

Dopamine lesions alter the striatal encoding of single-limb gait

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Reviewed Preprint

Published from the original preprint after peer review and assessment by eLife.

About eLife's process

Reviewed preprint posted

December 12, 2023 (this version)

Posted to bioRxiv

October 9, 2023

Sent for peer review

September 27, 2023

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

 Copyright information

Abstract

The striatum serves 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

In this manuscript, the authors 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. This **important** 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 pathways, however, the reviewers also point out that the evidence supporting the conclusion that striatal neurons encode single-limb gait is **incomplete**.

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^{1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100}. 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^{2,4}. 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**).

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**)²⁶, 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**). The phase locking strength did not show a spatial gradient along the depth of the dorsal striatum, or a bias for either ipsi- or contra-lateral limbs (**Figure S1**). The mean phase angle varied by approximately 180° between the front and rear limb on each side of the body (**Figure 2J,K**). This phase offset appears to reflect the anticorrelated motion of these limbs during walking (see **Figure 1F**).

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 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).

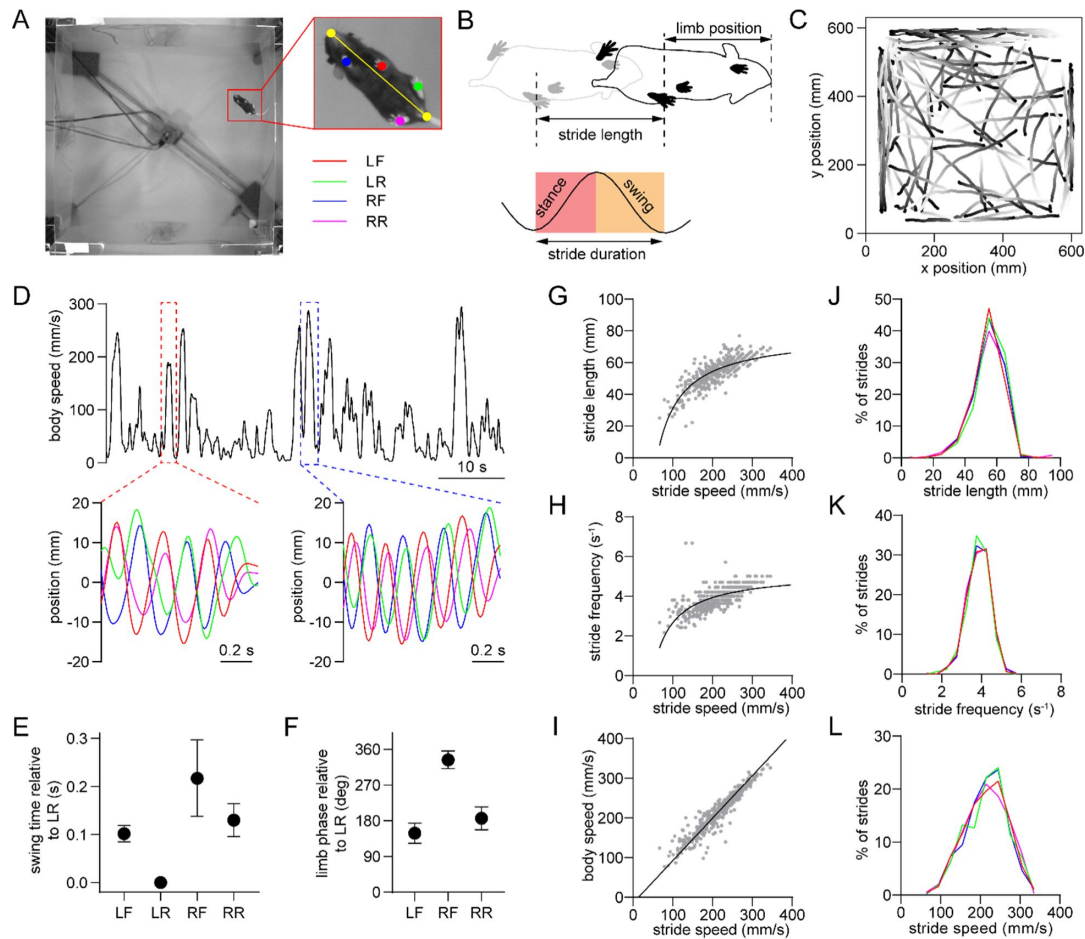


Figure 1.

Single-limb gait measurements in freely behaving mice.

(A) Video frame showing the bottom-up view of a mouse walking in the 60 cm × 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◊LF◊RR◊RF). Data represent mean ± 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 ± 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.

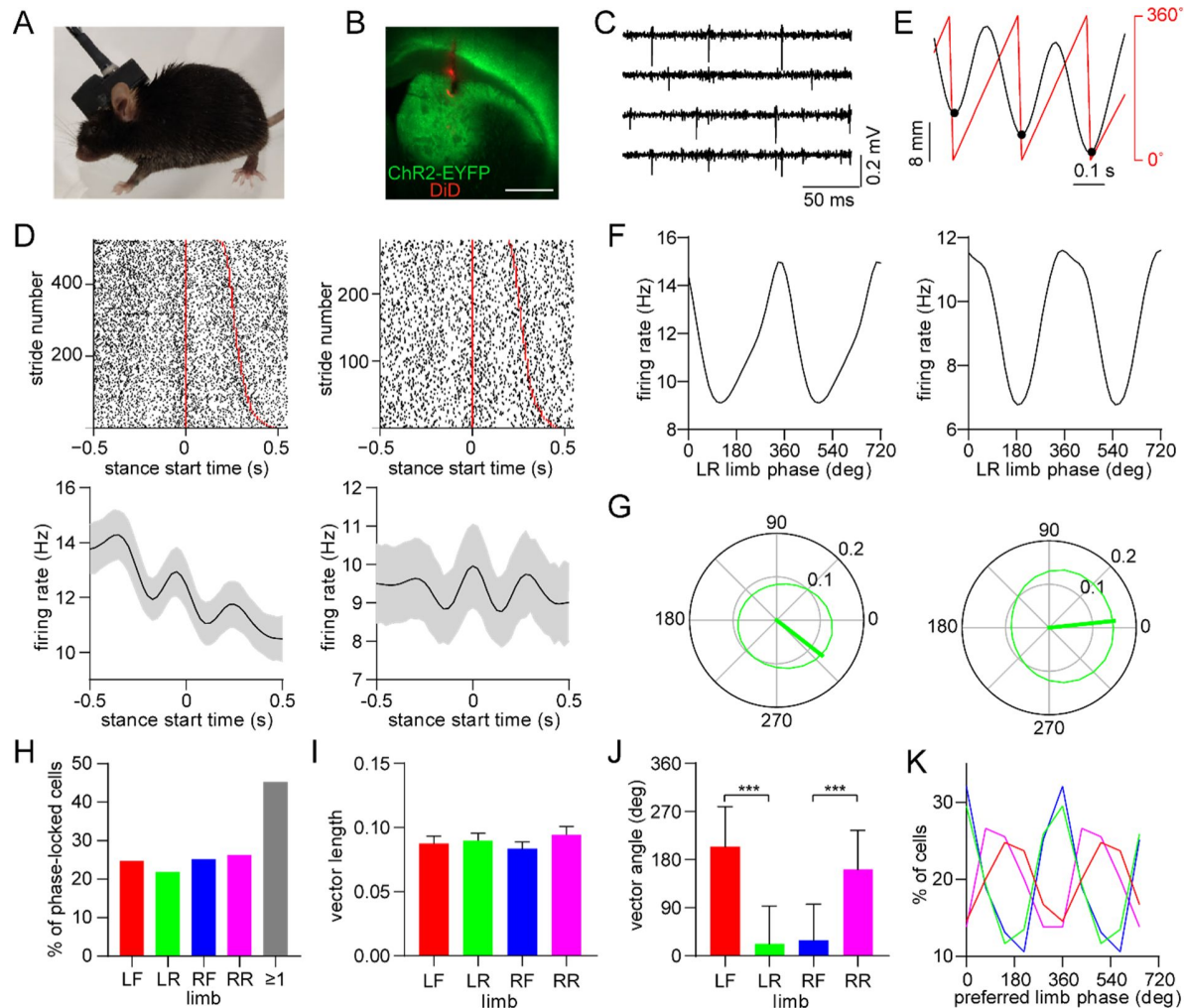


Figure 2.

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 $^\circ$) 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 significant different between front and rear limb (angular permutation test adjusted for 6 multiple comparisons, LF-LR: $p < 0.001$; LF-RF: $p < 0.001$; RF-RR: $p < 0.001$; LR-RR: $p < 0.05$, $***p < 0.001$). Data represent mean $\pm$ SD.

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

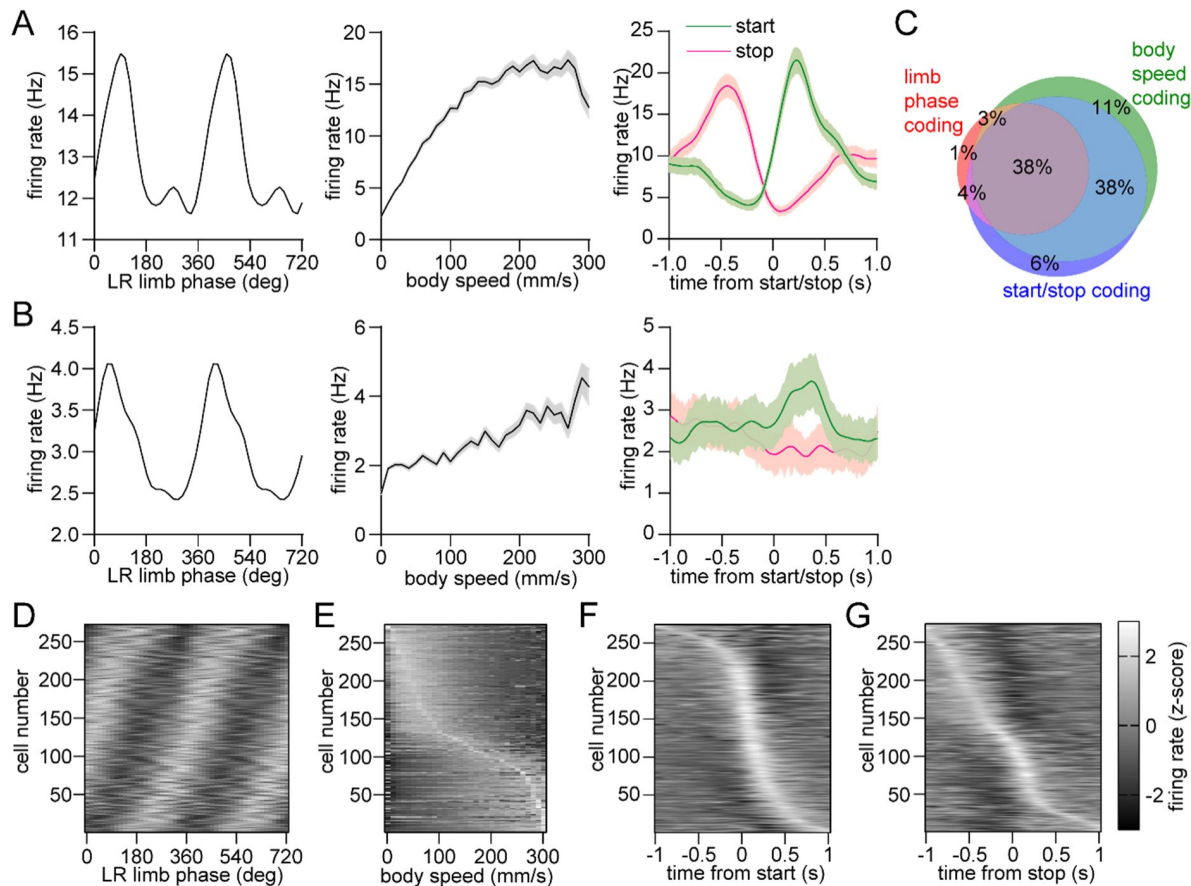


Figure 3.

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.

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, a subset of which displayed rhythmic firing patterns in relation to single-limb phase (**Figure 4D**). Across all identified cells there was no significant difference between D1 and D2 MSNs in either the mean vector length or angle (**Figure 4E,F**). These results suggest that there is normally a balanced level of D1 and D2 MSN activity coupled to the gait cycle. 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 4G**). Neural responses to movement cessation and speed were similar between D1 and D2 MSNs (**Figure 4H**). 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 S2A**). 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**)^{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 5H**). Additional changes in gait included lower coordination between different pairs of limbs, and higher variability in the length and speed of individual strides (**Figure S2B-F**). 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.

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**). However, this originally balanced activity was disrupted in 6OHDA-lesioned animals, which exhibited a significantly higher average spike-limb phase vector length among the D2 MSN population (**Figure 6D,E** and **S3A**). The average vector angle remained

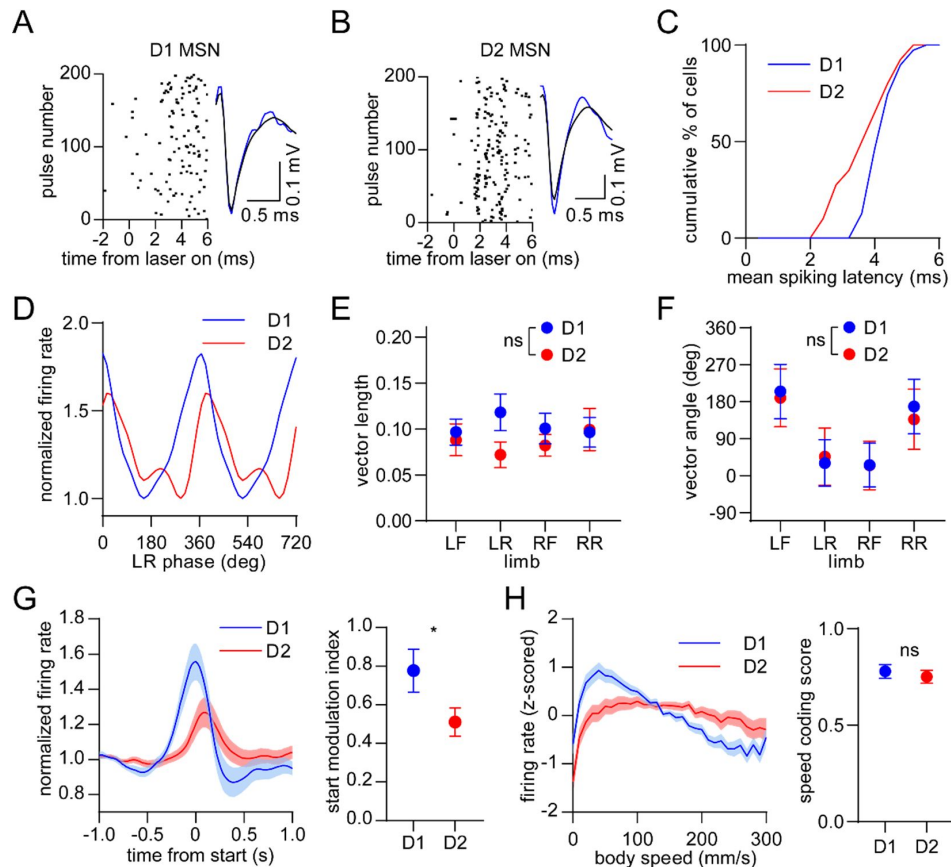


Figure 4.

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) 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°) for visual clarity.

(E) No significant difference in spike-limb phase vector length between D1 and D2 MSNs ($n = 39$ D1 and 40 D2 MSNs, two-way ANOVA, $F_{1,308} = 2.1$, $p = 0.14$).

(F) No significant difference in mean vector angle between D1 and D2 MSNs (angular permutation test corrected for 4 multiple comparisons, $p > 0.99$).

(G) 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, $p = 0.048$).

(H) 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.

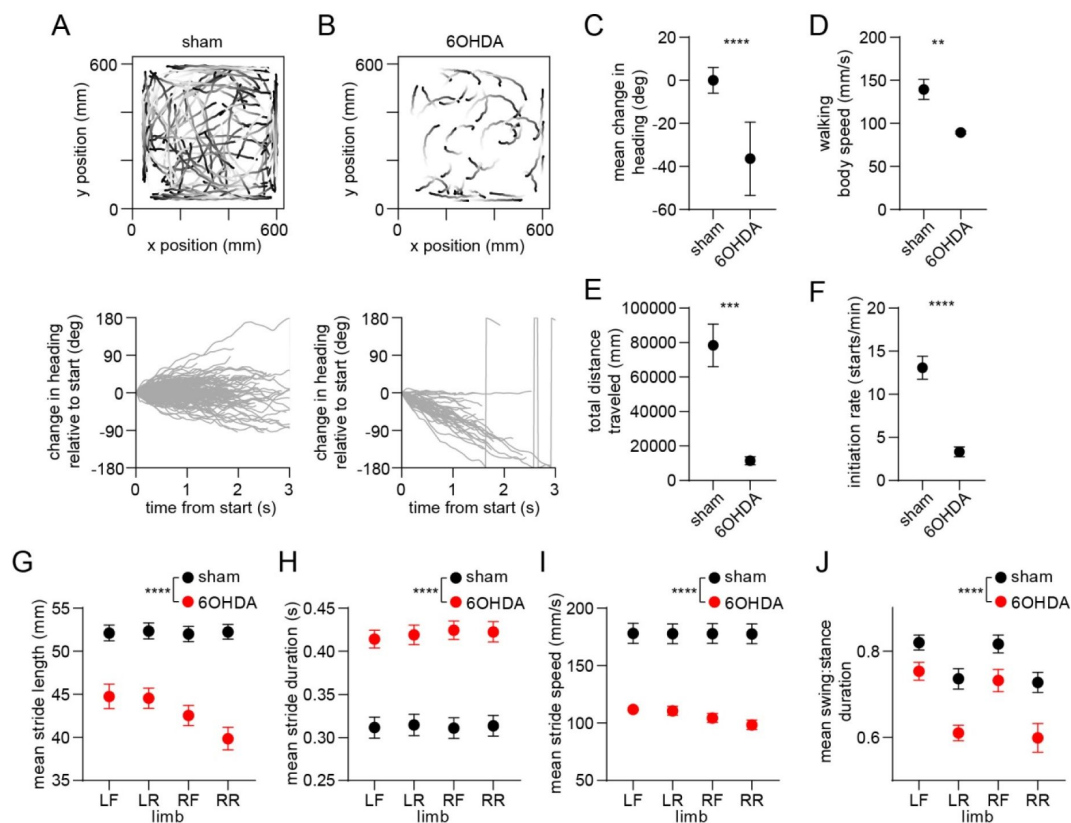


Figure 5.

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/ipsiversive 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.

similar between the two MSN subtypes in the 6OHDA group (**Figure 6F**). Thus, dopamine lesions strengthen the coupling of D2 MSNs to the gait cycle, whereas the coupling of D1 MSNs is unaltered. 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 6G,H**). In contrast, dopamine lesions attenuated this bias, with both MSN subtypes now showing similar levels of start-related activity (**Figure 6I,J**). 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 S3B-E**). 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.

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 (~40%) 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, 35–37}. 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 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. An open question is whether the observed gait phase coding phenomenon reflects a direct motor command, a motor efference copy, or a sensory feedback signal. However, all of these types of signals may serve important, though distinct roles in regulating locomotion³⁸. Furthermore, the dorsal striatum receives synaptic input from motor and sensory regions of cortex and thalamus³⁹, and provides feedback onto those areas via cortico-basal ganglia-thalamocortical loops⁴⁰. At least one of these areas – the primary motor cortex – has been shown to exhibit rhythmic spiking activity consistent with gait phase coding²⁷, suggesting a shared mechanism underlying the production of this code.

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, 41}, as well as a variety of habitual behaviors and action sequences^{14, 17–19, 42}. 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, 43}. However, the most parsimonious interpretation of our and others' data is that both discrete and continuous actions are represented⁴⁴. Behavioral studies provide further evidence that striatal neurons serve a role in both initiating and maintaining locomotion and other actions⁴⁵. 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}.

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

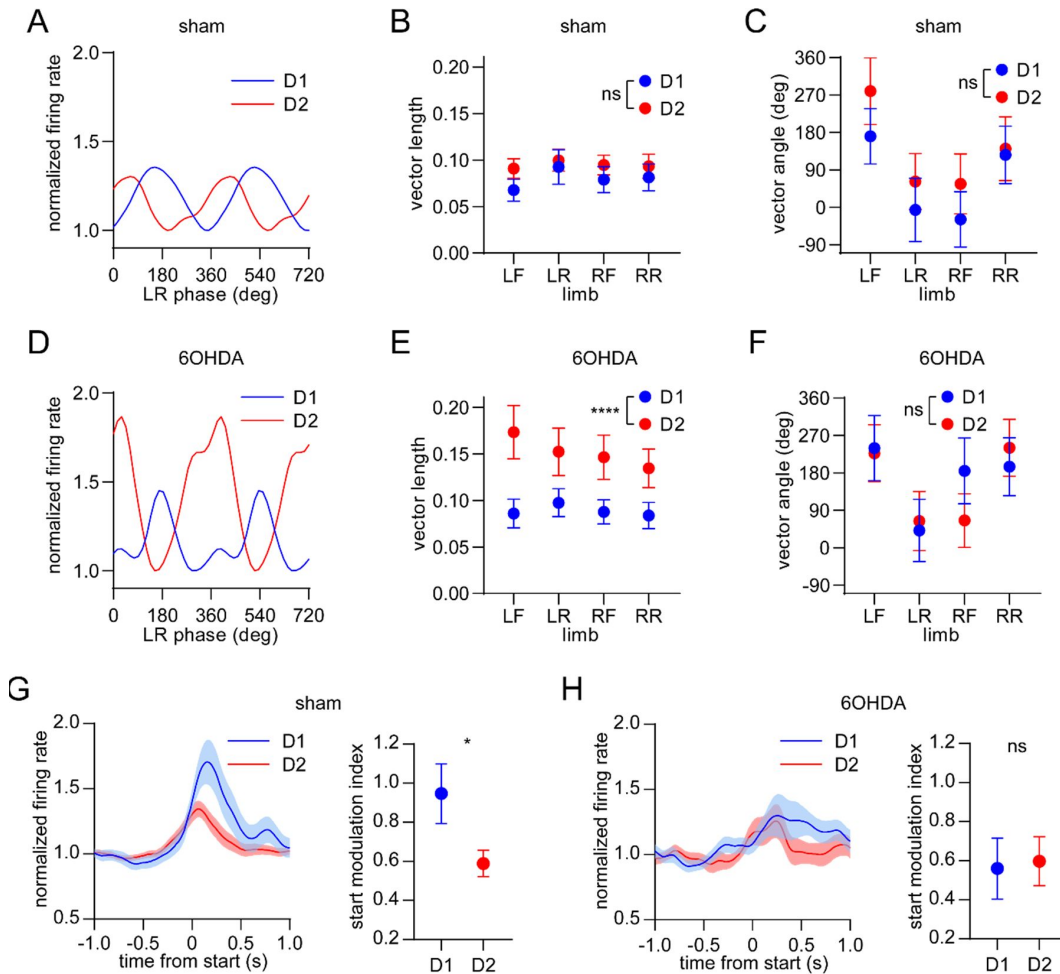


Figure 6.

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

(E) 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, $F_{1,228} = 20$, $p < 0.0001$).

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

(G) 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, $p = 0.016$).

(H) 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.

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^{8,9,15,30,42,46}. 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. We also note that the significant bias toward higher D1 MSN start activity was not reported in previous studies which relied on measuring calcium dynamics^{8,9,15}. A speculative explanation is 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 movements⁴⁷, a response that may have been missed by slower temporal resolution measurement techniques.

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 6-OHDA 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.

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. 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 × 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 Kilosort⁴⁸, and manually curated with Phy. 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.

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 coupling⁴⁹, 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 Ref⁵⁰[\[40\]](#). 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.8303087>[\[41\]](#).

Code for analyzing the data in this study is available at: <https://github.com/LongYang10/Gait-Analysis-of-Freely-Walking-Mouse>[\[42\]](#)

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.

Supplementary figures

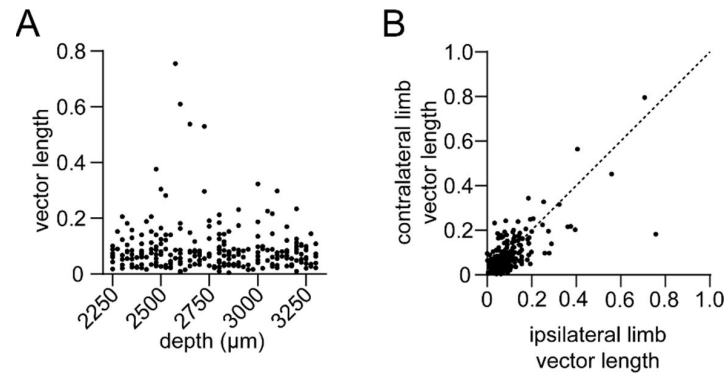


Figure S1.

Single-limb phase locking strength in dorsal striatal neurons show no spatial or limb preference.

(A) 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.

(B) 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.

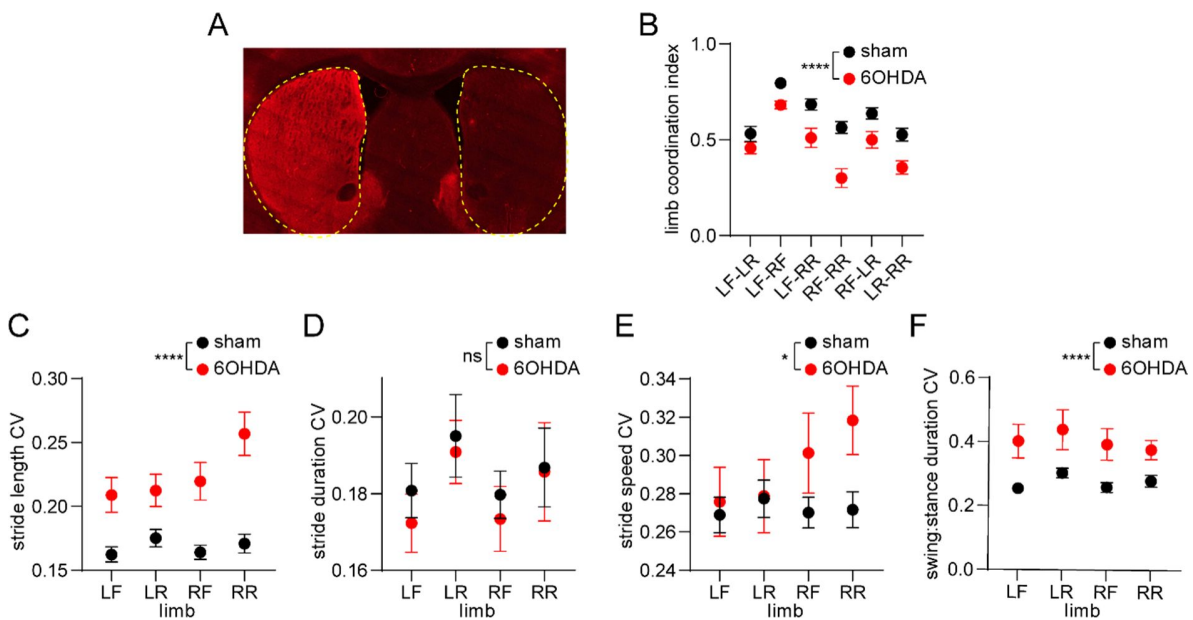


Figure S2.

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

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

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

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

(F) 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.

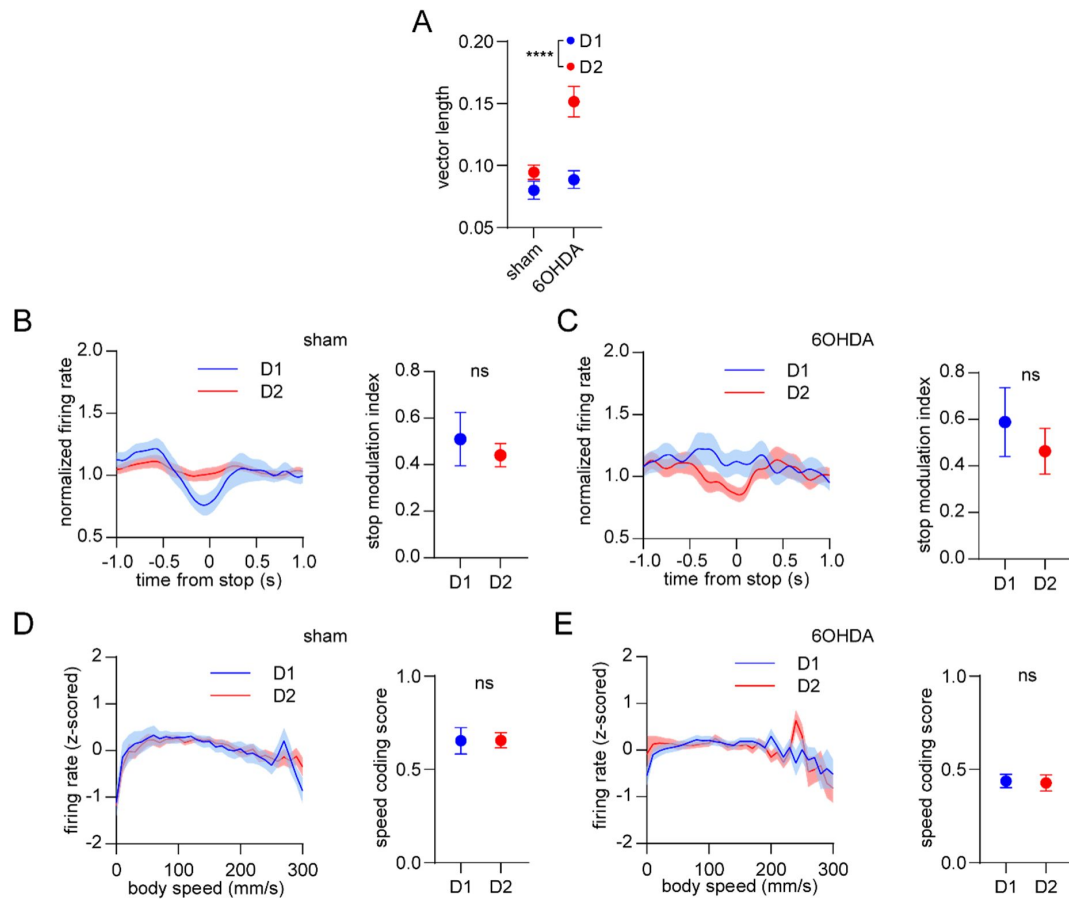


Figure S3.

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: $F_{1,548} = 21$, $p < 0.0001$, group factor: $F_{1,548} = 15$, $p = 0.0001$, interaction: $F_{1,548} = 8$, $p = 0.004$).

(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.

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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.

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.

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.

4. It looks like the data presented in Figure 4 D-F comes from all opto-identified D1- and D2-MSNs. 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.

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).

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).

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).

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.

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.

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.

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.

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.

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.

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.