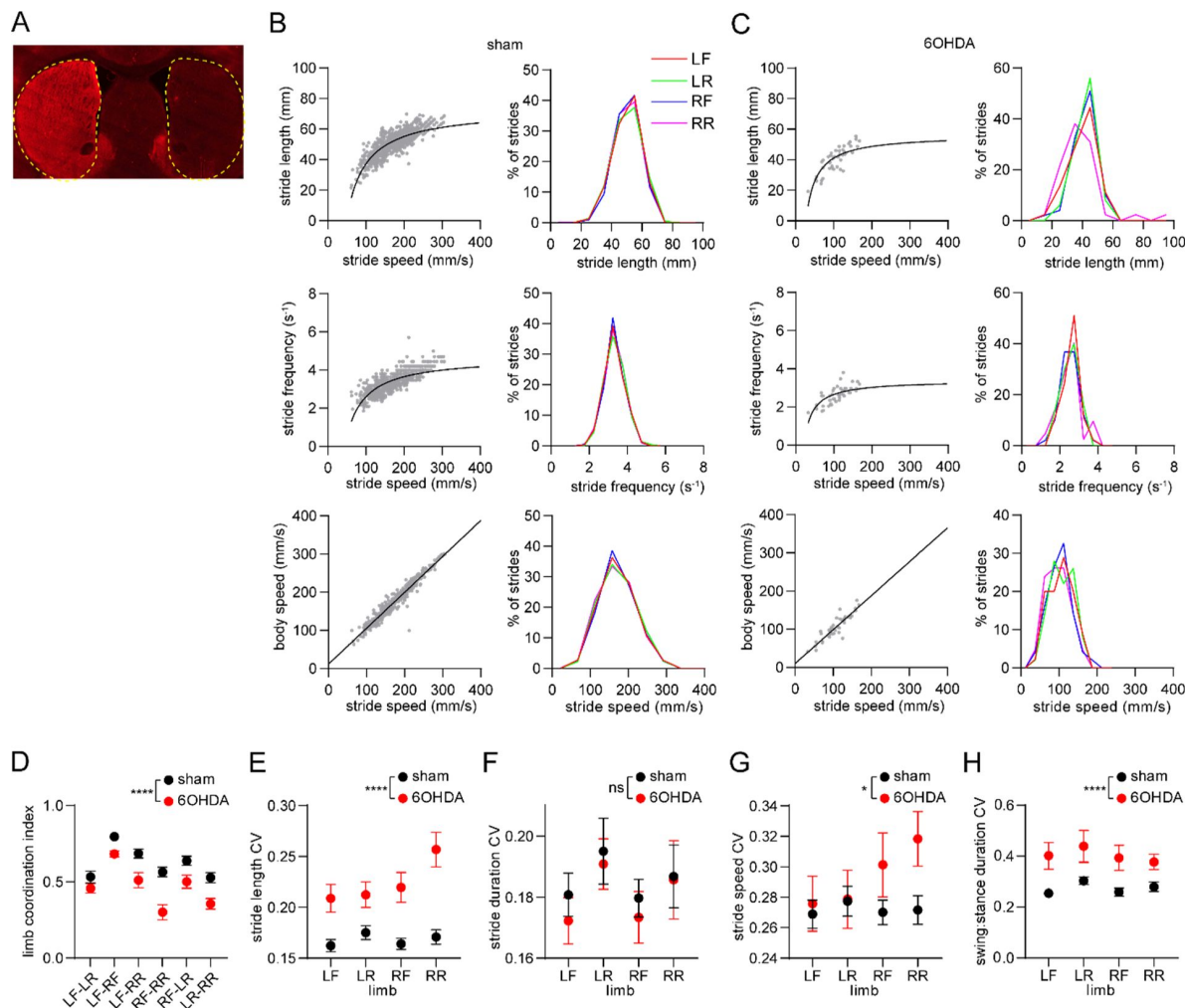


Figure 5 – Figure Supplement 1.

Limb coordination and gait variability are altered by dopamine lesions.

(A) Fluorescence image of a brain section from a 6OHDA-lesioned mouse, immunostained for tyrosine hydroxylase. Dashed yellow lines demarcate the striatum.

(B) Limb stride parameters from one recording session in the sham group. Gray dots represent individual LF limb strides. Black lines represent the best polynomial or linear fit.

(C) Limb stride parameters from one recording session in the 6OHDA group.

(D) The coordination between different limb pairs is significantly reduced in the 6OHDA group ($n = 10$ 6OHDA and 14 sham-lesioned mice, two-way ANOVA, $F_{1,132} = 61$, $p < 0.0001$).

(E) Mean coefficient of variation (CV) in stride length is significantly increased in the 6OHDA group (two-way ANOVA, $F_{1,88} = 61$, $p < 0.0001$).

(F) Mean CV in stride duration does not significantly change in 6OHDA lesioned mice (two-way ANOVA, $F_{1,88} = 0.6$, $p = 0.45$).

(G) Mean CV in stride speed is significantly increased in the 6OHDA group (two-way ANOVA, $F_{1,88} = 5$, $p = 0.03$).

(H) Mean CV in the stride swing:stance duration is significantly increased in the 6OHDA group (two-way ANOVA, $F_{1,88} = 31$, $p < 0.0001$). All data are represented as mean $\pm$ SEM.

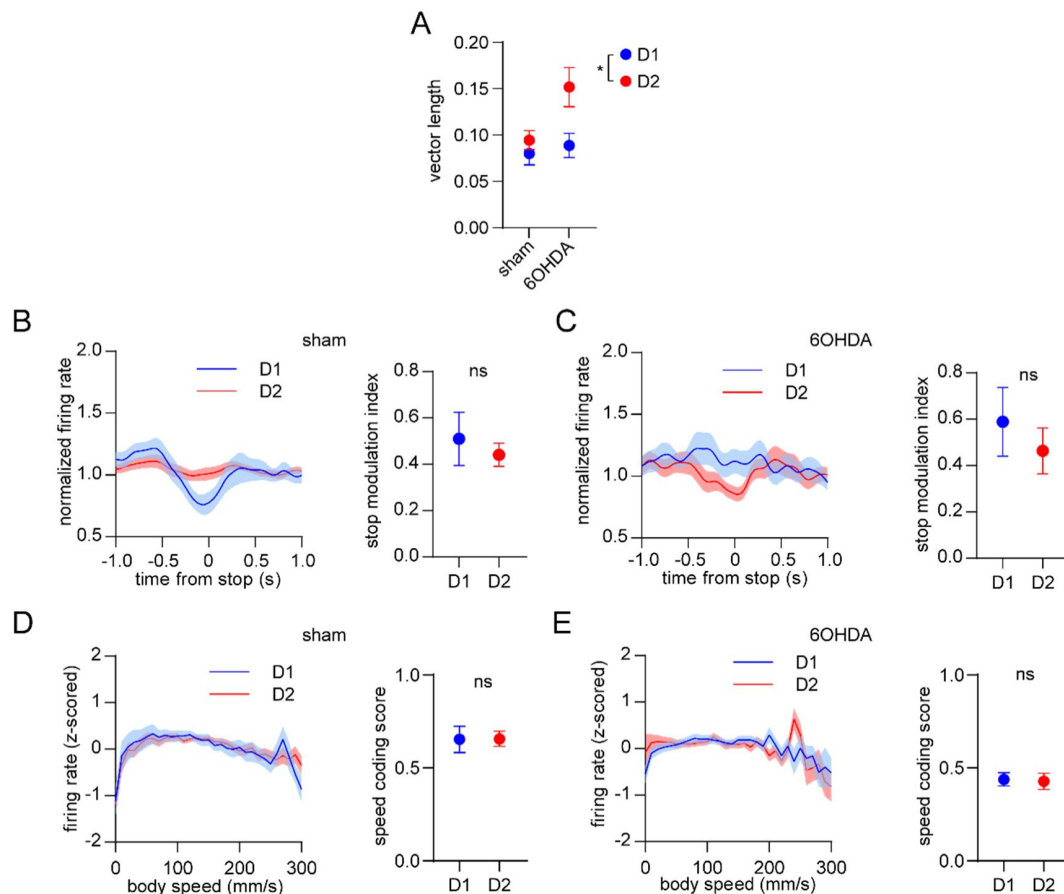


Figure 6 – Figure Supplement 1.

Dopamine lesions do not alter the balanced D1/D2 MSN encoding of movement cessation and body speed.

(A) Cell type (D1 or D2 MSN), group (sham or 6OHDA) and the interaction between them have a significant effect on the average vector length across the four limbs ($n = 22$ D1 and 57 D2 MSNs in the sham group, 31 D1 and 28 D2 MSNs in the 6OHDA group, two-way ANOVA, cell type factor: $F_{1,134} = 7$, $p = 0.01$, group factor: $F_{1,134} = 5$, $p = 0.03$, interaction: $F_{1,134} = 3$, $p = 0.1$).

(B) Left: normalized firing rate relative to the cessation of movement averaged across all D1 and D2 MSNs in the sham group. Data are normalized to the mean firing rate in a pre-stop period. Right: No significant difference in the stop modulation index (fractional change in firing in stop period relative to pre-stop) between D1 and D2 MSNs ($n = 22$ D1 and 57 D2 MSNs, unpaired t-test, $p = 0.52$).

(C) Left: normalized firing rate relative to the cessation of movement averaged across all D1 and D2 MSNs in the 6OHDA group. Data are normalized to the mean firing rate in a pre-stop period. Right: No significant difference in the stop modulation index between D1 and D2 MSNs ($n = 31$ D1 and 28 D2 MSNs, unpaired t-test, $p = 0.5$).

(D) Left: firing rate (z-scored) as a function of body speed averaged across all D1 and D2 MSNs in the sham group. Right: No significant difference in speed coding score (absolute Pearson r of firing rate in relation to speed) between D1 and D2 MSNs ($n = 22$ D1 and 57 D2 MSNs, unpaired t-test, $p = 0.97$).

(E) Left: firing rate (z-scored) as a function of body speed averaged across all D1 and D2 MSNs in the 6OHDA group. Right: No significant difference in speed coding score between D1 and D2 MSNs ($n = 31$ D1 and 28 D2 MSNs, unpaired t-test, $p = 0.93$). All data are represented as mean $\pm$ SEM.

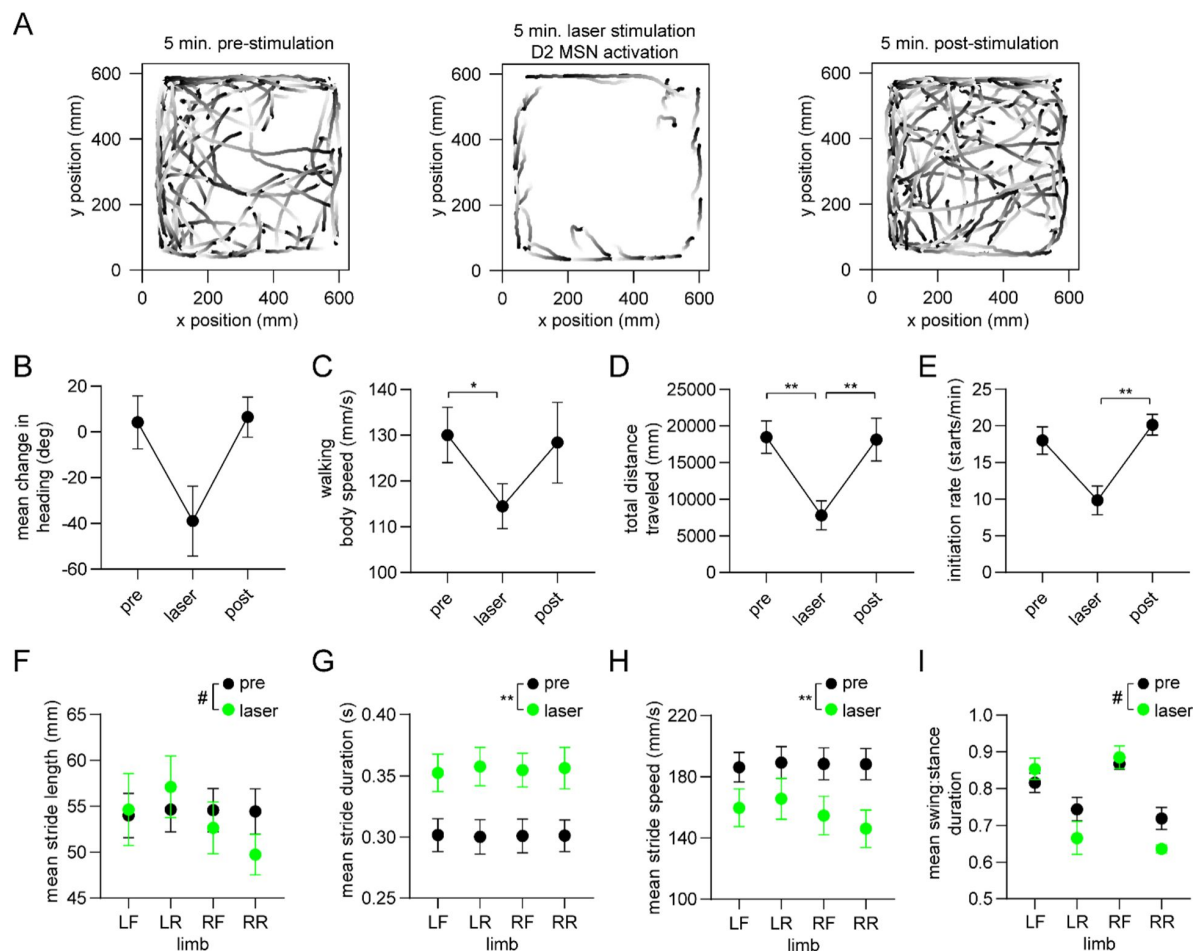


Figure 6 - Figure Supplement 2.

Optogenetic activation of D2 MSNs alters whole-body movement and single-limb gait.

(A) Walking bout body trajectories from one 15 minute recording session during which the middle 5 minutes coincided with continuous optogenetic activation of striatal D2 MSNs in the right hemisphere. Light color represents the start of movement.

(B) Mean change in movement direction in the pre-stimulation, laser, and post-stimulation periods. Negative angular values indicate ipsiversive turning ($n = 4$ mice, angular permutation test adjusted for 3 comparisons, $p > 0.05$).

(C) Mean body speed in the pre-stimulation, laser, and post-stimulation periods ($n = 4$ mice, one-way RM ANOVA, $p = 0.01$).

(D) Total distance covered in the pre-stimulation, laser, and post-stimulation periods ($n = 4$ mice, one-way RM ANOVA, $p < 0.0001$).

(E) Rate of initiating movements in the pre-stimulation, laser, and post-stimulation periods ($n = 4$ mice, one-way RM ANOVA, $p = 0.03$).

(F) Mean stride length per limb in the pre-stimulation and laser periods ($n = 4$ mice, two-way ANOVA, pre vs laser: $F_{1,3} = 0.9$, $p = 0.42$. Post-hoc multiple comparison tests revealed a significant difference for the RR limb: $\#p = 0.03$).

(G) Mean stride duration per limb in the pre-stimulation and laser periods ($n = 4$ mice, two-way ANOVA, pre vs laser: $F_{1,3} = 83$, $p = 0.003$).

(H) Mean stride speed per limb in the pre-stimulation and laser periods ($n = 4$ mice, two-way ANOVA, pre vs laser: $F_{1,3} = 161$, $p = 0.001$).

(I) Mean stride swing:stance ratio per limb in the pre-stimulation and laser periods ($n = 4$ mice, two-way ANOVA, pre vs laser: $F_{1,3} = 1.7$, $p = 0.28$. Post-hoc multiple comparison tests revealed a significant difference for the LR and RR limbs: $\#p = 0.02$).

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Editors

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Reviewer #1 (Public Review):

Summary:

The authors combined high-speed video tracking of the limbs of freely moving mice with in vivo electrophysiology to demonstrate how striatal neurons encode single-limb gait. They also

examine encoding other well-known aspects of locomotion, such as movement velocity and the initiation/termination of movement. The authors show that striatal neurons exhibit firing phase-locked with mouse gait at the single limb but also multi-limb level. Moreover, they describe gait deficits induced by severe unilateral dopamine neuron degeneration, and associate these deficits with a relative strengthening of gait-modulation in the firing of D2-expressing MSNs. Although the source and function of this gait-modulation remain unclear, this manuscript uncovers an important physiological correlate of striatal activity with gait, which may have implications for gait deficits in Parkinson's Disease.

Strengths:

While some previous work has looked at the encoding of gait variables in the striatum and other basal ganglia nuclei, this paper uses more careful quantification of gait with video tracking, comparing healthy and 6-OHDA-treated mice in the open field. The authors have collected a relatively large dataset of optically-identified striatal recordings to shed light on similarities and differences in the encoding of gait by striatal direct and indirect pathway neurons

Weaknesses:

There are some caveats to the interpretation of the analyses presented here, including how to compare encoding of gait variables when animals have markedly different behaviors (eg comparing sham and unilaterally 6-OHDA treated mice). The authors now address this caveat in the Discussion.

In an effort to causally link striatal firing to gait, the authors have added data from N=4 mice in which D2-expressing MSNs are optogenetically activated, and measured the resulting changes in gait parameters. As the authors note, this experiment does not directly get at the question of whether gait modulation of firing in the striatum contributes to the kinematics of gait (an experiment in which they altered the pattern of firing, to reduce modulation, would likely be needed). Given that this experiment has very low N and there are no included controls (eg mice expressing a control construct with optical stimulation), I do not think this data should be included in the manuscript. I think commenting in the Discussion that causal experiments will be needed in the future is adequate.

Many of the examples, as well as the average firing rates shown, are higher than typical for MSNs as reported in the literature. This is true even of the optically identified units that are shown in Figure 4. This may reflect the inclusion of neurons with interneuron-type properties (the authors report that there were some optically identified units with interneuron properties), the inclusion of some multi-unit activity in some recordings, or differences in recording/spike sorting techniques.

<https://doi.org/10.7554/eLife.92821.2.sa2>

Reviewer #2 (Public Review):

Yang et al. recorded the activity of D1- and D2-MSNs in the dorsal striatum and analyzed their firing activity in relation to single-limb gait in normal and 6-OHDA lesioned mice. The authors provided evidence that the striatal D1- and D2-MSNs were phase-locked to the walking gait cycles of individual limbs, and dopamine lesions led to enhanced phase-locking between D2-MSN activity and walking gait cycles.

Comments on revised version:

The authors addressed my largest concern, which questioned if D1 and D2 MSNs phase-locked to single limbs better than the global gait cycles.

As to my second major concern, which questioned the causal significance of single limb gait coding in D1 and D2 MSNs on gait control, they performed additional optogenetic experiments to establish evidence that D2 activity is causally relevant for gait pattern control. The additional experiments also closed the logic gap between dopamine lesion, D2 activity and gait control, supporting the hypothesis that dopamine affects gait control and global movement pattern via increasing D2 MSN activity.

<https://doi.org/10.7554/eLife.92821.2.sa1>

Reviewer #3 (Public Review):

In this study, Yang et al. address a fundamental question of the role of dorsal striatum in neural coding of gait. The authors study the respective role of D1 and D2 MSNs by linking their balanced activity to detailed gait parameters. In addition, they put in parallel the striatal activity related to whole-body measures such as initiation/cessation of movement or body speed. They are using an elegant combination of high-resolution single-limb motion tracking, identification of bouts of movements and electrophysiological recordings of striatal neurons to correlate those different parameters. Subpopulations of striatal output neurons (D1 and D2 expressing neurons) are identified in neural recordings with optogenetic tagging. Those complementary approaches show that a subset of striatal neurons have phase-locked activity to individual limbs. In addition, more than a third of MSNs appear to encode all three aspects of motor behavior addressed here, initiation/cessation of movement, body speed and gait. This activity is balanced between D1 and D2 neurons, with a higher activity of D1 neurons only for movement initiation. Finally, alterations of gait, and the associated striatal activity, is studied in a mouse model of Parkinson's Disease, using 6-OHDA lesions in the medial forebrain bundle (MFB). In the 6OHDA mice, there is an imbalance toward D2 activity.

Strengths:

The study combines elegant approaches to correlate cell-specific striatal activity with specific aspects of motion and how it is affected in a PD model. The results are convincing, and the methodology supports the conclusions presented here.

Weaknesses:

All the data were not fully exploited or explained in the first version of the manuscript and the present version has been significantly improved.

There is a long-standing debate on the respective role of D1 and D2 MSNs on the control of movement. This study goes beyond prior work by providing detailed quantification of individual limb kinematics, in parallel of whole-body motion, and showing high proportion of MSNs to be phase-locked to precise gait cycle and also encoding whole-body motion. The temporal resolution used here highlights preferential activity of D1 MSN at the movement starts, where previous studies described a more balanced involvement. Finally they reveal neural mechanisms of dopamine depletion induced gait alterations, with a preponderant phase-locked activity of D2 neurons.

<https://doi.org/10.7554/eLife.92821.2.sa0>

Author Response

The following is the authors' response to the original reviews.

We sincerely thank the reviewers for their constructive feedback. We have revised our manuscript to address some important concerns. The main changes are summarized as follows:

(1) A major concern as reflected in the eLife assessment and reviewer comments, was that the “evidence supporting the conclusion that striatal neurons encode single-limb gait is incomplete.” We have now provided an expanded analysis of gait phase-locking to different limbs in Figure 2 – figure supplement 1. The analysis reveals three key new insights: 1) most striatal neurons are significantly entrained to only one or two limbs; 2) for neurons entrained to two limbs, most limb pairs are diagonal pairs, whose phases are closely aligned; 3) the strength of phase-locking, as measured by the mean vector length, is biased toward a single limb. From these results we conclude that striatal neurons are indeed better correlated with single-limb (as opposed to multiple limbs’) gait. However, we speculate that because of the inherently correlated motion across limbs, some neurons also display significant phaselocking to multiple limbs, particularly to diagonal pairs.

(2) Reviewer 2 noted the lack of a manipulation experiment which would help establish the striatum’s relationship to gait control. We have therefore included the results of new experimental data in Figure 6 – figure supplement 2, in which we show that optogenetically activating D2 MSNs alters both some measures of whole-body motion and single-limb gait. We recognize that these experiments are not ideal, for example, the optical stimulation was not entrained to limb phase. Nevertheless, they hopefully allay any concern that the striatum is incapable of influencing gait performance.

(3) We have further characterized the relationship between vector length and firing rate, and firing rate between D1 and D2 MSNs. We now show that: 1) vector length is negatively correlated with session-wide firing rate (Figure 2 – figure supplement 1E); 2) session-wide firing rates are similar between D1 and D2 MSNs in both healthy and dopamine lesioned animals (Figure 4D and Figure 6H). Thus, the imbalance in the vector length between D1 and D2 MSNs following dopamine lesions is unlikely to be explained by changes in the overall firing rates of these cells.

(4) We have added new data similar to Figure 1 with distributions of stride frequency, duration, and length to illustrate the difference between sham and 6OHDA mice (Figure 5 – figure supplement 1B,C).

(5) We have expanded the Discussion section to discuss a number of important points raised by the reviewers. These include: 1) speculating on the origins of gait coding in the striatum; 2) discussion of some literature which reported similar levels of D1/D2 MSN start coding in contrast to our results in healthy mice; 3) discussion of the finding that almost all phase-locked cells also have a firing rate related to speed or start/stop signals; 4) discussion of one of the limitations of the unilateral 6OHDA model, namely, the strong turning bias, and its potential implications for our results.

Public Reviews:

Reviewer #1 (Public Review):

Summary:

Yang et al combine high-speed video tracking of the limbs of freely moving mice with in vivo electrophysiology to demonstrate how striatal neurons encode single-limb gait. They also examine encoding other well-known aspects of locomotion, such as movement velocity and the initiation/termination of movement. The authors show that striatal neurons exhibit rhythmic firing phase-locked with mouse gait, while mice engage in spontaneous locomotion in an open field arena. Moreover, they describe gait deficits

induced by severe unilateral dopamine neuron degeneration and associate these deficits with a relative strengthening of gait-modulation in the firing of D2-expressing MSNs. Although the source and function of this gait-modulation remain unclear, this manuscript uncovers an important physiological correlate of striatal activity with gait, which may have implications for gait deficits in Parkinson's Disease.

Strengths:

While some previous work has looked at the encoding of gait variables in the striatum and other basal ganglia nuclei, this paper uses more careful quantification of gait with video tracking. In addition, few if any papers do this in combination with optically-labeled recordings as were performed here.

Weaknesses:

The data collected has a great richness at the physiological and behavioral levels, and this is not fully described or explored in the manuscript. Additional analysis and display of data would greatly expand the interest and interpretability of the findings.

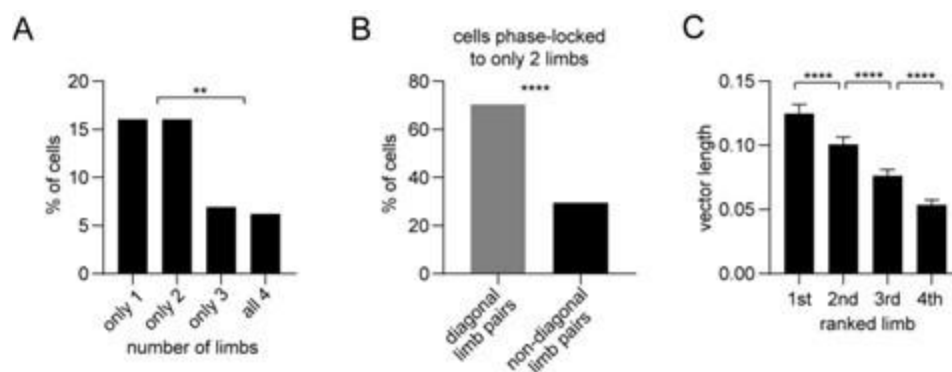
There are also some caveats to the interpretation of the analyses presented here, including how to compare encoding of gait variables when animals have markedly different behaviors (eg comparing sham and unilaterally 6-OHDA treated mice), or how to interpret the loss of gait modulation when single unit activity is overall very low.

(1) The authors use circular analysis to quantify the degree to which striatal neurons are phase-locked to individual limbs during gait. The result of this analysis is shown as the proportion of units phase-locked to each limb, vector length, and vector angle (Fig 2H-K; Fig 4E-F; Fig 6E-F). Given that gait is a cyclic oscillation of the trajectories of all four limbs, one could expect that if one unit is phase-locked to one limb, it will also be phase-locked to the other three limbs but at a different phase. Therefore, it is not clear in the manuscript how the authors determine to which limb each unit is locked, and how some units are locked to more than one limb (Fig 2H). More methodological/analytical detail would be especially helpful.

We thank the reviewer for raising this important issue, which was not sufficiently explored in our original manuscript. This relates to a major concern that “evidence supporting the conclusion that striatal neurons encode single-limb gait is incomplete.” We have now prepared a new figure supplement to address whether neurons are preferentially entrained to only one or multiple limbs (Figure 2 – figure supplement 1, panels A-C).

Author response image 1.

Panels A-C. Phase-locking to different limbs.



Panel A shows the percentage of striatal neurons (all neurons including untagged cells) with significant phase-locking to only 1, 2, 3, or all 4 limbs. The results indicate that most phaselocked cells are entrained to either only 1, or only 2 limbs, as opposed to 3 or all 4 limbs. We next looked more closely at the cells which were entrained to only 2 limbs: Panel B shows that a significant majority of those cells were coupled to diagonal limb pairs. This finding is insightful because diagonal limb pairs move at nearly the same phase during walking, thus some overlap in phase-locking to these limbs is to be expected. Finally, Panel C shows the mean vector length per neuron ranked from the highest to lowest value. The results reveal that the vector length is significantly biased toward the highest ranked limb. This bias would be absent if neurons were entrained to all 4 limbs with similar strength. Together, these results support the conclusion that striatal neuron spiking is preferentially coupled to single limbs as opposed to multiple limbs. However, we speculate that because of the inherently correlated motion across limbs, some neurons also display significant phase-locking to multiple limbs, particularly to diagonal pairs.

(2) In Figures 2 and 3, the authors describe the modulation of striatal neurons by gait, velocity, and movement transitions (start/end), with most of their examples showing firing rates compatible with rates typical of striatal interneurons, not MSNs. In order to have a complete picture of the relationship between striatal activity and gait, a cell type-specific analysis should be performed. This could be achieved by classifying units into putative MSN, FS interneurons, and TANs using a spike waveform-based unit classification, as has been done in other papers using striatal single-unit electrophysiology. An example of each cell type's modulation with gait, as well as summary data on the % modulation, would be especially helpful.

We appreciate the reviewer's suggestion to analyze our data after classifying units into different putative cell types (MSN, FSI, TAN). Indeed, we have frequently adopted this practice in our other publications (e.g., Bakhurin & Masmanidis 2016, 2017; Lee & Masmanidis 2019). However, this study already relies on a more rigorous method – optogenetic tagging – to identify D1 and D2 MSNs. We felt that adding a second, more subjective and therefore less rigorous identification method based on spike waveforms would add unnecessary confusion in how the results are presented and interpreted. For example, we were unsure how to address the situation where an opto-tagged D1 or D2 MSN may be classified as a putative FSI or TAN according to spike waveform criteria. For this reason, we decided not to perform an analysis by putative MSN, FSI, and TAN. Finally, we have made all our electrophysiological data available should someone want to perform this analysis themselves.

(3) By normalizing limb trajectories to the nose-tail axis, the analysis ignores whether the mouse is walking straight, or making left/right turns. Is the gait-modulation of striatal activity shaped by ipsi- and contralateral turning? This would be especially important to understand changes in the unilateral disease model, given the imbalance in turning of 6-OHDA mice.

This is an important question, which our data are unfortunately underpowered to address. Lesioned mice turn sharply for nearly the entire duration of walking, while healthy mice walk in a nearly straight line, with occasional brief turning bouts. Thus, we do not have sufficient stride numbers during healthy turning to enable a rigorous analysis of gait phase locking during left/right turns. This raises some questions about the interpretation of the higher D2 MSN vector length in dopamine lesioned mice – does the higher vector length relate to the impaired gait, or the higher incidence of turning in this PD model? We have acknowledged this issue in the Discussion section as a limitation of the unilateral 6OHDA model. And, in future work we hope to investigate turning effects in more detail using behavioral arenas which force animals to turn left or right at specific locations.

(4) It looks like the data presented in Figure 4 D-F comes from all opto-identified D1- and D2MSNs. How many of these are gait-modulated? This information is missing (line 110). Pooling all units may dilute differences specific to gait-modulated units, therefore a similar analysis only on gait-modulated units should be performed.

The reviewer is correct that the data presented in Figure 4 comes from all optogenetically tagged cells. We have now included a new panel, Figure 4H, which shows the proportion of D1 and D2 MSNs which encode limb phase, body speed, or start/stop. The reviewer suggested that a similar analysis only gait-modulated units should be performed. We prefer to stick to our current approach (of using all cells, regardless of whether they show significant gait modulation) because it is less biased. For example, even cells which do not pass our threshold for statistical significance may display weak but visible gait modulation.

(5) Since 6-OHDA lesions are on the right hemisphere, we would expect left limbs to be more affected than right limbs (although right limbs may also compensate). It is therefore surprising that RF and RR strides seem slightly shorter than LF and LR (Fig 5G), and no differences in other stride parameters (Fig 5H-J). Could the authors comment on that? It may be that this is due to rotational behavior. One interesting analysis would be to compare activity during similar movements in healthy and 6-OHDA mice, eg epochs in which mice are turning right (which should be present in both groups) or walking a few steps straight ahead (which are probably also present in both groups).

Unilateral 6OHDA lesions are associated with ipsiversive turning (in this case, toward the right). The reviewer noted that the stride length is shorter for the two right compared to the two left limbs (Figure 5G), which is consistent with a right turning bias. In line with this observation, the stride speed for the right limbs also seemed slower than for the left limbs (Figure 5I), though we agree this is a bit difficult to see in the plot due to the choice of y-axis range. We appreciate the reviewer's suggestion to analyze activity during similar movements in healthy and lesioned mice. As discussed in reply to their third comment above, our data did not contain sufficient bouts of straight walking in lesioned mice, or turning in healthy mice, to make such analysis possible. We have acknowledged this issue in the Discussion section as a limitation of the unilateral 6OHDA model. And, in future work we hope to investigate turning effects in more detail using behavioral arenas which force animals to turn left or right at specific locations.

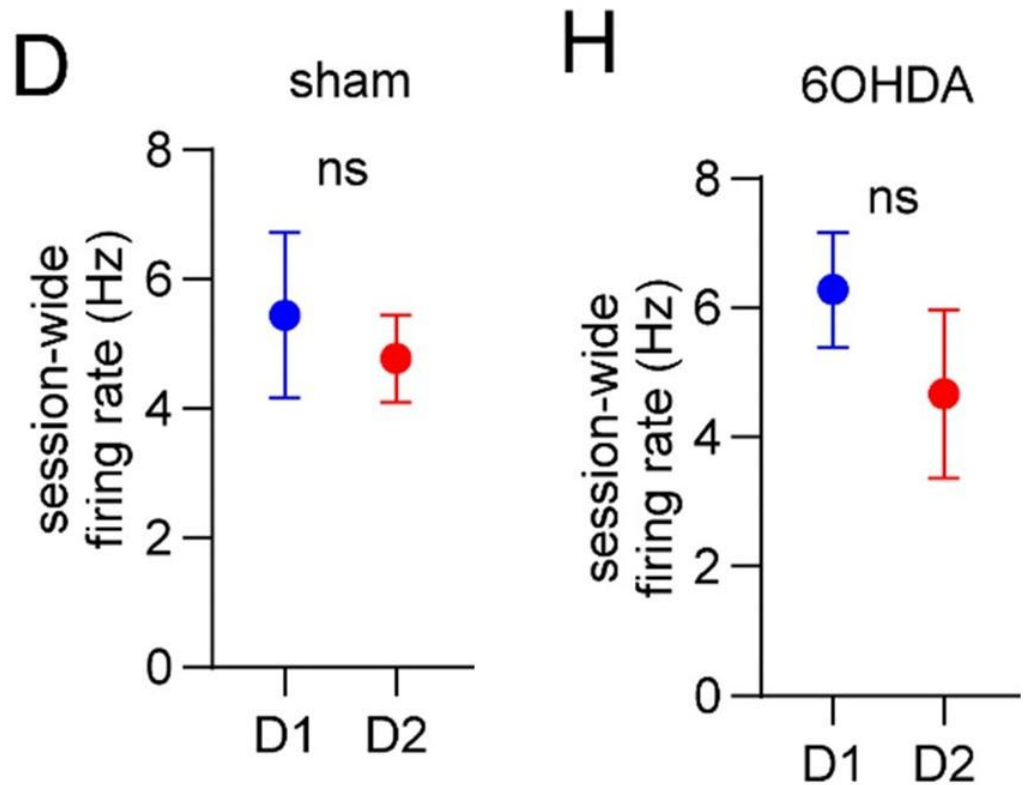
(6) Multiple publications have shown that firing rates of D1-MSN and D2-MSN are dramatically changed after dopamine neuron loss. Is it possible that changes observed in gait-modulation might be biased by changes in firing rates? For example, dMSNs have exceptionally low overall activity levels after dopamine depletion (eg Parker...Schnitzer, 2018; Ryan...Nelson, 2018; Maltese...Tritsch, 2021); this might reduce the ability to detect modulation in the firing of dMSNs as compared to iMSNs, which have similar or increased levels of activity in dopamine depleted mice. Does vector length correlate with firing rate? In addition, the normalization method used (dividing firing rate by minimum) may amplify very small changes in absolute rates, given that the firing rates for MSN are very low. The authors could show absolute values or Z-score firing rates (Figure 6 A, D).

The reviewer asked a number of important questions here. First, is it possible that changes in gait modulation are biased by changes in firing rates? We have included a new analysis comparing the average session-wide firing rate of D1 and D2 MSNs (Figure 6D & 6H). This showed that firing rates were statistically similar between D1 and D2 MSNs for both sham and dopamine lesioned mice. Thus, it seems unlikely that the imbalance in vector length is purely due to changes in firing rate. The reviewer referenced some literature (e.g. Parker & Schnitzer; Ryan & Nelson; Maltese & Tritsch) which does appear to show significant changes

in the relative firing levels of D1/D2 MSNs after dopamine lesions. While we can only speculate about the reason for the discrepancy (e.g., differences in measurement method, behavioral task, or analysis method), we note that not all prior literature has reported such changes (e.g., Ketzef & Silberberg 2017).

Author response image 2.

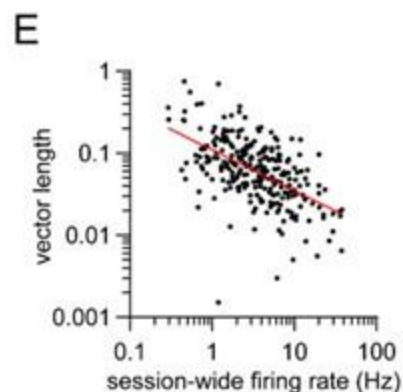
Panels D & H. No difference in firing between D1 and D2 MSNs.



Second, does vector length correlate with firing rate? Interestingly, we found that indeed it does. We now show that vector length is negatively correlated with firing rate (Figure 2 – figure supplement 1E), implying that cells with higher overall firing rates tend to have weaker phaselocking to the gait cycle. Though not shown in the manuscript, we found a similar negative correlation for D1 and D2 MSNs in both healthy and dopamine lesioned mice.

Author response image 3,

Panel E. Vector length is negatively correlated to firing rate.



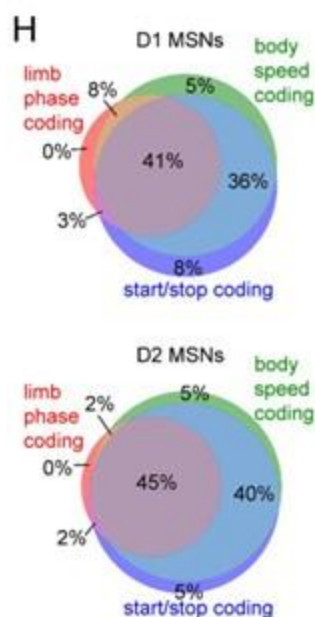
Third, the reviewer asked about our normalization method in Figure 6A etc, in which we divide by the minimum rate. We would like to clarify that this normalization method was only used for visualizing our data, but not for calculating the vector length. Therefore, we chose to leave the plots as they are.

(7) The analysis shown in Fig 3C should also be done for opto-identified D1- and D2-MSNs (and for waveform-based classified units as noted above).

We have now performed the same analysis for optogenetically tagged D1 and D2 MSNs from healthy mice (Figure 4H). As with our original analysis, both populations showed a similar proportion of neurons which encoded limb phase, start of movement, body speed, and the combination of these. We did not perform this analysis for waveform-based classified units as per our reason outlined in reply to the reviewer's second comment above.

Author response image 4.

Panel H. Venn diagrams showing the percentage of D1 and D2 MSNs with significant responses to limb phase of at least one limb, body speed, and start and/or stop of motion.



(8) Discussion: the origin of the gait-modulation as well as the possible mechanisms driving the alterations observed in 6-OHDA mice should be discussed in more detail.

Our Discussion section includes the following paragraph speculating on the origin of gait modulation: “Movement-related neural activity is widespread in many brain areas, and it is plausible that the striatum receives both motor and sensory signals involved in gait generation. For example, the primary motor cortex, which projects to dorsal striatum, has been shown to exhibit rhythmic spiking activity consistent with gait phase coding (Armstrong & Drew 1984), suggesting a shared mechanism underlying the production of this code.” We appreciate the request to also discuss the possible mechanisms driving the alterations in 6OHDA mice. But this is a very complex topic which our study is not aimed at addressing. The range of possible mechanisms uncovered in the literature is vast – from synaptic changes in striatal microcircuits, to altered intrinsic excitability of D1/D2 MSNs, and network-level alterations. Therefore, we preferred to keep the discussion focused on gait and movement coding.

Reviewer #2 (Public Review):

Summary:

Yang et al. recorded the activity of D1- and D2-MSNs in the dorsal striatum and analyzed their firing activity in relation to single-limb gait in normal and 6-OHDA lesioned mice. Although some of the observations of striatal encoding are interesting, the novelty and implications of this firing activity in relation to gait behavior remain unclear. More specifically, the authors made two major claims. First, the striatal D1- and D2-MSNs were phase-locked to the walking gait cycles of individual limbs. Second, dopamine lesions led to enhanced phase-locking between D2-MSN activity and walking gait cycles. The second claim was supported by the increase of vector length in D2-MSNs after unilateral 6-OHDA administration to the medial forebrain bundle. However, for the first claim, the authors failed to convincingly demonstrate that striatal MSNs were more phase-locked to gait with single-limb and step resolution than to the global gait cycles.

We thank the reviewer for their feedback and for their comment that “the authors failed to convincingly demonstrate that striatal MSNs were more phase-locked to gait with single-limb and step resolution than to the global gait cycles.” We now present new analysis demonstrating that neurons are more phase-locked to single-limb gait rather than multiple limbs (Figure 2 – figure supplement 1, panels A-C). These results are discussed in detail in response to Reviewer #1’s first comment. For conciseness we will not repeat the same response here but instead refer the reviewer to Reviewer #1, comment #1.

Strengths:

It is a technically advanced study.

Weaknesses:

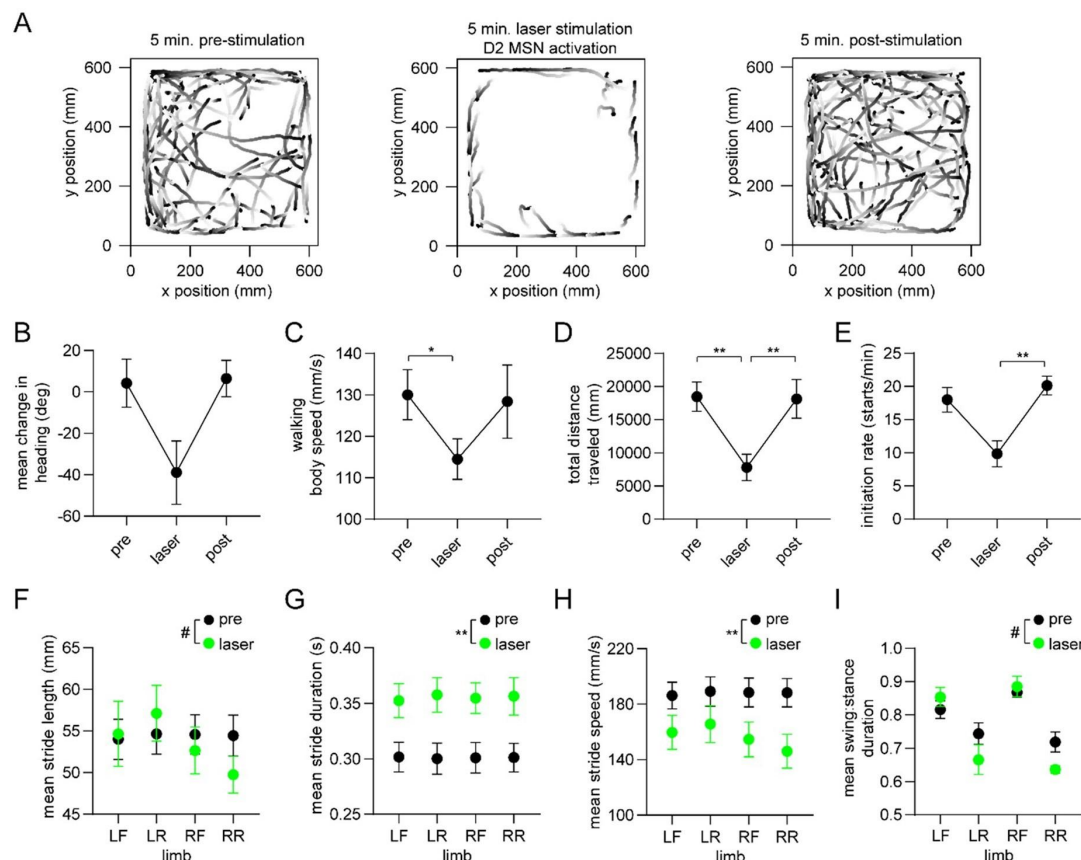
(1) The authors focused on striatal encoding of gait information in current studies. However, it remains unclear whether the part of the striatum for which the authors performed neuronal recording is really responsible for or contributing to gait control. A lesion or manipulation experiment disrupting the part of the striatum recorded seems a necessary step to test or establish its relationship to gait control.

We agree that our study – like many others which employ recordings – is largely correlative, and that a direct causal relationship was lacking. We have therefore decided to present some

data which, despite some caveats, shows that the striatum is in principle capable of altering gait performance (Figure 6 – figure supplement 2).

Author response image 5.

Optogenetic activation of D2 MSNs alters whole-body movement and single-limb gait.



These new results are from healthy mice (n=4) receiving optogenetic stimulation of D2 MSNs over a 5 minute period. Panels A-E show changes in a variety of whole-body measures of motion, mostly replicating the results of Kravitz & Kreitzer 2010. Panels F-I show changes (statistically significant or trending) in a variety of gait parameters, with the greatest effects found on the single-limb stride duration and stride speed. Interestingly, Kravitz & Kreitzer 2010 actually examined effects of this stimulation on gait; quoting from their paper: “we examined gait parameters in D1-ChR2 and D2-ChR2 mice in response to illumination, using a treadmill equipped with a high-speed camera. We quantified multiple gait parameters with the laser on and off, and found no significant differences in the average or variance of stride length, stance width, stride frequency, stance duration, swing duration, paw angle and paw area on belt for either line....This indicates that activation of direct and indirect pathways in the dorsomedial striatum regulates the pattern of motor activity, without changing the coordination of ambulation itself.” We wonder therefore if the reviewer’s comment about causality may have stemmed from the negative result in Kravitz & Kreitzer 2010. In any event, we now present results which firmly show a link between striatal D2 MSNs and gait. To be clear, we are not claiming that Kravitz & Kreitzer’s study was fundamentally flawed, but that perhaps their ability to resolve gait changes using a commercial treadmill system, or their choice of dorsomedial as opposed to more lateral regions of the striatum may have contributed to the negative result.

It is also important to acknowledge a limitation of our optogenetic stimulation experiment. Our optical stimulation was not phase-locked to the gait cycle; thus, technically, we did not address whether the phase code per se is involved in producing gait. We mention this caveat in the manuscript. Despite this, we believe the new data address the reviewer's concern about lack of causality.

(2) The authors attributed one of the major novelties to phase-locking of striatal neural activities with single-limb gait cycles. The claim was not clearly supported, as the authors did not demonstrate that phase-locking to single-limb gaits was more significant than phase-locking to global walking gait cycles. In rhythmic walking, the LR and RF limbs were roughly anti-phase with the LF and RR limbs (Fig. 1D, E). In line with this relationship, striatal neurons were mainly in-phase with LR and RF limbs and anti-phase with LF and RR limbs (Fig. 2J, K). One could instead interpret this as the striatal neurons spanned all the phases of the global walking gait cycles (Fig. 3D). To demonstrate phase-locking with individual limb movements, the authors need to show that neural activities were better correlated with a specific limb than to the global gait cycles.

We sincerely appreciate the reviewer's comment. As described above we now present new analysis demonstrating that neurons are more phase-locked to single-limb gait rather than multiple limbs (Figure 2 – figure supplement 1, panels A-C). These results are discussed in detail in response to Reviewer #1's first comment. For conciseness we will not repeat the same response here but instead refer the reviewer to Reviewer #1, comment #1.

(3) The observation of the enhancement of coupling between D2 MSN firing and the gait cycles was interesting, but the physiological interpretation was not clear (as the authors also noted in the Discussion), which hampers the significance of the observation.

In the Discussion we comment on the potential behavioral significance of our findings, keeping in mind the reviewer's earlier concern about the correlative nature of recordings. For example, we speculate that the increase in D2 MSN limb phase-locking strength contributes to bradykinetic symptoms, specifically the production and maintenance of a normal gait cycle and rhythm. We respectfully disagree with the reviewer about the limited significance of the observations, as this is the first study to describe striatal gait phase coding in detail, noting that gait impairments are a major motor symptom in PD. We believe that progress in better understanding and eventually treating PD will be made through a combination of correlative observations (i.e., neural recordings) and causal manipulations. There are both advantages and disadvantages to correlative as well as causal experiments.

(4) Due to the lack of causality experiments as mentioned in the first comment above, the observations of coupling between striatal neuronal activity and gait control might well result from a third brain region/factor serving as the common source to both, whether in normal or dopamine lesioned brain. If this is the case, the significance and implications of current findings will be greatly limited.

As mentioned above we have included new data to address this concern (Figure 6 – figure supplement 2). Please refer to Reviewer #2, comment #4 for a detailed discussion of these results and their caveats.

Reviewer #3 (Public Review):

In this study, Yang et al. address a fundamental question of the role of dorsal striatum in neural coding of gait. The authors study the respective roles of D1 and D2 MSNs by linking their balanced activity to detailed gait parameters. In addition, they put in parallel the striatal activity related to whole-body measures such as initiation/cessation

of movement or body speed. They are using an elegant combination of high-resolution single-limb motion tracking, identification of bouts of movements, and electrophysiological recordings of striatal neurons to correlate those different parameters. Subpopulations of striatal output neurons (D1 and D2 expressing neurons) are identified in neural recordings with optogenetic tagging. Those complementary approaches show that a subset of striatal neurons have phase-locked activity to individual limbs. In addition, more than a third of MSNs appear to encode all three aspects of motor behavior addressed here, initiation/cessation of movement, body speed, and gait. This activity is balanced between D1 and D2 neurons, with a higher activity of D1 neurons only for movement initiation. Finally, alterations of gait, and the associated striatal activity, are studied in a mouse model of Parkinson's Disease, using 6-OHDA lesions in the medial forebrain bundle (MFB). In the 6OHDA mice, there is an imbalance toward D2 activity.

Strengths:

There is a long-standing debate on the respective role of D1 and D2 MSNs on the control of movement. This study goes beyond prior work by providing detailed quantification of individual limb kinematics, in parallel with whole-body motion, and showing a high proportion of MSNs to be phase-locked to precise gait cycle and also encoding whole-body motion. The temporal resolution used here highlights the preferential activity of D1 MSN at the movement starts, whereas previous studies described a more balanced involvement. Finally, they reveal neural mechanisms of dopamine depletion-induced gait alterations, with a preponderant phase-locked activity of D2 neurons. The results are convincing, and the methodology supports the conclusions presented here.

Weaknesses:

Some more detailed explanations would improve the clarity of the results in the corresponding section. Analysis of the 6OHDA experiments could be expanded to extract more relevant information.

Recommendations for the authors:

Reviewer #1 (Recommendations For The Authors):

(1) Panels I and J from Figure 6 are referred to in the text (line 158) but they don't exist.

Thank you, we have corrected this in the text.

(2) For the classification of striatal units into putative MSN, FS interneurons, and TANs, see Gage et al. DOI: 10.1016/j.neuron.2010.06.034 or Thorn et al. DOI: 10.1523/JNEUROSCI.178213.2014.

As explained in the Public Reviews, Reviewer #1 comment #2 we opted not to perform an analysis by putative MSN, FSI, and TAN. We have performed analysis of different putative cell types in several of our other publications (e.g., Bakhurin & Masmanidis 2016, 2017; Lee & Masmanidis 2019). However, this study already relies on a more rigorous method – optogenetic tagging – to identify D1 and D2 MSNs. We felt that adding a second, more subjective and therefore less rigorous identification method based on spike waveforms would add unnecessary confusion in how the results are presented and interpreted. For example, we were unsure how to address the situation where an opto-tagged D1 or D2 MSN may be classified as a putative FSI or TAN according to spike waveform criteria. For this reason, we decided not to perform an analysis by putative MSN, FSI, and TAN. Finally, we have made all our electrophysiological data available should someone want to perform this analysis themselves.

(3) The discussion section could be improved by elaborating on the origin and function of these gait signals in the striatum, as well as the mechanisms underlying changes in the 6-OHDA model. In addition, it would be important to discuss the limitations of this model, since unilateral 6-OHDA lesions may not accurately recapitulate parkinsonian gait deficits, as it results in a very asymmetric gait.

Our Discussion section includes a paragraph speculating on the origin of gait modulation in the striatum, and another paragraph addressing the limitation that unilateral 6OHDA lesions induce gait asymmetry. We appreciate the request to also discuss the possible mechanisms driving the alterations in 6OHDA mice. But this is a very complex topic which our study is not aimed at addressing. The range of possible mechanisms uncovered in the literature is vast – from synaptic changes in striatal microcircuits, to altered intrinsic excitability of D1/D2 MSNs, and network-level alterations. Therefore, we preferred to keep the discussion focused on gait and movement coding.

Reviewer #2 (Recommendations For The Authors):

(1) The authors denoted the limb movement sequences as LR-LF-RR-RF, with limbs on the same left/right side moving first. However, considering multiple gait cycles, the sequence could also be described as RF-LR-LF-RR, with movements of the diagonal limbs temporally closer to each other, which was more intuitive from the visual inspection of Fig. 1D. The LR-LF-RR-RF denotation would make more sense if the authors could demonstrate that a walking bout almost always started from LR, as seen in the two examples in Fig. 1D.

We designated the sequence as LR-LF-RR-RF to illustrate the lateral sequence pattern. But the reviewer is correct that a shifted version of this sequence, such as RF-LR-LF-RR, is also valid. We are not making any claim that the LR limb is always the first to move in a walking bout, but rather, that limbs on the same side of the body move one after the other, followed by the limbs on the opposite side. We have edited the text to hopefully clarify this point: “Mice walked with a lateral sequence gait pattern (e.g., LR-LF-RR-RF), with the limbs on the same side of the body moving one after the other, followed by movement of limbs on the opposite side (Figure 1E).”

(2) The study identified a biased D1-MSN activation at movement initiation, which was not reported in previous studies that relied on measuring calcium dynamics. The authors attributed the difference to the temporal resolution of electrophysiological versus optic methods. The authors would probably notice that in some previous studies that relied also on optic-tagging and electrophysiological recordings, start/stop activity was not found to be different between direct and indirect pathway MSNs. The authors should discuss these studies and offer some possible explanations.

This is an oversight on our part, and we thank the reviewer for noting this. We are aware of one such study (Jin & Costa 2014); we apologize if other studies were missed. The Discussion has been updated as follows to discuss this paper: “We also note that another study employing optogenetic tagging did not find significant D1/D2 MSN differences in start/stop activity (Jin & Costa 2014). However, the movement being measured was an instrumental action (reward-guided lever pressing), as opposed to self-initiated motion examined in our work. This suggests either that imbalances between D1 and D2 MSN start activity may be more pronounced under specific behavioral conditions, or that results vary depending on how movement initiation and cessation events are identified.”

(3) The authors could add some denotations to the peak firing rates in Fig. 3D to aid visualization, so that readers could get a sense of the distribution of neurons preferring

each phase of the movements.

We appreciate this suggestion. We tried adding various colored lines to denote the peak firing rates, but ultimately, we felt the lines were not helpful and potential deleterious for some readers. We thus decided not to add any lines to the plot.

(4) Although the relative strength of D1/D2-MSN coding of body speed and movement cessation was found after dopamine lesion, it seemed that D1-MSNs cessation coding, as well as D1- and D2-MSN speed coding, were all altered after dopamine lesion (Fig. S3). The authors could mention these to avoid misunderstandings.

We thank the reviewer for their observation. In the Results, we now mention that “while speed coding remained balanced between D1 and D2 MSNs, there was a substantial reduction in the speed coding score of both cell types after dopamine lesions.” The stop modulation index did not change appreciably.

Reviewer #3 (Recommendations For The Authors):

(1) A suggestion would be to put more emphasis in the title on the first parts of the study, i.e. detailed correlation between striatal activity and quantified motion, and not only focus on the dopamine depletion model.

We considered other titles, but felt that our current choice is appropriate given that the study's climax is with the dopamine lesion results in Figures 5 & 6.

(2) The calculation and the significance of the vector length should be more detailed in the results as it is used all along as a measure of "the strength of neural entrainment to the gait cycle".

We have added the following statement in the Results section to clarify the significance of vector length: “The vector length is a unitless parameter which can theoretically vary from 0 to 1, with 0 representing a neuron whose spikes occur at random limb phases, and 1 representing a neuron which always spikes at the same phase. Thus, higher vector length indicates a stronger entrainment of spiking activity to a specific limb phase.” For details on how vector length is calculated we refer readers to our Methods, specifically the section entitled “Gait phase coding analysis.”

(3) There is no difference in the ipsi- or contralateral limbs while recordings are made only in the right hemisphere. Given that MSNs receive inputs from IT and PT neurons from the motor cortex, would it not be expected to have differences in the phase-locked activity to right versus left limbs? This is a question also with the dopamine depletion model which is performed with unilateral 6OHDA injections.

This is something we also wondered and were somewhat surprised by the lack of a contralateral bias in the phase locking vector length, as shown in Figure 2 – figure supplement 1D. We have two hypotheses as to why there is no ipsi/contra-lateral bias. First, it is possible that striatal neurons receive similar levels of synaptic input signaling ipsi/contralateral limb movements. Second, the strongly correlated motion of diagonally opposed limbs may give the appearance that neurons that are phase-locked to one limb (e.g., LF) are also locked to the diagonally opposite limb (i.e., RR). We see evidence of this diagonal limb coupling in Figure 2 – figure supplement 1B.

(4) Among the 45% of striatal neurons that display significant phase-locking to at least one limb, it would be interesting to describe the % of neurons being phase-locked to

several limbs and whether they are specific subtypes. Are there animals with more phase-locked cells in several limbs?

This is indeed a very interesting and important point which relates to the major concern that “evidence supporting the conclusion that striatal neurons encode single-limb gait is incomplete.” As described above we now present new analysis demonstrating that neurons are more phaselocked to single-limb gait rather than multiple limbs (Figure 2 – figure supplement 1, panels AC). These results are discussed in detail in response to Reviewer #1’s first comment. For conciseness we will not repeat the same response here but instead refer the reviewer to Reviewer #1, comment #1. With regard to whether there are specific subtypes, we performed the same analysis on optogenetically identified D1/D2 MSNs and found similar trends, but did not show these results in the manuscript to avoid redundancy.

(5) The Venn diagram in Fig. 3C shows ~40% of striatal cells encoding body speed, single-limb and start/stop information. Nevertheless, this percentage is limited by the number of single-limb phase-locked cells as almost all have a firing rate related to body speed and start/stop signals. This could be discussed.

This is a very interesting observation. Basically, the reviewer is noting that almost all the phaselocked cells also encode start/stop and/or speed. We have now updated the Discussion to specifically discuss this observation: “We found a different percentage of striatal neurons which encoded limb phase, movement initiation or cessation, and speed (Figure 3). Among these three categories, limb phase coding cells represented the smallest population with ~45% of neurons, as opposed to ~90% for start/stop or speed. In addition, nearly all phase coding cells were also significantly responsive to start/stop or speed, whereas a sizable proportion of start/stop or speed coding cells were not entrained to limb phase. It is unclear, however, whether these population size differences reflect a proportionally smaller role for the striatum in regulating single-limb gait as opposed to whole-body movement initiation, cessation or speed.”

(6) D1/D2 analysis:

For optogenetic identification of D1 and D2 neurons, 39 D1 neurons and 40 D2 neurons were extracted from the total of 274 recorded neurons while 222 neurons were optogenetically tagged according to the mat and meth. Were there any technical difficulties that made it difficult to identify more neurons?

The low yield of optogenetic tagging is quite common in the literature due to the rigorous criteria which must be satisfied in order to qualify as a tagged neuron (e.g., Kvitsiani & Kepecs 2013). The number 222 neurons quoted in the methods reflects the entirety of optogenetically tagged neurons in this study. Our study contained 33 mice, thus the average number of tagged units per animal was $222/33 \sim 6.7$ units/animal. This is actually comparable to or slightly better than the yield reported in some other striatal literature (see for example, Figure 1 of Ryan & Nelson 2018).

It is mentioned that "a subset" of these were phase-locked to a single limb. It would be interesting to specify the exact percentage of those neurons for D1 and D2 populations.

Phase-locking of D2 neurons seems less sharp than D1 neurons, with a lower firing rate (Fig. 4D), please comment. Also difference in vector length for LR while none for other limbs, why? There is a balanced activity of D1 and D2 MSNs during walking (speed) and single-limb movements, but more D1 MSNs active at movement initiation. Is it also true for stop signals? Are they separated based on the speed threshold of 20 mm/s?

As mentioned above, our new analysis specifically examines the percentage of all neurons which are phase locked to a single limb (Figure 2 – figure supplement 1, panels A-C). We have performed the same analysis on optogenetically tagged D1/D2 MSNs and found similar trends, but not show these results in the manuscript to avoid redundancy. With regard to whether phase-locking of D2 is less sharp than D1 MSNs, the “sharpness” of phase-locking is characterized by the mean vector length. And we show that on average, the vector length is statistically the same for D1 and D2 MSNs in healthy mice (Figure 4F). The reviewer noted that the D2 vector length in Figure 4F appears visibly higher for LR while not for other limbs, however, this difference is not statistically significant. With regard to whether more D1 MSNs are active during movement cessation, we show that both sham and dopamine lesioned mice have similar levels of D1/D2 MSN activity during stop (Figure 6 – figure supplement 1, panels A & B). Details of how start, stop, and speed are calculated are provided in the Methods.

The relationship between firing and body speed (Fig. 4H) displays differences between D1 and D2. If a speed inferior to 20 mm/s, corresponds to "start or stop signal" as mentioned in the mat and meth, then early difference would correspond to start, but still there is a difference between 20 and 100 mm/s and after 150 mm/s. These results should be commented on.

The reviewer is correct that in the plot of firing rate vs body speed (Figure 4J), there visibly appears to be a difference between D1 and D2 MSNs at low speeds. However, according to our pre-determined measure of speed coding which relies on the correlation coefficient between firing rate and speed, D1 and D2 MSNs have similar speed coding indices. Since there is a precedent for using the correlation coefficient to quantify speed coding (Fobbs & Kravitz 2020; Kropff & Moser 2015), we prefer to stick with this measure despite some caveats. Furthermore, the apparent difference between D1 and D2 MSNs in Figure 4J is not seen in either sham or dopamine lesioned mice (Figure 6 – figure supplement 1, panels D & E). Taken together, we do not believe the apparent speed coding difference in Figure 4J rises to the level of a consistent result.

(7) The timing of normalized firing rate in relation to start/stop signals might be also quite interesting to comment on. D1 neurons have stronger activation for start signals and it seems that it is also earlier, with D2 activated after the onset of the movement (Fig. 4G).

We appreciate the observation that D1 neurons appear to fire a little earlier than D2 neurons in Figure 4I. However, this did not rise to the level of a statistically significant result by our attempted quantitative analysis (not shown). Furthermore, the earlier timing of D1 is not apparent in sham lesioned animals in Figure 6I, thus overall we cannot make any confident statements about earlier timing of D1 start signals.

In dopamine lesion experiments, in sham mice, it seems that both D1 and D2 have higher activity after the onset of the movement and that the peak of D2 activity is earlier (Fig. 6G). In 6OHDA mice, both peaks are after the onset of the movement although they are much less clearly defined.

Both peaks become less sharp after 6OHDA lesions, but in terms of amplitude the main effect is a reduction in the D1 start signal. This is reflected in the reduced D1 start modulation index whereas the D2 index remains relatively constant.

(8) 6OHDA model displays much fewer walking bouts with lower speed and initiation rate. It would be important to include in the figure a similar representation to Fig.1 with distributions of stride frequency, duration, and length to illustrate the difference between

control and 6OHDA mice. On average, how many walking bouts were analyzed in control and 6OHDA animals?

We have added new data similar to Figure 1 with distributions of stride frequency, duration, and length to illustrate the difference between sham and 6OHDA mice (Figure 5 – figure supplement 1, panels B & C). We also added the following information on the number of walking bouts: “The mean number of walking bouts per session was reduced from 124 ± 42 in sham to 47 ± 19 in dopamine lesioned mice (mean $\pm$ SD).”

The initiation rate is particularly low in 6OHDA animals, 3-4 per minute, did the authors make longer behavioral recordings to extract enough initiation/stop signals for neural correlation analysis?

All of our recordings were of the same duration (30 minutes). This duration was pre-determined at the beginning of the study to ensure consistency.

The stride length seems smaller on the right limbs in 6OHDA mice and vector length in D2 neurons as well, while there is no change in D1 neurons. Is it a significant effect? If yes, it would be important to comment on this.

The ANOVA test in those figures was not designed to perform post-hoc multiple comparisons between different limbs. However, if one changes the ANOVA design then the effect for stride length is significant. This is probably related to the ipsiversive turning bias in the unilateral 6OHDA lesion model. Though we have not changed the ANOVA design, in the Discussion we do comment on the shorter stride length on the right limbs in 6OHDA mice in Figure 5G. There is no significant difference in D2 vector length between different limbs.