Dopamine neuron activity before action initiation gates and invigorates future movements

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Abstract

Deciding if and when to move is critical for survival. Loss of dopamine neurons (DANs) in substantia nigra pars compacta (SNc) causes deficits in movement initiation and slowness of movement. We found that a large proportion of SNc DANs, non-overlapping with reward-responsive DANs, increased their activity before self-paced movement initiation. This movement-related DAN activity is not action-specific, and is related to the vigour of the movements to be initiated. Consistently, inhibition of DANs when animals were immobile impaired the probability of movement initiation, and the vigour of future movements. Conversely, brief activation of DANs when animals were immobile increased the probability of movement and the vigour of future movements. Dopamine activity manipulations after movement initiation did not affect ongoing movements. Similar findings were observed for the initiation and execution of learned action sequences. These findings causally implicate DAN activity before movement initiation in the probability and vigour of future movements.

Deciding if and when to move is one of the most important functions of the brain. Actions can be triggered by particular stimuli, or initiated in a self-paced manner without explicit external triggers. Motor control centres in the brain are critical for triggering and executing specific actions. However, little is known about mechanisms that signal if an organism should initiate a movement, or when to do it. Although dopamine neurons (DANs) in the substantia nigra pars compacta (SNc) have been implicated in key brain functions such as reward-based learning[1] and plasticity[2], the most striking phenotype after the loss of these neurons in Parkinson's disease (PD)[3,4] is impairment in self-paced movement initiation, and slowness of movement (bradikynesia)[5]. Consistently, DAN activity has been proposed to modulate the motivation to move [6,7], i.e. to modulate the probability of moving and the vigor of movements. The most widely accepted view is that the role of DANs in self-paced movement is supported by their tonic or sustained activity, while their role in reward prediction is mostly attributed to phasic changes in activity[8,9]. However, some studies have challenged this view by showing transient changes in DAN activity prior or during self-paced movement initiation[10–13]. Initial studies suggested that a large proportion of DANs in the SNc significantly changed their activity during or before the onset of reaching movements[14]. This was confirmed using recordings from classified neurons, showing that a large proportion of SNC DANs increase their activity before or during lever pressing[10]. More recently, it was shown that transient activity of dopamine neuron terminals in the dorsal striatum precedes spontaneous movement initiation [13].

These studies suggest that activity in SNc DANs is perfectly poised to modulate the initiation of spontaneous self-paced movements. However, the necessity of this activity for spontaneous movement initiation has not been demonstrated. Moreover, it is not known whether this transient activity represents a general signal or is specific to particular actions, and how it relates to reward-related activity in SNc DANs. Finally, it is unclear if this activity is critical for initiation or also for ongoing movement, and how it relates to movement vigour[15,16].

The disambiguation of the role of the activity of SNC dopamine neurons in the different movement states requires high temporal resolution manipulations

(activation and inhibition) coupled with precise behavioural readouts of movement. We used high-resolution motion sensors to characterize in detail the moments of spontaneous movement initiation, the actions initiated, and the vigour of such actions in mice exploring an open field. Concomitantly, we employed optogenetically-aided recordings and endoscopic imaging to monitor the activity of dopamine neurons. In addition, we utilized temporally- and state-specific optogenetic manipulations to examine the role of dopamine activity before the initiation or during the ongoing execution of self-paced movements.

Self-paced movements can be complex, and be constituted of many elements. Therefore, we also investigated the dynamics and role of DAN activity in the initiation and execution of self-paced action sequences. We imaged the activity of SNc dopamine neurons while mice learned to execute a sequence of lever presses, and used optogenetic manipulations to explore the role of these neurons in the self-paced initiation and performance of this action sequence.

Transient activity of DANs in SNc precedes spontaneous movement initiation

In order to precisely determine changes in movement of the animals in 3 dimensions, we mounted inertial sensors that measure acceleration and angular velocity at high-resolution (200-1000 Hz sampling rate) in the heads of mice. This allowed us to precisely measure motion in mice spontaneously moving in an open field, without any external cues, food deprivation, or reward involved (**Fig. 1a,b**). The raw acceleration signal recorded using such sensors is a combination of a static component (determined by earth's gravitational pull) and a dynamic component (determined by the animal movement). Using a standard approach[17], we filtered the raw acceleration signal using a low pass filter to separate these two components, and found that the dynamic component tracked the animal movement accurately, and correlated well with pixel change in video measurements (**Supplementary Fig. 1b**, $r=0.74$, $p<0.05$). We uncovered that the distribution of movements in an open field was not a gradual continuum between arrest and motion, but rather a bimodal distribution (**Fig.1c**). This allowed us to separate the distributions using the minimum value of acceleration between the two peaks of the bimodal distribution (**Fig. 1c**),

and verify that values from the low acceleration distribution corresponded to periods of immobility (with the possible exception of small and slow postural adjustments), while values from the high acceleration distribution corresponded to periods of overt mobility (**Fig. 1d**, **Supplementary Fig. 1a,b**). This permitted the establishment of a threshold to precisely separate periods of immobility from periods of mobility, and to identify episodes of spontaneous movement initiation with great accuracy. To investigate the activity of photoidentified SNc dopamine neurons in relation to movement initiation, we implanted 16 channel movable electrode bundles coupled to a fibre optic cannula placed just above the SNc (**Fig. 2a**). We implanted these bundles in six Th-Cre mice[18] (line FI12, 95.3 % specificity in the SNc, **Supplementary Fig. 2a-c**) crossed with Ai32 mice[19] to obtain expression of Channelrhodpsin2 (ChR2) in TH-positive neurons, and used photoidentification[20] to identify which single units were dopaminergic (**Supplementary Fig. 3**). We recorded 45 single units that were significantly activated by 10 ms pulses of blue light at 1 Hz. After analysing the average latency to activation and the relative increase in spike probability during light pulses, we considered neurons with a latency ≤ 7 ms and an increase of 30% or more in spiking probability as photoidentified dopamine neurons (n=25 neurons, **Fig. 2b, Supplementary Fig.** 3, see methods for details).

To examine whether these neurons were modulated by movement initiation, we built peri-event time histograms (PETH) of their activity aligned to spontaneous movement initiations, defined as transitions between periods of at least 300 ms below the threshold (immobility) followed by at least 300 ms above the threshold (mobility). We found that the average activity of all the dopamine neurons recorded increased transiently before movement initiation (**Fig. 1d**). Consistently, we found that many photoidentified DANs were significantly modulated by movement initiation and that the majority of these were positively modulated (**Fig 1e).** To better characterize the activity of these neurons, we compared the firing rate during 50 ms bins with the firing rate at baseline (-1.5 to -1s in relation to movement onset) using a receiver operating characteristic (ROC) analysis[21]. The area under the ROC curve (auROC) was calculated for each time-bin, with values higher than 0.5 denoting an increased firing rate in relation to the baseline, and values lower than 0.5

denoting a decreased firing rate in relation to the baseline (**Fig. 1f**). We then used an affinity propagation algorithm[22] to cluster the traces that resulted from the ROC analysis (**Fig. 1f, see methods for details**). Besides segregating negatively modulated neurons from positively modulated neurons, this analyses further separated positively modulated neurons in three groups: transiently active before the initiation of movement (cluster 1), transiently active before the initiation of movement followed by inhibition after the initiation (cluster 2), and sustained increase in activity with movement initiation (cluster 3). Negatively modulated neurons were all clustered together (sustained decrease in activity, cluster 4). The latency for modulation preceded the initiation of movement for clusters of positively modulated neurons, but not for the cluster of negatively modulated neurons (**Fig. 1g**). These findings were corroborated using microendoscopic calcium imaging of SNc DANs in freely moving mice (**Supplementary fig 4**, 22 neurons, see methods and below for details), and confirm that a larger proportion of DANs is transiently active before spontaneous movement initiation.

The activity of individual DANs is not action-specific

We next examined whether the transient activity of individual DANs before action initiation was tuned to the initiation of specific actions or represented a more general signal before the initiation of all actions. To characterize precisely different spontaneous movement initiations, we used a combination of three variables obtained from the motion sensors: total body acceleration; the angular velocity of the axis most parallel to the dorsal-ventral axis of the mice and the gravitational acceleration of the same axis. The total body acceleration provided us with a measure of movement initiation vigour, the angular velocity characterized the direction of the movement (**Fig. 2a,b**) and the gravitational acceleration was sensitive to postural changes like rearing. We used the combination of these three variables to describe trajectories in the motion sensor space for each movement initiation (**Fig. 2a,b**), and used affinity propagation to cluster different initiations. To perform this clustering analysis we created a vector for each initiation by

concatenating the distribution of each of the three motion sensor variables during the first second of an initiation trajectory (**Fig. 2c**). We found that none of the positively modulated dopamine neurons were active only during one specific initiation cluster, but rather that most neurons were broadly tuned and responded before very different initiations. Furthermore, we found that initiation trajectories preceded by increased activity of each DAN were as variable as all other initiation trajectories (**Fig. 2e**). These data strongly indicate that each positively modulated dopamine neuron is active before the initiation of a wide range of movements, and hence not action-specific.

DAN activity before initiation is related to the vigour of future movements

Previous studies in patients with Parkinson's disease[16] and animal models[15] have shown that dopamine depletion leads to less vigorous movements. We therefore investigated whether the activity of dopamine neurons before movement initiation encoded any information about the vigour of the movement about to be initiated. We used the mean acceleration during the first 500ms of each spontaneous initiation as a measure of movement vigour, and separated trials into low acceleration trials (lower tertile), medium, and high acceleration trials (higher tertile). We verified that overall, the positive transients in dopamine activity 300ms before movement initiation were significantly related to the vigour of future movement (**Fig. 2f**). When doing per trial analyses comparing all the lower vigour with the higher vigour initiations, we found that 38.5 % of the neurons had significantly higher activity before higher vigour movements (paired t-test comparing firing rate of low and high acceleration trials $P < 0.05$, **Fig. 2g**).

Transient inhibition of dopamine neuron activity decreases the probability of action initiation and the vigour of future movements

To achieve temporally controlled inhibition of dopaminergic neuron activity, we expressed archaerhodopsin (ArchT)[23] specifically in SNc neurons (AAV2/1. CAG.Flex.ArchT-GFP injected into Th-Cre mice). We verified in vivo that ArchT was

efficiently silencing SNc dopamine neurons by using 16 channel movable electrode bundles coupled to a fiber optic cannula (**Fig. 3a**). Optic fibers were positioned 300 µm above the SNc. Light intensity was ~35 mw at the tip of the fiber, corresponding to an estimated[24] irradiance of 70-206 mw/mm^2 at SNc depth (200 to 400 um from the fiber tip). Neural activity was recorded daily and the electrodes were moved 50µm at the end of each recording session. We were thus able to record neural activity from different depths (-3.90 mm to -4.60 mm from the brain surface), with neurons being recorded above, within and below the SNc. We recorded from 140 units (see methods) and observed that at depths were the SNc is located more than 60% of recorded units were inhibited, (**Supplementary Fig. 5**), while above and below very few neurons were modulated. Furthermore, we only observed one single unit that was modulated by light at the depth closest to the fibre where light intensity is higher (-3.9:-3.95mm, 0.7% of all units recorded, 7.7% of all units recorded at this depth), indicating that light delivery per se was not sufficient to change neural activity at this power.

In order to test if inhibiting dopamine neuron activity would affect movement initiation, we expressed ArchT in the SNc of 11 Th-Cre mice and YFP in the SNc 9 Th-Cre mice (control group), and delivered light unpredictably for periods of 15 seconds (**Fig 3a**). Inhibition of SNc dopamine neurons, increased the probability of mice being immobile (**Fig. 3 b, c**). This was not observed in YFP controls **(Fig. 3b,c).** To investigate more specifically whether inhibiting SNc dopamine neurons affected movement initiation or impaired ongoing movement, we analysed separately trials in which the animal was immobile when the inhibition started (mice immobile for at least 300 ms), or mobile when the inhibition started (mice already moving for at least 300 ms, **Fig. 3d-h**). Acceleration decreased during inhibition, and there was an interaction between inhibition and mobility state when inhibition started (**Fig. 3ef**, see extended data table for statistics). To explore this interaction we compared mean acceleration between mobile and immobile trials, and found a significant impairment in movement initiation when SNc DANs were inhibited during immobility (**Fig. 3 g**). The effect of inhibition was relatively rapid with a significant difference between light and no light after 2.4 seconds (**Fig. 3 2**). Consistently, we also tested

that 5 seconds of inhibition was sufficient to impair movement initiation (**supplementary Fig. 6**).

On the contrary, there was no significant change in mean acceleration when inhibition happened after movement onset (**Fig. 3g**). There was no chance in the vigour of movement when SNc dopamine neurons were inhibited after movement initiation and the mice did not stop during the 15 seconds of inhibition (**Fig 3h**). Furthermore, in trials where animals stopped during the 15 seconds (≥ 300 ms immobile), there was no difference in acceleration between laser-on and laser-off trials prior to the first stop. However, there was clear difficulty in movement initiation after the first stop (**Fig. 3i).** This was not observed in YFP controls **(Fig. 3j).**

We analysed in more detail the deficits in movement initiation when the inhibition was triggered while mice were immobile. We found that there was a significant decrease in the probability of initiating movement during the 15 seconds of inhibition (**Fig. 3k**). Furthermore, even in trials where mice were able to initiate movements, the latency to initiate was significantly higher than in laser-off trials, and importantly, the initiated movements were less vigorous (**Fig. 5l,m**). Taken together, these data indicate that dopamine neuron activity before movement initiation modulates the probability and vigour of future movements, but activity in these neurons does not appear to be critical for the maintenance and vigour of ongoing movements.

Activation of DANs during arrest promotes movement initiation and invigorates future movements

Next we investigated whether brief activation of dopamine neurons when animals are immobile would be sufficient to promote movement initiation. To test this, we expressed ChR2 in SNc DANs using a similar Cre-dependent strategy as described above (local injection of AAV2/1 viruses containing DIO ChR2-YFP in 7 mice, and control DIO EYFP in 5 mice). Stimulation at 20 Hz for 500 ms, using a 456 nm blue light (3 mW light intensity at the tip of the fiber), resulted in robust firing by SNc dopamine neurons (Fig. 4a). We found that brief transient activation of SNc

dopamine neurons when mice were immobile was sufficient to produce overt movement that lasted several seconds, while similar activation when mice were overtly moving did not affect ongoing acceleration (**Fig. 4 a-c**). No significant differences were found in the YFP group.

To further corroborate this finding, we performed an online closed loop experiment in which mice received stimulation if they were immobile for at least 900ms, but only in 50% of the trials. Trials where light was not delivered were used as within-animal control (laser-off trials). We found that brief activation of SNc dopamine neurons in laser-on trials resulted in increased probability of movement initiation (**Fig. 4d-h**), with no effect in laser-off trials compared to YFP controls. Average acceleration during the first second after the closed loop trigger was higher during laser-on than during laser-off trials in the ChR2 group (**Fig. 4f**). Moreover, the latency to initiate movement when SNc dopamine neurons were briefly activated was almost three times shorter than in laser-off trials (**Fig. 4g**). Also, the percentage of trials in which movement was initiated during the first second was higher during laser-on than during laser-off trials (**Fig. 4h**). We found no evidence that this closed-loop SNc DAN activation had a reinforcement effect because immobility states did not become more frequent in ChR2 mice (interval between immobility periods: Chr2, 254.5±116.5 s; YFP, 150.5±65.8 s; t=1.74, *P*=0.12). To investigate whether dopamine activation promotes the initiation of more vigorous movements, we calculated the maximum acceleration during the first second after movement initiation for laser-on and laser-off trials. We found that movements initiated during laser-on trials were more vigorous than movements spontaneously initiated during laser-off trials (**Fig. 4i**).

In summary, the results of ChR2 experiments were symmetric to those obtained in the ArchT experiments. Brief activation of SNc dopamine neurons when animals were immobile was sufficient to promote and invigorate future movements.

SNc DANs become preferentially active before the initiation of action sequences

The results presented above highlight a specific role for transient dopamine activity in gating self-paced movement initiation but not in modulating ongoing movements. Based on these findings, one prediction would be that if individual spontaneous movements are concatenated or chunked into sequences of movements, then dopamine neuron activity would become preferentially active before sequence initiation but not during the execution of the concatenated elements within the sequence. To test this prediction, we trained mice in a self-paced operant task in which eight lever presses led to a sucrose 20% solution reward (fixed ratio eight task, FR8), without any explicit stimuli signalling the availability of reward[10] (**Fig. 5a**). To investigate the relation between dopamine neuron activity and the learned action sequence, we implanted a gradient index (GRIN) lens (500 µm diameter, 8.2 mm) just above the SNc of four Th-Cre mice (**Supplementary Fig. 7d**) and injected an AAV viral vector that expressed GCaMP6f[25] in a Cre-dependent manner[26] (AAV2/5.CAG.Flex.GCaMP6f). We then used a miniaturized epifluorescence microscope[27] to image calcium transients in genetically identified SNc dopamine neurons while mice were performing the FR8 task. Although calcium imaging using miniscopes allows the imaging of neurons in deep structures in freely moving mice, it is a challenge to extract neuronal signals without background contamination. To deal adequately with this problem we used constrained non-negative matrix factorization for endoscopic data (CNMF-E)[28], an adaptation of the recently described CNMF Algorithm[29]. CNMF-E can reliably deal with the large background changes from multiple sources in the data, allowing the accurate source extraction of cellular signals (see methods). We created PETHs using normalized fluorescence traces (z-score of ΔF) aligned to six different events during the sequence (first, second, third, third to final, second to final and final press of each sequence). We quantified positively modulated neurons for each of the sequence events that were significantly different ($\geq 3\sigma$) from baseline (-5 to -2s before first press, see also methods for details). Similarly to previous findings[10], we found that the proportion of modulated neurons was different between press events with the highest proportion of neurons being modulated by the first press (**Fig. 5d-f**). As predicted, this higher proportion of neurons related to first press was not apparent early in training, and developed with sequence learning (**Supplementary Fig.8)**[10].

Different SNC DANs are modulated by movement versus reward

Recent studies claimed that SNc neurons are more modulated by movement, while VTA neurons are more modulated by reward[13]. Therefore, we investigated whether neurons in the SNc would respond positively to reward consumption (aligning traces to the first lick with reward available). We found that ~ 35% of neurons in the SNc responded to reward (**Fig. 5e**). This is almost similar to the percentage of neurons modulated during sequence initiation (~40% first press neurons, **Fig. 5d,e**). Importantly, there was little overlap between reward and first press modulated neurons, and the overlap was not significantly different than what would be expected by chance (**Supplementary Fig. 9**). These data indicate that SNc DANs can be activated by both movement and reward, and that these populations do not have much overlap.

Transient inhibition of DANs before action sequences impairs sequence initiation

Next we tested whether SNc dopamine neuron activity was necessary for sequence initiation. To achieve this we trained Th-Cre mice expressing ArchT (n=11) or YFP (n=9), in the FR8 task for 12-14 days. With training mice develop a structured behaviour with predictable sequence initiations and trajectory after reward consumption[31] (**Fig. 5a**). We triggered the laser based on mice breaking an infrared beam right next to the reward magazine (**Fig. 5g,h**), thus inhibiting SNc neurons before sequence initiation, and at the moment of minimal DAN activity (before the increase in activity of first press neurons, **Fig. 5g**). We compared a block of inhibition (laser-on block) with a previous block without inhibition (laser-off block) during the same session. Inhibition during 5 seconds before the first lever press resulted in a significant increase in the latency to initiate the action sequence when compared to laser-off trials (**Fig. 5h**). Moreover, the probability of initiating a sequence decreased during the 5 seconds of dopamine neuron inhibition (**Fig. 5i**). Consistently with the

experiments presented before, when the inhibition happened after sequence initiation (triggered by the first press), the inter-press interval and number of presses during the 5-second inhibition were not altered (**Fig. 5j**). No effects were observed in YFP controls (**Fig. 5h-j**). These results were replicated using DAT IRES-cre mice using a different inhibitory opsin (Jaws[32]) and inhibiting pseudorandomly 30% of the trials (**Supplementary Fig. 10**). Taken together, these results indicate that SNc dopamine activity before action sequence initiation modulates the probability and latency of sequence initiation, but is not critical for the execution of ongoing sequences.

Discussion

In this study, we found that a large proportion of SNc DANs are transiently active during self-paced movement initiation. The majority of these DANs increase their activity before movement initiation, but return to baseline activity after initiation. This movement initiation related activity is not action-specific, suggesting that SNc DANs express a general signal preceding the initiation of self-paced movements. We showed, using state-dependent optogenetic manipulations, that SNc DAN activity modulates self-paced movement initiation. Inhibition of DANs while animals are immobile reduces dramatically and rapidly the probability of spontaneous movement initiation, while brief activation of DANs quickly increases the probability of movement initiation. Importantly, these precisely-timed and state-dependent optogenetic manipulations did not change ongoing movements, indicating a role for SNc DAN activity especially in initiation. These results where corroborated using more complex movement sequences. Most DANs became active before the initiation of a sequence of movements, and inhibition of DAN activity before sequence onset impaired initiation. However, inhibition of DAN activity before sequence onset did not impair performance. These results reveal a role for DAN activity before movement in the probability of movement initiation.

We also found that SNc DAN activity before movement was correlated with the vigour of the movements about to be initiated. Furthermore, the state-dependent optogenetic manipulations revealed that DAN activity before movement initiation

modulates the vigour of future movements, but changes in DAN activity after movement onset have little impact in the vigour of ongoing movement. These findings are extremely interesting in light of the bradykinesia observed in PD. It has been proposed that dopamine release in the dorsal striatum is important for the regulation of movement vigour[8,16 15], but it was thought that this effect was mostly due the ongoing tonic levels of dopamine release. Our results indicate that it is the activity before movement onset that determines movement vigour, and suggest that this could be the reason why PD patients select less vigorous movements to initiate[16]. It is also in accordance with recent studies showing that activity of DAN terminals in the dorsal striatum preceded spontaneous movement initiation but did not precede and even followed acceleration bursts of ongoing movement[13].

Our results show that DAN activity is not action-specific and is related to the vigour of future movements. These data argue that the gating of self-paced initiation presumably happens by permissive effects of dopamine on downstream striatal circuits that would receive information about which plans to execute from other inputs. This suggests a model in which dopamine modulates the excitability of striatal medium spiny neurons, which are also critical for self-paced action initiation[31,33], by gating action-specific glutamatergic inputs from motor and pre-motor cortices and thalamus.

Furthermore, although our results show that transient changes in the activity of SNc DANs are relevant for gating and invigourating self-paced movement, we do not claim that this gating occurs exclusively through transient changes in DAN activity. There is extensive literature showing modulation of self-paced movement initiation by sustained changes in the levels of striatal dopamine[15,34,35]. We hypothesize that transient changes can function as a fast system working on top of tonic release to adjust the concentration of striatal dopamine thus increasing the probability (and vigour) of initiation of movements planned at that exact time. More sustained changes in DAN activity could represent states in which the “gate” would be more permissive, increase the probability of action initiation during longer periods of time, therefore promoting movement[36]. This would translate into more movement variability or exploration action space, which could be important in situations of uncertainty or learning.

Conciliating the roles of DANs in movement and reward-based learning has been a challenge. Recent studies attempted to resolve it by proposing that motor-related signals in DAN axons originated from SNc neurons, while reward-related signals originated mainly from VTA neurons[13]. However, we found a similar percentage of action initiation and reward modulated DANs in the SNc (largely non-overlapping). This discrepancy might be due to methodological differences (eg. head fixed vs freely moving different behavioural tasks), to mechanisms of local control of dopamine release at terminals, or even anatomical segregation of the projections arising from these different neuron types[37]. However, the results presented here clearly show that DANs in the SNc can respond to reward, and suggest that there might be subpopulations of SNc neurons (modulated by reward or movement onset), receiving different inputs. Supporting this, it was recently shown that manipulation of PPN cholinergic inputs into the ventral SNc affects locomotion but does not condition mice in a conditioned place preference paradigm[38].

Dopamine depletion in PD is chronic. Studies of the role of dopamine in movement in chronic models of depletion are very valuable, but have limited our understanding of the dynamic role of SNc DAN activity in downstream circuits. Not surprisingly, current treatments are focused mainly on increasing dopaminergic effects in a sustained way, with potential undesired effects. Our work, by highlighting the relevance of transient activity of these neurons before self-paced movement initiation suggests that it could be beneficial to pursue treatments aimed at providing transient modulations of basal ganglia circuitry when patients initiate movements, e.g. via closed loop deep brain stimulation[39] triggered by activity in cortical areas related to motor planning.

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Author contributions

J.A.S. and R.M.C. designed the experiments and analyses and wrote the study, J.A.S. performed all experiments and analyses, F. Tecuapetla helped with optogenetic and recording experiments, V.P. helped with accelerometer experiments and accelerometer data analyses.

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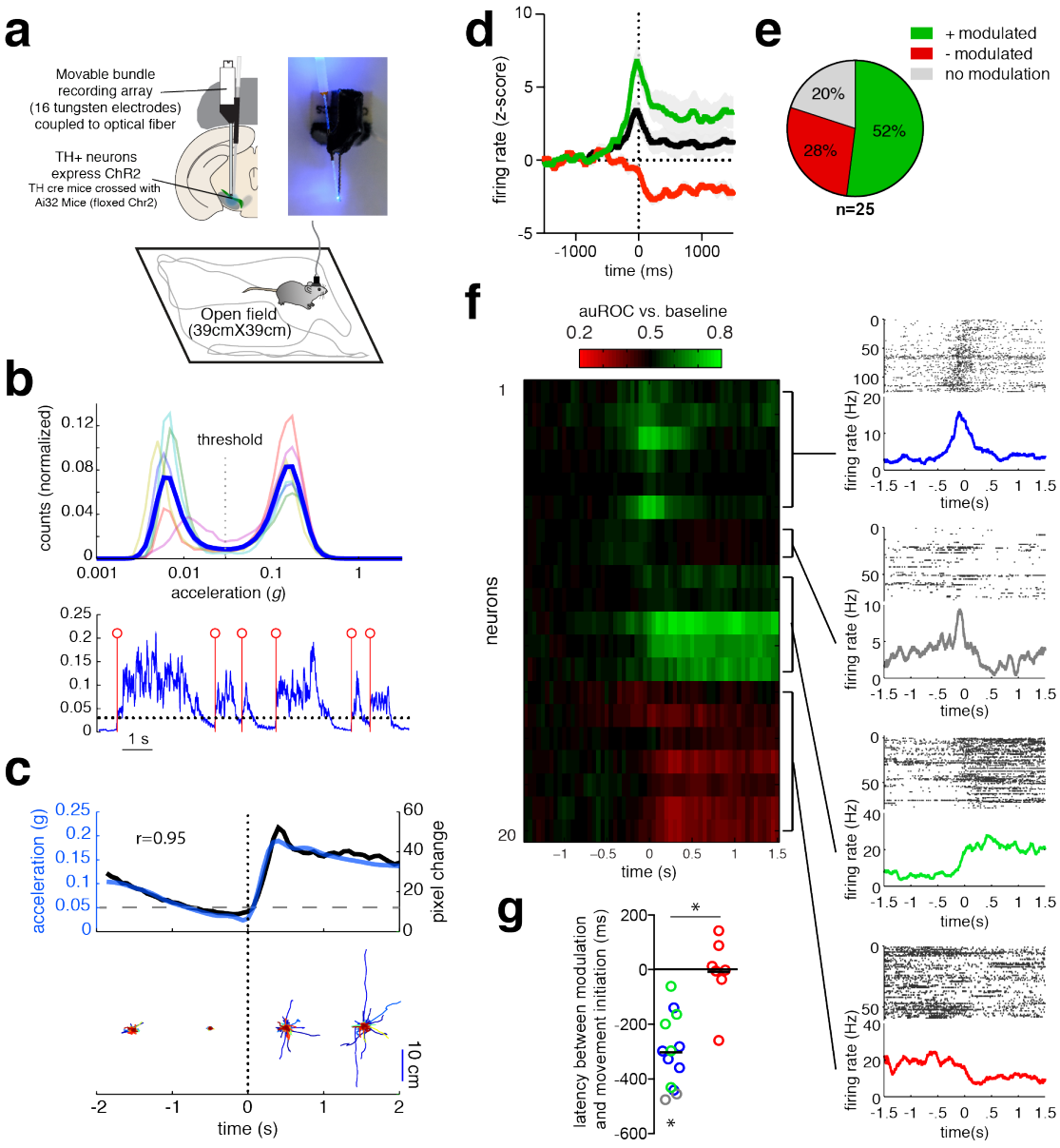

Figure 1. Movement initiation is preceded by increased activity of dopamine neurons. a, Top left; schematics of the method used to record and photoidentify SNc dopamine neurons. Top right; Sixteen channel movable bundle electrode and fibre optic cannula used in PINP experiments. Bottom, open field setup. **b**, Top; distribution of total dynamic acceleration in the open field. Blue thick line represents the mean distribution of all mice and the faded color lines represent the distribution for each mouse (obtained from a mean of 3±2.2 one hour open field sessions per mouse). Bottom; the red lines represent examples of movement initiation trials

determined using the threshold represented in the top panel overlaid on the total dynamic acceleration trace. **c**, Top; comparison between a video derived motion measure (pixel change) and total dynamic acceleration aligned to movement initiation determined using the acceleration threshold (n=454 initiations obtained from 3 mice during a total of 13 open field sessions). Bottom, Representation of the movement of mice (based on centre of mass) during each trial within the time intervals denoted on the x axis. The trajectories were aligned to the center of mass of the last frame of each 1 s interval. Different colours denote individual trials. **d**, Mean trace for all neurons (including non-modulated, n=25, black), positively modulated neurons (n=13, green), and negatively modulated neurons (n=7, red) ,aligned to movement initiation (93.45 ± 35.99 mean spontaneous initiations per neuron). Grey shadow denotes S.E.M. **e**, Proportion of modulated and non-modulated neurons. **f**, Left; responses from all modulated neurons aligned to movement initiation, sorted according to affinity propagation clustering (green increase from baseline, red decrease from baseline). Right; representative neuron for each cluster. **g**, latency of each neuron to be significantly modulated in relation to movement initiation (negative indicates modulation preceding movement onset and positive indicates modulation after movement onset).

* $P<0.05$

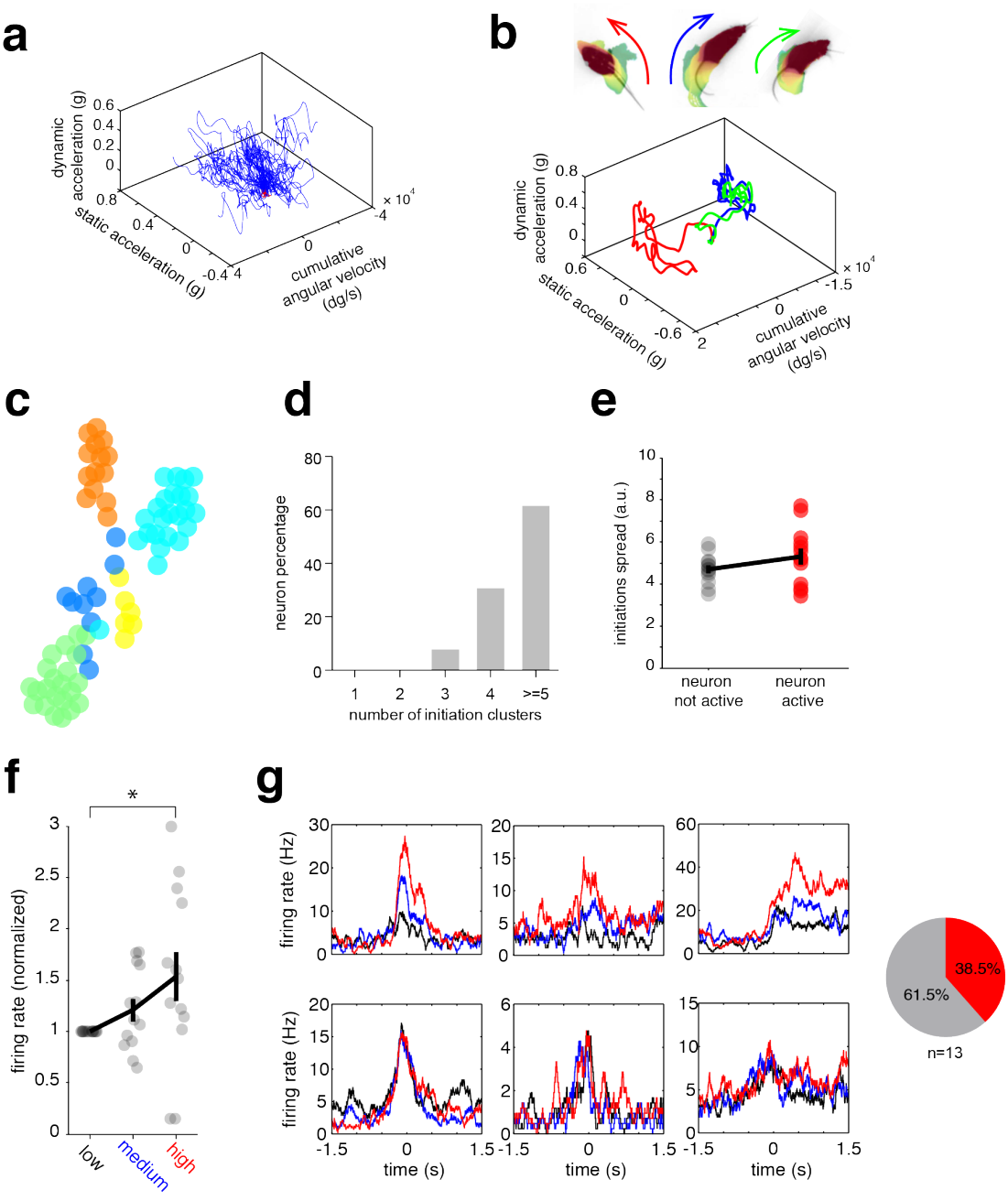

Figure 2. Initiations preceded by increased activity of dopamine neurons are more vigorous but not more similar to each other. a, Example of 20 randomly selected initiation trials of one session represented in a motion sensor 3d space determined by total dynamic acceleration, static acceleration and cumulative angular velocity of the sensor axis most parallel to mice's dorsal-ventral axis. The red portion of each

trajectory represents 300 ms of immobility before the initiation of movement and the blue portion represents the 1.5 s from movement initiation onward. **b**, Video frames time series of three examples of movement initiations (frames corresponding to the first 1.5 seconds after movement initiation) and their representation in the same motion sensor space shown in a. **c**, Representation of the initiations of one example session using t-SNE[36] dimensionality reduction. Different colours represent different clusters of initiations determined using affinity propagation clustering. **d**, Number of initiation clusters in which each positively modulated neuron was significantly activated (n=13, 30.4% ± 0.19 of initiations were preceded by significant increase in neuron activity, see methods). **e**, Comparison of the spread of initiations (mean distance to every other initiation) between initiations not preceded by increased activity and initiations preceded by increased activity for positively modulated neurons. **f**, Firing rate normalized (firing rate/firing rate for low acceleration trials) of positively modulated neurons for low, medium and high acceleration trials. Error bars denote S.E.M. **g**, examples of PETH of vigor-related neurons (top) and non-vigor related neurons aligned to movement initiation separated for high, medium and low acceleration initiations (red, blue and black respectively). The proportion of vigor related (red) and non-related neurons (gray) is quantified in the pie chart on the right.

* $P<0.05$

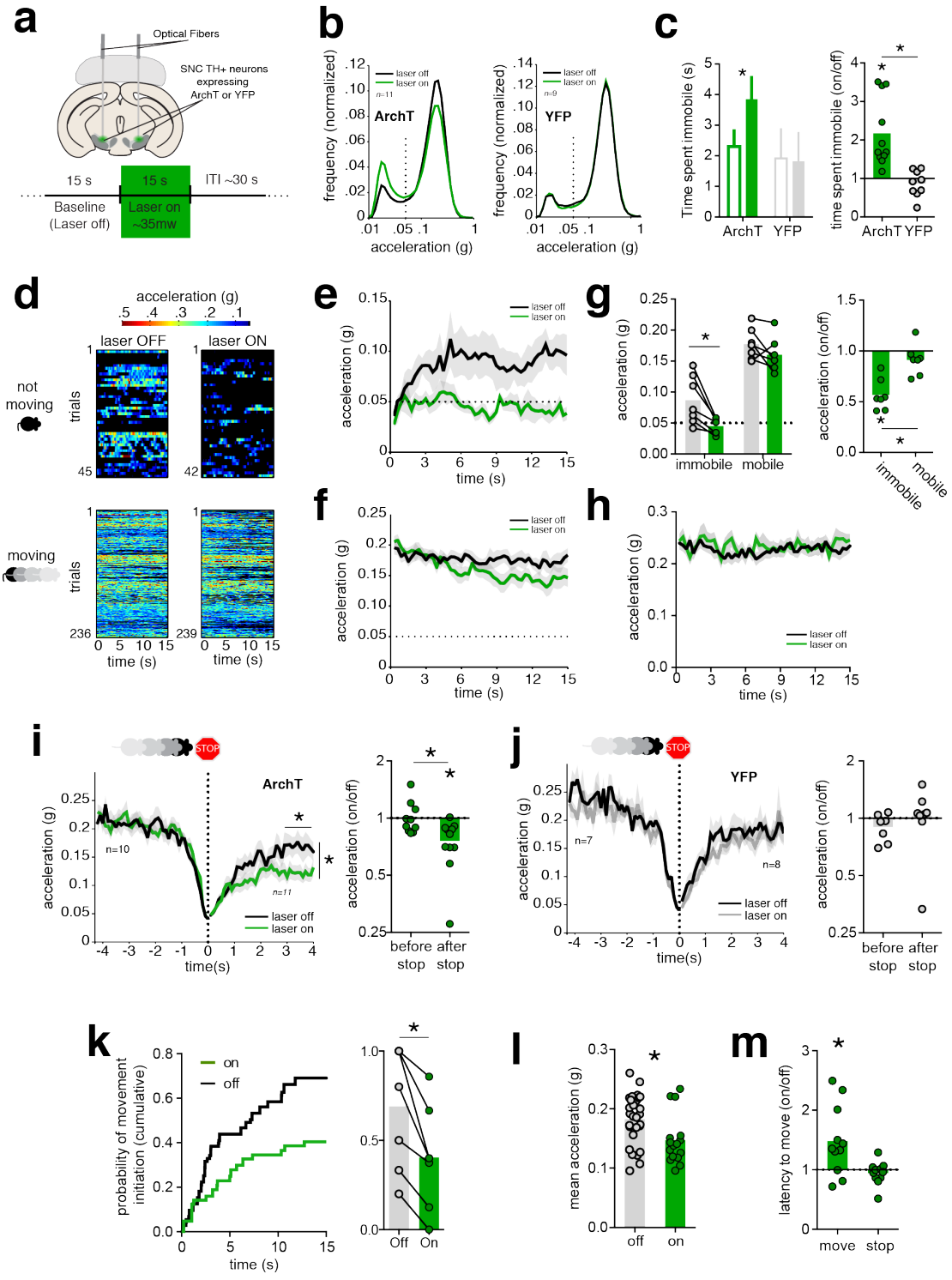

Figure 3. Inhibition of SNc dopamine neurons impairs movement initiation, but not ongoing movement. **a**, Schematics showing fibre positioning and trial structure. **b**, Distribution of acceleration in the open field during laser-on and -off for ArchT (left) and YFP (right) groups. Vertical dashed line denotes the threshold used to distinguish immobile from mobile state. **c,** Time spent immobile during laser-off and laser-on

periods (left). Time spent immobile during laser-on normalised by the baseline value (right). Clear bars indicate laser-off and filled laser-on. **d**, Heat maps of acceleration data of all trials where the mouse was immobile (top) or mobile (bottom) prior to trial start, in laser off (left) and laser on (right) conditions (trials obtained from n=11 ArchT mice). **e**, Acceleration during laser-off and laser-on trials when ArchT mice were immobile before trial start (n=7 mice). **f**, Acceleration during laser-off and laser-on trials when ArchT mice were mobile before trial start (n=7 mice). Horizontal dotted line denotes the threshold used to classify acceleration state. **g**, Left, mean values of the data plotted in a and b. Grey bars represent laser-off data while green bars represent laser on data. Right, data shown in left panel normalised (laser-on/laser-off). **h**, Mean acceleration per mouse for trials where mice were already mobile when photoinhibition was triggered, and never stopped during the trial (n=10; two way repeated measures anova, Main effect laser: $F(1,9)=0.77$, $P=0.40$; Main effect time: $F(49,441)=1.52$, $P=0.016$; interaction: $F(49,441)=1.04$, $P=0.40$). **i**, Left; mobile trials aligned to the first stop. Right; normalised mean acceleration (laser-on/laser-off) before (-4:-3s) and after (3:4s) the first stop for ArchT mice. **j,** Same as in i for the YFP group. **k**, left, mean cumulative probability of initiating movement for immobile trials, for laser on (green) and laser off (black) trials. Right, mean probability to initiate movement per mouse for laser on and laser off trials (n=7 ArchT mice). **l**, Mean acceleration for initiations that occurred during immobile trials for laser on (green, n=16) and laser off trials (black, n=30 trials) of ArchT mice. **m**, Normalised mean latency to initiate movement (laser on/laser off; n= 9 YFP mice, 11 ArchT mice).

Error bars and shaded areas denote S.E.M. * $P<0.05$

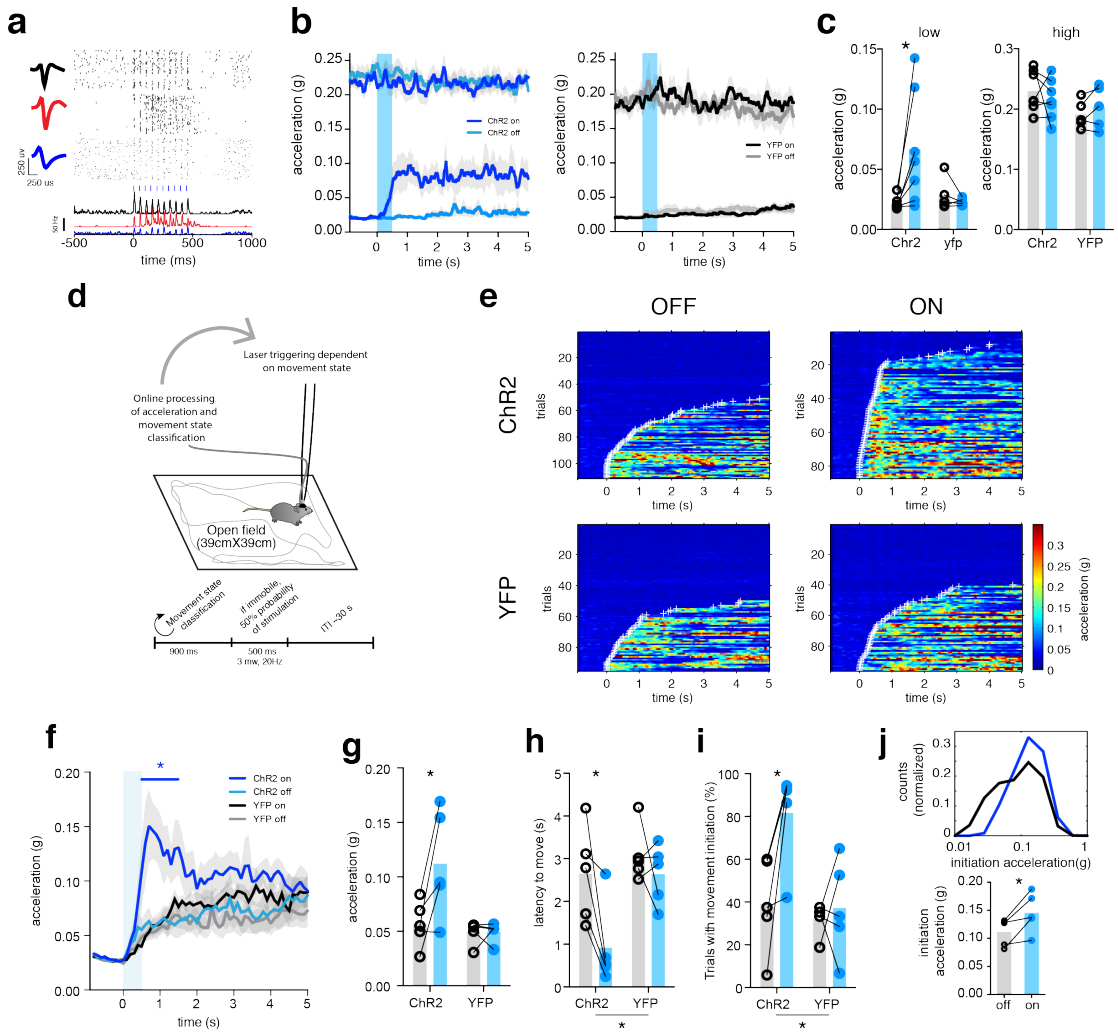

Figure 4. Transient SNc dopamine neuron activation promotes movement initiation. a, Example of three SNc dopamine neurons (single units) expressing ChR2, following stimulation - 10 pulses at 20 Hz. Vertical black lines represent spikes and the traces in the bottom correspond to the PETH for each neuron. **b**, Mean acceleration during laser-off and laser-on trials, depending on the acceleration state of ChR2 and YFP mice before the trial. **c**, mean acceleration from 0 to 1s depending on laser state and group for trials were animals were immobile (left) and trials where animals were moving (right) before laser trigger. **d**, experimental design for closed loop experiments. **e**, Heat maps of acceleration data of all trials for both ChR2 and YFP groups. Laser-trigger criteria was reached at time 0. White crosses signal the onset of movement for a given trial. Blue light was delivered from 0 to 0.5s in laser-on trials (right panels). **f**, Mean acceleration for ChR2 and YFP groups during laser-on and laser-off closed loop trials. Light blue area indicates laser-on period, n= 5 mice

per group. **g**, mean acceleration from 0 to 1s depending on laser state and group. **h**, Latency to initiate movement depending on laser state and group. **i**, Percentage of trials per mouse with movement initiation between 0 and 1s depending on laser state and group. **j**, Top, mean distribution of acceleration during the first second after movement initiation for laser-on and laser off (n=5 ChR2 mice). Bottom, mean acceleration during the first second of movement initiation during laser off and laser on trials (n=5 ChR2 mice).

Clear bars indicate laser-off and filled laser-on, error bars and grey shaded areas represent S.E.M., * $P<0.05$

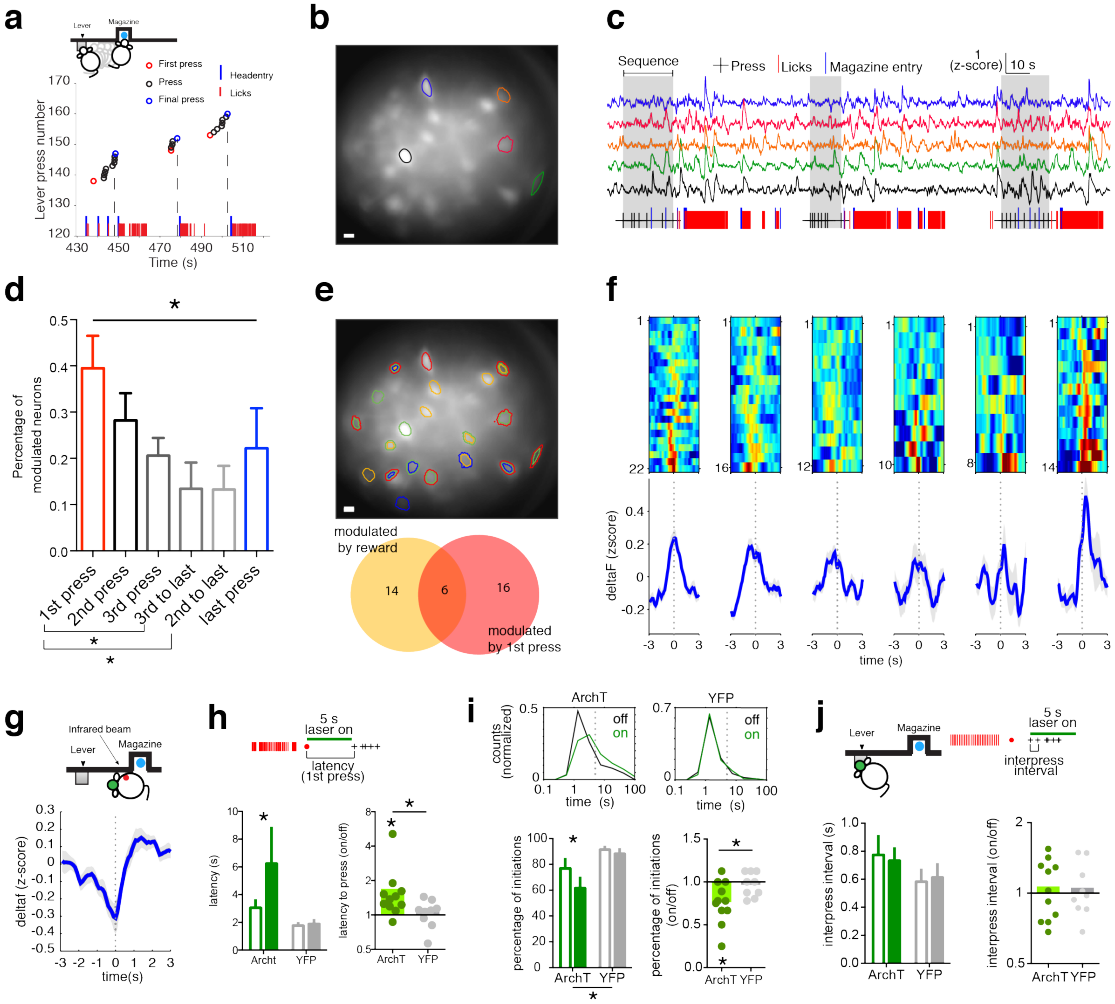

Figure 5. SNc dopamine neurons are transiently active at sequence initiation, and when inhibited impair sequence initiation but not sequence performance. a, Example of the microstructure of the behaviour of a mouse performing the FR8 task late in training. The dashed vertical lines denote when the reward was delivered. **b**, Example of a field of view of a Th-Cre mouse expressing GCaMP6f in the SNc. Horizontal white bar denotes 20 µm. Pixel intensity represents the standard deviation of that pixel during the recorded session. Coloured contours indicate the ROIs used to obtain the same colour traces in c**. c**, Example of traces obtained using the CNMF-E algorithm and task related behaviours for one session. Colour of traces matches the ROI's shown in b. **d**, Percentage of neurons modulated by the different presses within a sequence. **e**, Top, Example of the map of significantly modulated ROIs for one mouse. Red, green and blue represent ROIs modulated by the first, middle and last press correspondingly. Orange ROIs correspond to neurons

modulated by reward. Bottom, Venn diagram representing the overlap of reward related and first press related neurons. **f**, Heatmap of the PETH of positively modulated neurons for each press event (top panel) and the average of these PETHs (bottom panel). Grey shadow denotes s.e.m. **g**, Activity of first-lever press responsive neurons aligned to the moment when mice cross from the magazine in direction of the lever, before the first lever press. This is the same event that triggers dopamine neurons inhibition in the ArchT experiments (n=22). **h**, latency to initiate lever press sequence for laser-off trails and trials with inhibition starting just before sequence initiation for both ArchT (n=11) and YFP (n=9) groups. Right plot shows the mean latency to sequence initiation during laser-on trials normalised by the latency during laser-off trials. **i**, Left, distribution of latencies for laser on and laser of trials, for the ArchT and YFP groups. Right, percentage of late initiations (latency >5 s) for laser-off trails and trials with inhibition starting just before sequence initiation for both ArchT (n=11) and YFP (n=9) groups. **j**, Press rate in trials with no light delivery and trials with light delivery starting after the first press for both ArchT (n=11) and YFP (n=9) groups. Right plot shows the mean press rate during laser-on trials normalised by mean press rate during laser-off trials.

* $P<0.05$. Clear bars indicate laser-off and filled laser-on blocks in panels g,h and I. Error bars represent S.E.M.