Opposing roles of the dorsolateral and dorsomedial striatum in the acquisition of skilled action sequencing

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Abstract

The shift in control from dorsomedial to dorsolateral striatum during skill and habit formation is well established, but whether striatal subregions orchestrate this shift co-operatively or competitively remains unclear. Cortical inputs have also been implicated in the shift towards automaticity. Do cortical inputs mirror their downstream striatal targets across this transition? We addressed these questions using a five-step heterogeneous action sequencing task that is optimally performed by automated chains of actions. By optimising automatic responding, we discovered that loss of function in the dorsomedial striatum accelerated acquisition. In contrast, loss of function in the dorsolateral striatum impeded acquisition of sequencing, demonstrating functional opposition within the striatum. Unexpectedly the medial prefrontal cortex was not involved, however the lateral orbitofrontal cortex was critical. These results shift current theories about striatal control of behaviour to a model of competitive opposition, where the dorsomedial striatum acts in a gating role to inhibit dorsolateral-driven behaviour.

Keywords

Striatum, prefrontal cortex, orbitofrontal cortex, cortical, skill, habit, action.

INTRODUCTION

Automaticity of actions is characteristic of both skilled behaviours and habits. Initiation and execution of chunked motor sequences reduces the need for cognitive resources though forfeiting options of flexibility and control (Graybiel & Grafton, 2015; Robbins & Costa, 2017). The transition to automaticity is paralleled by a well-documented shift in control from the dorsomedial (DMS) to dorsolateral (DLS) striatum (Ashby, Turner, & Horvitz, 2010; Graybiel & Grafton, 2015; Kupferschmidt, Juczewski, Cui, Johnson, & Lovinger, 2017; Thorn, Atallah, Howe, & Graybiel, 2010; Yin et al., 2009). Yet, it is unknown how this transition occurs and how these regions co-ordinate the control of actions (Bergstrom et al., 2018; Kupferschmidt et al., 2017).

Early in instrumental conditioning, goal-directed behaviour depends on DMS function, giving way to control by the DLS if conditions support habitual responding (Balleine, Liljeholm, & Ostlund, 2009; Yin & Knowlton, 2006; Yin, Knowlton, & Balleine, 2004, 2005, 2006; Yin, Ostlund, Knowlton, & Balleine, 2005). Similarly, in skill learning there is an early learning phase where actions are variable and slow but as they become refined and efficient then control shifts from the DMS to DLS (Kupferschmidt et al., 2017; Lehericy et al., 2005; Miyachi, Hikosaka, & Lu, 2002; Yin et al., 2009). Skilled behaviour refers to efficient sequences of actions that are often goal-directed, while stimulus-response habits may also refer to 'chunked' sequences of actions however they are autonomous of the goal (Dezfouli & Balleine, 2012; Jin & Costa, 2015; Robbins & Costa, 2017). Neural recordings indicate the DMS and DLS operate in parallel during this transition with some degree of interdependency (Bergstrom et al., 2018; Gremel & Costa, 2013; Kupferschmidt et al., 2017; Vandaele et al., 2019; Yin et al., 2009). In fact, the DLS appears to be engaged from the beginning of conditioning and only after initial experience does the goal-directed system start driving behaviour. However, it is unclear whether the DMS and DLS act via a

co-operative or competitive relationship (Balleine et al., 2009; Smith & Graybiel, 2016). One dual control account suggests these two processes both contribute to an animal's behaviour with the relative influence shifting with extended training (Balleine, 2019; Dickinson, 1985; Robbins & Costa, 2017). Alternatively, habits may form early but remain latent or inhibited unless required (Hardwick, Forrence, Krakauer, & Haith, 2019). Similar accounts may apply to the relative neural contribution of the DMS and DLS.

If these regions operate independently, then loss of function should impair only that region's function, however if they operate co-operatively then suboptimal performance would be expected in both functions. In contrast, an 'opponent' model would predict that loss of function in one region would favour the alternate structure's function. The role of cortical inputs may be critical in arbitrating this striatal balance (Daw, Niv, & Dayan, 2005; Peak, Hart, & Balleine, 2019). A problematic issue when addressing this question in habits has been the "zero-sum" interpretation as habits are defined by a lack of goal-directed features (Robbins & Costa, 2017; Schreiner, Renteria, & Gremel, 2020). Identifying habits also often imposes an element of flexible or goal-directed behaviour (e.g. a choice test after devaluation) to determine whether actions are sensitive to changes in value or contingency. Habits are rarely measured as the optimal response. Hence, we developed a novel rodent task using a series of heterogeneous actions where rigid, automated responding would lead to superior performance to test models of striatal control during the development of automaticity. We hypothesised that DMS loss of function would causally accelerate, whereas DLS loss of function would impair, the development of behavioural automaticity.

RESULTS

A novel five-step action sequencing task in rats

Using a multiple-response operant chamber (Carli, Robbins, Evenden, & Everitt, 1983), rats made a nose poke response in each of five holes from left to right to receive a reward pellet in the magazine (Figure 1A). After brief training, rats could initiate self-paced sequences during a daily 30 min session (Figure 1C). The only experimental cues provided were the magazine light signalling reward delivery, and a 5s house light indicating time out after an incorrect response. Preliminary studies found that incorporating a timeout after an error occurred prevented elongated sequence attempts and improved automatisation. To maximise rewards, rats should complete fluent sequences as rapidly, as well as accurately as possible. Improved speed and accuracy are two features of skill learning that are not typically captured by measures of habit, despite their relevance to automaticity. As there was little need for flexible variation, automatisation would reduce the use of cognitive resources and thus promote efficient action sequencing. Acquisition was observed over 15 sessions that were grouped into five blocks of three sessions. Response times across the five actions in the first acquisition block were comparable but following further training a ballistic response pattern developed. This pattern was characterised by an extended initiation pause prior to the first element that led into a rapid escalating response pattern from holes 2-4, being completed with a concatenation pause following the terminal element. Here the rat anticipates and prepares the next motor sequence - reward retrieval. Data from treatment-naïve rats (n=36) indicated that from the first to last block there was a significant change in nose poke duration at each location (interaction $F_{2,82}=19.80$, $p<0.001$; pairwise comparisons p's<0.025) (Figure 1B). The ballistic response pattern began to emerge in the first block with relatively equivalent variation at each step. By the last block, each action in the sequence became increasingly faster and less variable, indicative of refined and automated action sequencing. This response pattern, particularly the initiation and termination delays, are characteristic of

motor sequence chunking (Abrahamse, Ruitenberg, de Kleine, & Verwey, 2013; Sternberg, Monsell, Knoll, & Wright, 1978).

Importantly, the sequential nose poke task was self-initiated and not cued. This required the acquisition and then retrieval of a planned motor sequence, of which the first four actions were never immediately rewarded. The removal of cues also ensured that the sequence required internal representation where enhanced performance was due to an improved representation and retrieval of the sequence rather than an improved ability to detect stimuli (Yin, 2010). With five individual steps required in sequential order to receive a reward, it is highly unlikely to be performed by chance. Following repeated reinforcement, it was expected that these five individual actions would be chunked into a more efficient unitary motor program.

DMS-lesioning improved acquisition of action sequencing, while DLS-lesioning impaired efficient sequencing

Initial training

To determine if the DMS and DLS work cooperatively or in opposition, subregion-specific loss of function was required throughout training and acquisition (Figure 2A, B). Lesions made via discrete fiber-sparing quinolinic acid infusions avoided any overlap between the DMS and DLS. Following recovery, rats were food restricted and trained on the sequencing task (see Figure 1C for schedule). DLS-lesioned rats took significantly longer to reach training criteria (Figure 2C; Lesion: $F_{2,23}$=7.80, p=0.003) than DMS-lesioned (p=0.045) or sham treated rats (p=0.001). Rats then moved to sequence acquisition where only correct 5-step sequences were rewarded.

Sequence acquisition

We compared performance measures during acquisition to quantify action sequence refinement, with a focus on changes between the first (sessions 1-3) and last blocks (sessions 12-15). Across acquisition, DMS-lesioned rats initiated more trials (Figure 2D; Lesion: $F_{2,23}$=6.94, p=0.004) than either DLS-lesioned (p=0.002) or sham (p=0.005) rats. The number of trials initiated was equivalent between groups in the first block (p>0.3). However, by the last block there were opposing effects detected between groups (Lesion: $F_{2,23}$=11.59, p<0.001). DLS-lesioned rats initiated significantly fewer trials than sham rats (p=0.09), while DMS-lesioned rats completed significantly more trials than sham rats (p=0.025); with a substantial difference between DMS and DLS groups (p<0.001). This acquired divergence between DMS and DLS lesioned rats demonstrated that DMS-lesions enhanced, while DLS-lesions impaired, initiation of action sequences. As sessions were time limited, performing more trials indicated greater speed and opportunity for reward, however these trials could have been either correct or incorrect.

The number of correct sequences increased for all groups across acquisition, indicating all groups were able to learn the five-step sequence. Opposing effects of striatal lesions were again also observed in the total number of correct sequences. There was no difference between groups on the first block, yet there was a clear divergence between DMS and DLS lesioned rats across acquisition (Figure 2E). On the last block the main effect of Lesion was not significant ($F_{2,23}$=2.67, p=0.09) but given our a priori hypothesis that DMS and DLS lesions would have opposing effects, a comparison between the lesion groups found that DMS-lesioned rats completed nearly twice as many correct sequences as DLS-lesioned rats at the end of acquisition (DMS = 117±12, DLS = 67±13; t_{13}=2.79, p=0.015). Despite the dissociation between groups in both the number of sequences initiated and correct sequences, there was no difference in the number of incorrect sequences made by each group (Figure 2F; $F_{2,23}$=0.16, p=0.85) and all groups showed a significant reduction in erroneous sequences

from the first to last block (p's<0.01). These results support a model of sequence learning where the DMS and DLS have opposing roles in the development of automated behaviours.

Sequence timing

We next investigated how striatal lesions influenced the timing of actions within sequences. Across sequence acquisition, sequence duration significantly reduced (Figure 2G; $F_{4,92}$=6.74, p<0.001), indicating increased sequencing efficiency with experience. This is important as faster execution is considered one of the hallmarks of skill learning and sequence chunking. Throughout acquisition, DLS-lesioned rats took longer to execute complete sequences (Lesion: $F_{2,23}$=4.59, p=0.021) than sham rats (p=0.007) and DMS-lesioned rats; however, this comparison failed to reach significance (p=0.059). All groups completed sequences significantly faster from the first to last block of acquisition (sham, t_{10}=2.33, p=0.042; DMS, t_6=4.78, p=0.003; DLS, t_7=2.83, p=0.026). In the final block, DLS-lesioned rats took significantly longer to complete sequences (Lesion: $F_{2,23}$=5.87, p=0.009) than sham (p=0.004) and DMS-lesioned rats (p=0.013), supporting the conclusion that DLS lesions impaired the development of refined action sequencing. In addition, we examined each individual rat's standard deviation of sequence duration to determine if variability reduced with training, as another hallmark of skill learning and automaticity. Only the DMS-lesioned rats had a significant reduction from the first to last block in their individual sequence duration variability (Supplementary Figure 1C), in agreement with other measures indicating enhanced automatisation of sequencing with DMS lesions.

As the task utilised five spatially heterogeneous responses, the timing of each action within the sequence was then compared across the initiation (hole 1), execution (holes 2-4) and terminal (hole 5) responses as well as the nose poke duration within each hole. Nose poke duration became faster across acquisition (Figure 2H; Block: $F_{4, 92}$=19.57, p<0.001) and developed the characteristic accelerating response pattern (Block X Hole: $F_{16, 368}$=9.07,

p<0.001). There were no significant differences between groups in the first block of acquisition (Figure 2H; $F_{2,23}$=0.67, p=0.52). However, by the last block, nose poke duration had stratified to a ballistic response pattern and the variance in timing had reduced as the movement became stereotypical. On the last block, there was a main effect of Hole ($F_{2,49}$=67.84, p<0.001) and the Hole X Lesion interaction approached statistical significance ($F_{4,49}$=2.51, p=0.051). Given our interest in whether there was divergence between DMS and DLS groups, planned post-hoc comparisons found DLS-lesioned rats paused significantly longer than DMS-lesioned rats on the first two actions of the sequence (hole 1, t_{13}=2.28, p=0.040 and hole 2, t_{13}=2.92, p=0.012) but not the latter half of the sequence (p's>0.7). These results demonstrate that while DLS-lesioned rats were capable of extremely fast nose poke responses (see hole 4) and therefore were not exhibiting general motor impairments (also see locomotion data in Supplementary Figure 1G), they were significantly delayed in initiating the sequence. These results indicated that the DLS is important for action selection or retrieval. However, once the sequence was engaged, its execution was not dependent on intact DLS function.

The inter-poke interval between correct nose pokes also speeded with training (Supplementary Figure 1A, B) indicating improved efficiency. There was a u-shaped pattern across the curved wall, likely reflecting ambulation requirements ($F_{3, 45}$=44.88, p<0.001) but there was no effect of lesion ($F_{2, 23}$=1.04, p=0.37) or interactions with treatment groups (p's>0.1). There was also no effect of group on the latency from leaving the magazine to starting at hole 1 (Supplementary Figure 1D), indicating all groups were equally as motivated to initiate sequences. There were also no significant changes in magazine nose poke duration (Supplementary Figure 1F) or reward collection latency (Supplementary Figure 1E) over acquisition or between groups suggesting training and lesions did not alter reward motivation.

Given the groups were equal at the beginning these results suggest that DMS lesions accelerated the shift towards automatisation, while DLS lesions impaired the development of efficient action sequencing. Delayed sequence initiation but not execution, suggests difficulties in loading the motor program particularly if distal sequence elements are under stimulus-response control. Cortical inputs to the striatum play an important role in both adaptive and compulsive responding therefore we sought to determine whether subregions within the prefrontal cortex influence the acquisition of action sequencing. We hypothesised that cortical regions with inputs into the DLS would impair sequence acquisition, while those with inputs to the DMS may enhance acquisition.

Lateral OFC but not medial OFC lesions impair sequencing

We first examined the role of the medial (mOFC) and lateral (lOFC) orbitofrontal cortex, which projects to medial and lateral regions of the dorsal striatum, respectively. Lesions to the mOFC result in habitual responding via an inability to retrieve outcome value in the devaluation test (Bradfield, Dezfouli, van Holstein, Chieng, & Balleine, 2015; Bradfield, Hart, & Balleine, 2018). In contrast, the lOFC is well known for its role in flexible responding in reversal learning, outcome prediction and devaluation (Gremel et al., 2016; Gremel & Costa, 2013; Hervig et al., 2019; Izquierdo, 2017; Panayi & Killcross, 2014; Turner & Parkes, 2020). However, it was unclear whether these regions would enhance automated behaviour in this task.

Acquisition of sequencing

Using the same procedure, we determined if the mOFC and lOFC were required for action sequencing (Figure 3A, B). During training, there was no effect of lesions on the number of sessions required (Figure 3C). Yet across sequence acquisition, there was a significant interaction between treatment groups for the number of trials initiated (Figure 3D;

Lesion X Block, $F_{8, 180}$=2.72, p=0.024) with lOFC-lesioned rats starting significantly fewer trials than the mOFC group in the final two blocks. There was no difference between groups in the first block, but by the last block lOFC-lesioned rats initiated fewer trials than mOFC-lesioned rats (main effect Lesion, $F_{2,27}$=4.49, p=0.021; post-hoc comparison p=0.006). lOFC-lesioned rats were also the only group to show a significant *reduction* in trials completed from the first to last block (t_9=3.17, p=0.011). A reduction in trials initiated occurs early when rats learn to suppress incorrect sequences. Rats then increase the number of trials as their efficiency to produce correct sequences increases.

There was a main effect of Lesion on the number of correct sequences completed (Figure 3E; $F_{2, 27}$=3.55, p=0.043) with lOFC-lesioned rats producing significantly fewer correct sequences than sham treated rats (p=0.014) throughout acquisition, suggesting a more general sequencing deficit rather than delayed acquisition. There was also a significant Lesion X Block interaction for the number of incorrect sequences produced (Figure 3F; $F_{5, 108}$=2.59, p=0.034). This was most evident in the early blocks with more errors from lOFC-lesioned rats in block 2 (p=0.026); and both lOFC and mOFC-lesioned rats in block 3 (mOFC p=0.042, lOFC p=0.055) compared to the sham group.

Sequence timing

There was an overall significant reduction in total sequence duration across acquisition (Figure 3G; $F_{4, 108}$=11.11, p<0.001), however only the mOFC-lesioned group showed a significant reduction in duration from the first to last block (sham: t_7=1.52, p=0.17; mOFC: t_{11}=5.28, p<0.001; lOFC: t_9=1.14, p=0.29). Rats became significantly faster at executing nose pokes from the first to last block ($F_{1,27}$=26.28, p<0.001) with a significant Block X Hole interaction (Figure 3H; $F_{4, 108}$=22.33, p<0.001) as response times shifted to a ballistic response pattern with training. Between treatment groups, there was a significant Lesion X Hole ($F_{5,64}$ =2.68, p=0.032) interaction with both lesion groups making faster

responses in the middle of the sequence than sham rats (hole 3 p's<0.003), yet lOFC-lesioned rats were significantly delayed on the terminal action in the sequences compared to mOFC-lesioned rats (p=0.011). There was no significant change in the duration of time spent in the magazine (Supplementary Figure 2F) or latency to collect the reward (Supplementary Figure 2E) either over training or between groups. The inter-poke intervals were also not significantly different for lesioned rats, although they appeared slower on the first block leading to a significant Block X Lesion interaction following the shift to sham levels by the final block (Supplementary Figure 2A, B). The lOFC-lesioned rats were highly efficient at mid-sequence execution but had relatively elongated terminal nose pokes, when rats usually pause to detect cues associated with pellet delivery and start the next motor plan - reward collection.

Prelimbic and infralimbic cortex lesions do not alter sequence acquisition.

To further understand the role of the prefrontal cortex, we next examined the effects of excitotoxic lesions of the prelimbic (PrL) and infralimbic (IL) cortex. These regions are associated with goal-directed and habitual behaviour respectively, with the PrL having strong inputs to the DMS and the IL into the ventral striatum (Coutureau & Killcross, 2003; Hart, Leung, & Balleine, 2014; Heilbronner, Rodriguez-Romaguera, Quirk, Groenewegen, & Haber, 2016; Mailly, Aliane, Groenewegen, Haber, & Deniau, 2013).

Acquisition of sequencing

Identical procedures were implemented in PrL and IL lesioned rats (Figure 4A, B). All groups reached criteria before moving onto the sequence acquisition (Figure 4C). The number of correct sequences significantly increased across acquisition (Figure 4E; $F_{2, 51}$=26.57, p<0.001) and incorrect sequences significantly decreased (Figure 4F; $F_{2, 41}$=58.93,

p<0.001) with no effect of treatment or interactions on trials initiated (Figure 4D) or the number correct or incorrect sequences (Figure 4E, F).

Sequence timing

While there was a main effect of Block (Figure 4G; $F_{3, 64}$=2.95, p=0.041) on total sequence duration where rats became significantly faster at executing the sequence with training there was no significant difference between lesion groups for nose poke duration across sequence or magazine (Supplementary Fig 3F), inter-poke intervals between holes (Supplementary Fig 3E), or interval from hole 5 to the magazine (Supplementary Fig 3D). Nose poke duration did reduce from first to last block ($F_{1, 22}$=7.61, p=0.011) across all lesion groups and a significant Block X Hole interaction (Figure 4H; $F_{4, 2}$=8.64, p<0.001) identified a ballistic-like response pattern with training. These results indicated that the PrL and IL cortex are not critical for the acquisition of action sequencing.

DISCUSSION

Our results provide the first direct causal evidence that the loss of function in the DMS and DLS have opposing roles on the skilled acquisition of sequencing behaviour. Loss of DLS function impaired, while loss of DMS function enhanced the acquisition of action sequencing. These results build on previous studies in rodents suggesting that disengagement of the DMS predicts skill learning by allowing the DLS to take control (Kupferschmidt et al., 2017), and that the DMS gates habit formation in the T-maze, suggesting that although the DLS is active early during learning it only gains control when DMS activity subsides (Thorn et al., 2010). Here, we demonstrate that DMS lesions enhance acquisition of rigid, automatic behaviour that is impaired by DLS-lesions. Furthermore, while the lOFC was required for efficient sequencing, the results of dorsal striatum lesions were not reproduced when following lesions of upstream prefrontal subregions, however the role of the anterior

cingulate cortex and motor cortex (M1 and M2) remains to be tested. Together, these results demonstrate that disengagement of the DMS to facilitate the acquisition of DLS-dependent skills and habits is not a simple product of reduced top-down drive from cortical inputs. However, given that these key cortical regions did not mirror the effects observed by striatal manipulations, it is possible (and perhaps likely) that the source of arbitration between these parallel corticostriatal loops occurs downstream of the dorsal striatum. Concurrent activity observed within the DMS and DLS across numerous tasks and training stages also supports this suggestion (Kupferschmidt et al., 2017; Thorn et al., 2010; Thorn & Graybiel, 2014). Where and how this arbitration process elicits actions remains to be elucidated.

Opposing roles of the dorsal striatum in the acquisition of action sequences

Previous studies have shown habitual responding can be acquired despite DMS lesions (Gremel & Costa, 2013; Hilario, Holloway, Jin, & Costa, 2012), suggesting a DMS-dependent goal-directed acquisition phase is not required for the development of habits. We provide evidence for this hypothesis by demonstrating not only that DMS-lesioned rats were capable of performing automatised action sequences, but that they show *enhanced* acquisition and reduced variability of this habit-like response pattern. These results not only indicated that DMS-dependent learning was not critical for efficient task acquisition, but that DMS functions to oppose the development of an optimal but automatic sequence. In contrast, rats with a dysfunctioning DLS were impaired in acquiring action sequencing.

The results are consistent with the findings of Moussa, Poucet, Amalric, and Sargolini (2011) for which the opposite pattern was shown for a T-maze task requiring *flexible* responding (in contrast to this sequencing task which required stable, automatic responding). They found DMS lesions impaired acquisition, but DLS lesions enhanced learning rate. A second example of striatal opponency is the demonstration by Bradfield and Balleine (2013)

that removing the influence of the DLS actually enhanced goal-directed control beyond the capacity of sham treated rats. A third example comes from a study of visual discrimination where silencing the DLS during the choice phase led to faster learning, again highlighting that removing DLS activity enhances adaptive behaviours beyond those seen when both regions are functional (Bergstrom et al., 2018). Together with the current study, these results demonstrate competitive opponency between the DLS and DMS by utilising tasks optimised by flexible responding or automaticity, respectively (see Figure 5).

Habits, skills and automaticity

Here we capitalised on a task that is dependent on reduced behavioural variation (rather than overtraining) to examine the neural underpinnings of automatisation with relevance for both habits and skills. How the similarities and differences between habits and skills can be consolidated has been a topic of growing interest that remains largely unanswered (Ashby et al., 2010; Graybiel & Grafton, 2015; Hardwick et al., 2019; Robbins & Costa, 2017). While acknowledging that each is defined by specific characteristics, these results sit at the intersection of skills and habits and are therefore discussed in this broader context.

Automaticity is commonly measured in skill learning using tasks such as rotarod (Kupferschmidt et al., 2017; Yin et al., 2009) and action sequencing paradigms, including fixed ratio lever pressing or shorter two-step sequencing (e.g. L-R lever press) (Cui et al., 2013; Garr & Delamater, 2019; Jin, Tecuapetla, & Costa, 2014; Tecuapetla, Jin, Lima, & Costa, 2016; Yin, 2009, 2010; Yin, Ostlund, et al., 2005). A four-step (L-L-R-R) lever press task was recently developed using no experimental cues and a self-paced design (Geddes, Li, & Jin, 2018). However, to our knowledge, models of skill and habit formation have not been tested in rodent operant paradigms requiring more than two different response elements to

contrast initiation, execution and termination sequence elements as would be more common in real-world behaviours. This will be important for understanding how the DMS and DLS operate and contribute to action selection, execution and termination. We found that while DLS-lesions impair acquisition, the deficits predominately affected sequence initiation rather than execution, reflecting the habit-like nature of actions distal to rewards within action sequences observed by Balleine, Garner, Gonzalez, and Dickinson (1995). This also suggests the DLS deficit is one of selection or loading of motor plans, rather than motor execution, which is also evident in task bracketing patterns within the DLS (Jin & Costa, 2010; Smith & Graybiel, 2013; Sternberg et al., 1978). Isolating the role of striatal circuits within sequence performance is critical for understanding movement disorders such as Parkinson's disease (Agostino, Berardelli, Formica, Accornero, & Manfredi, 1992).

Yin et al. (2004) found DMS lesions increased response rates under a habit-inducing FI-20 schedule, aligning well with the increased sequencing behaviour observed in our study. In a subsequent study using a ratio schedule to maintain goal-directed action, DMS-lesioned rats were insensitive to devaluation (Yin, Knowlton, et al., 2005), however this may have either been due to enhanced habit formation or impaired goal-directed control. Goal-directed actions and habitual responding are typically measured simultaneously, however determining whether a change is due to a lack of one function or enhancement of the other is not easily determined. However, there is evidence that they are independent processes (Hardwick et al., 2019).

To focus on habit-like responding we developed this original sequencing task so that repetitive behaviours would be optimal, allowing examination of the divergent impacts of DLS and DMS lesions on the development of automaticity. The highly repetitive and accelerating execution of sequences observed here reflect qualities expected of an automatic and chunked behavioural repertoire. Reduced variation through rigid repetition may also be

critical conditions for the development of habits, which we observed as significantly reduced variation in sequence duration across acquisition in DMS-lesioned rats. Indeed, using an FR5 lever press task Vandaele and Janak (2021) recently reported that rats performed habitually under strict sequencing conditions (DT5), but that allowing rats to either make mid-sequence reward port entries or greater than five presses reverted behaviour to goal-directed control. This was accompanied by high DLS and low DMS activity during the DT5 task, but relatively similar activity across the striatum in the task variants. As pointed out by Dickinson (1985), *"contrary to popular belief, habit formation is not a simple consequence of over-training or practice. Rather it appears to arise because over-training typically tends to reduce the variation in behaviour..."* (page 76). Similarly, Daw et al. (2005) suggested that the shift from a model-free to model-based control is dependent on uncertainty, where even providing two choices will prevent model-free responding. Further, Drummond and Niv (2020) suggest that the level of *certainty* within the model-based and model-free estimates may determine which system becomes engaged. Experimental support for the notion that habits are not merely the product of overtraining was demonstrated across five studies that failed to produce habitual responding in humans (de Wit et al., 2018). In addition, evidence from Hardwick et al. (2019) suggests habits form easily, but their expression can be overruled by goal-directed control such that time to act is also critical factor in determining which is expressed. Action sequences are not only invariant but also performed with rapid pace, which may explain the contribution of automaticity in habit and skill formation.

Cortical functions in action sequencing

Cortical inputs may play a critical role in goal-directed learning, habit formation and skill development but less is known about how they operate across transitions (Bassett, Yang, Wymbs, & Grafton, 2015; Bergstrom et al., 2018; Bradfield et al., 2018; Gremel & Costa,

2013; Killcross & Coutureau, 2003; Kupferschmidt et al., 2017; Smith & Graybiel, 2013; Turner & Parkes, 2020). A link between cortical disengagement and skill refinement has been observed using imaging in humans (Bassett et al., 2015) and recordings in rodents (Kupferschmidt et al., 2017). As these are correlational findings, reduction in cortical activity may not be required for skill refinement but appears as a consequence of changes in other regions within cortico-striatal loops. Previous research has associated PrL with goal-directed actions and the IL with habits. Shipman, Trask, Bouton, and Green (2018) suggested that control shifts from the PrL to IL with experience but prior to habit formation. This may be the reason that these highly implicated cortical regions had much subtler or no effect on acquisition of automatised behaviour compared to striatal manipulations. The lack of effect observed in the current study also highlights the importance of considering tasks that optimise automatic and habitual actions in parallel with tasks that favour goal-directed or flexible control of behaviour to understand cortico-striatal functions.

The PrL cortex is important for early stages of goal-directed learning but not for habit formation (Corbit & Balleine, 2003; Coutureau & Killcross, 2003; Hart, Bradfield, Fok, Chieng, & Balleine, 2018), so the lack of effect in this study where goal-directed control was minimised is consistent. The fact that PrL lesions did not *enhance* sequencing indicates that the PrL inputs to the DMS are not solely responsible for maintaining DMS functions or goal-directed interference on this task and the role of the PrL cortex is clearly separable. This independence of functions between the PrL cortex and DMS suggests the release of control from goal-directed to habitual responding within the dorsal striatum is not driven by PrL function.

Lesioning the IL did not impair sequence acquisition as would have been predicted from devaluation studies where IL-lesions result in goal-directed responding (Coutureau & Killcross, 2003). In contrast to the proposal by Smith and Graybiel (2013) that the IL and

DLS operate to establish habits, we found no IL-related deficit in sequence acquisition as was observed for DLS lesions. This suggests that the IL was not required for the automatisation or chunking of action sequences in a task with a minimal goal-directed phase. It is important to note that there are differences between the electrophysiological signatures of DLS and IL in habits (e.g. after devaluation), and there are no direct IL-DLS projections, suggesting they have independent roles in habitual responding (Smith & Graybiel, 2013). Haddon and Killcross (2011) found that the IL plays a role when goal-directed and habitual associations are in competition, but this was not the case in our study as flexible, goal-directed responding was not advantageous. Therefore, the results of this study support the argument that this competition, particularly in the context of extended goal-directed training, may be an important condition for IL-dependent habits, as with little-to-no competition, IL lesions did not influence action sequence acquisition.

In contrast, lOFC lesions reduced total sequences with fewer correct sequences (although increased incorrect sequences) and delayed sequence termination. While largely consistent with deficits in DLS-lesioned rats, two key differences emerged (i) lOFC lesioned rats were relatively slower to terminate sequences and (ii) had higher rates of incorrect responses. This is important given the lOFC has been implicated in perseverative and compulsive behaviours, which lack appropriate termination and do not obtain the goal (Burguiere, Monteiro, Feng, & Graybiel, 2013; Chudasama & Robbins, 2003).

Prior studies have found that large lOFC lesions produced similar effects to those seen in DMS-lesioned animals performing under both random ratio (RR) and random interval (RI) schedules (Gremel & Costa, 2013). The lack of devaluation sensitivity in both RR and RI contexts following lOFC loss of function was suggested to indicate its role in conveying action-value information. These results are in contrast to studies suggesting the lOFC is important for Pavlovian, but not instrumental, processes (Chudasama & Robbins, 2003;

Ostlund & Balleine, 2007). The terminal delay in our study, as well as the delayed reward collection latency in Hervig et al. (2019), may be due to the lOFC's role in predicting outcomes based on Pavlovian cues since the reward delivery is cued (Ostlund & Balleine, 2007; Panayi & Killcross, 2014). Furthermore, the role of the lOFC in using Pavlovian occasion setting cues may also explain the impairment in reducing incorrect responses, which are signalled by the illumination of the house light (Shobe, Bakhurin, Claar, & Masmanidis, 2017).

Conclusions

These findings provide the strongest evidence yet for competition between DMS and DLS functions in the development of behavioural automisation. We found medial prefrontal subregions were largely unnecessary for sequence acquisition, however lesions to the lOFC impaired action sequencing. Developing an innovative spatial heterogeneous action sequencing task, we were able to isolate initiation, execution and termination specific deficits. These results provide empirical support for a model where DMS activity limits the formation of automated behaviour, emphasising its role in gating the acquisition of skills and habits.

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Author contributions

KMT and TWR designed the experiments; KMT, ML, AS, CM performed the experiments; KMT wrote the first draft and ML, AS, CM, TWR reviewed and edited the manuscript.

Declaration of interests

The authors declare no competing interests.

FIGURE LEGENDS

Figure 1. The sequential nose poke task leads to ballistic responding.

(A) Rats were trained to make a five-step nose poke sequences to receive a food reward if they nose poked into each of the holes in order from left to right.

(B) Rats developed a ballistic response pattern across the five holes from the first to last block of training. Each nose poke was faster and with less variance as the sequence progressed.

(C) The training schedule included habituation to the magazine and nose poke training (see Methods). The nose poke cues were rapidly removed once rats were responding to each hole. From the beginning of the sequence acquisition period only correct five-step sequences were rewarded and errors were penalised by a brief time out period, after which the sequence had to be reinitiated.

Figure 2. DMS-lesioning improved acquisition of action sequencing, while DLS-lesioning impaired efficient sequencing.

(A) Rats received targeted bilateral lesions with extent illustrated for lesion groups; sham (open, n=11), DMS (blue, n=7), DLS (red, n=8).

(B) Striatal sections showing NeuN staining in sham (left), DMS (middle) and DLS (right) lesioned rats.

(C) DLS-lesioned rats required significantly more sessions to reach training criteria than sham or DMS-lesioned rats.

(D) Left: When acquiring sequencing behaviour, DMS-lesioned rats initiated more trials than either DLS-lesioned or sham rats. Right: There was no significant difference between groups in the first block, however by the last block, DLS-lesioned rats started fewer trials and DMS-lesioned rats completed more trials than sham.

(E) Left: Contrasting effects of lesions were also observed for the number of correct sequences. Right: DMS-lesioned rats completed nearly twice as many correct sequences than DLS-lesioned rats in the last block of acquisition.

(F) Incorrect sequences decreased across acquisition, demonstrating all groups learned to avoid errors.

(G) Left: Overall, DLS-lesioned rats took longer to complete sequences than sham rats. Right: All groups completed sequences significantly faster from the first to last block of acquisition and in the final block DLS-lesioned rats took significantly longer to complete sequences than sham and DMS-lesioned rats.

(H) Across acquisition, the duration of nose pokes became faster and developed a ballistic response pattern. Right: By the last block, DLS-lesioned rats paused significantly longer than DMS-lesioned rats on the first two actions of the sequence, but not the latter half of the sequence.

Data shown as group mean ± S.E.M. *$p<0.05$.

Figure 3. Lateral OFC but not medial OFC lesions impair sequencing.

(A) Rats received targeted bilateral lesions as shown for sham (open, n=10), mOFC (purple, n=8) or lOFC (green, n=12).

(B) Sections showing NeuN staining for sham (left), mOFC (middle) and lOFC (right) lesion groups.

(C) Sessions to reach training criteria was not different between groups.

(D) The number of trials initiated was not different in the first block, but signficantly reduced in lOFC- compared to mOFC-lesioned rats after acquisition.

(E) lOFC-lesioned rats producing significantly fewer correct sequences than sham rats across acquisition.

(F) All rats significantly reduced incorrect sequences over acquisition. During early acquisition, lOFC-lesioned rats continued to make more incorrect sequences in block 2 and both lesion groups made more errors in block 3 compared to the sham group.

(G) There was a trend for reduced sequence execution time across acquisition, however only the mOFC-lesioned group significantly reduced sequence duration from the first to last block.

(H) At the end of acquisition, nose poke duration was faster in lesioned rats than sham controls in the middle of the sequence, however lOFC-lesioned rats were slower at terminating the sequences compared to mOFC-lesioned rats.

Data shown as group mean ± S.E.M. *$p<0.05$.

Figure 4. Prelimbic and infralimbic cortex lesions do not alter sequence acquisition.

(A) Rats received targeted bilateral lesions as shown for sham (open, n=9), PrL (cyan, n=9) or IL (orange, n=7).

(B) Sections showing NeuN staining in sham (left), PrL (middle) and IL (right) lesion groups.

(C) The number of trials initiated was not different between groups.

(D) The number of correct sequences significantly increased without an effect of lesion.

(E) Incorrect sequences significantly decreased, and this was also not different between groups.

(F) Rats became significantly faster at executing the sequence with training with no significant differences between groups.

(G) Total sequence duration reduced across the acquisition period but was not different between groups.

(H) Nose poke duration shifted to the characteristic accelerating pattern with no effect of PrL or IL lesion.

Data shown as group mean ± S.E.M. *p<0.05.

Figure 5. Competitive parallel control by the dorsomedial and dorsolateral striatum.

(A) Studies of adaptive behaviour have found DMS lesions impair performance as anticipated given the role of the DMS in goal-directed behaviours. However, three studies have also found DLS-lesioned rodents showed enhanced learning compared to shams, suggesting a competitive influence of DLS functions on DMS-dependent behaviours (Bergstrom et al., 2018; Bradfield & Balleine, 2013; Moussa et al., 2011). We build on this model by demonstrating that the converse is true for automatisation of actions. DLS lesions unsurprisingly impaired performance where the task demands habit-like behaviour. However, we found that DMS lesions enhanced acquisition, suggesting this competitive relationship is bidirectional.

(B) Based on these findings we propose a model of opponency between the DMS and DLS. In situations where adaptive or goal-directed behaviours are critical, DMS control dominates and results in performance of individual and slower actions that can be easily modified. Lesioning the DLS biases behaviour in this direction. We suggest that just as a purple colour gradient can be made bluer through either adding more blue (enhanced DMS activity) or not adding as much red (DLS lesioning); the relative balance is critical such that the loss of one region's function enhances expression of the other. Parallel development of both pathways incorporates redundancy such that either region can take control as situations change.

(C) Tasks requiring automatised actions, such as action sequencing and chunking, thrive under DLS-dominated control. Disengagement of the DMS to allow DLS domination has been proposed in the transition from goal-directed to habitual action and in skill refinement (Kupferschmidt et al., 2017). This study demonstrates that habit-like

behaviours can also be expedited via DMS loss of function, indicative of functional opponency.

METHODS

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Three experiments were conducted in separate cohorts of rats examining the effect of pre-training lesions of the (1) DMS and DLS; (2) mOFC and lOFC; and (3) PrL and IL on acquisition of action sequencing. Methods were the same across these experiments, with exceptions detailed below.

Animals and Housing

Adult male Lister-hooded rats weighing 280-300g (Charles River, UK) were housed in groups of four on reversed 12-h light cycle (off at 07:00) within a temperature (21°C) and humidity-controlled environment in open top cages with aspen bedding, wood block and tube. A week after arriving, rats were food-restricted to no less than 90% of free-feeding weight with unrestricted access to water and were exposed to reward pellets. All procedures were conducted in accordance with the United Kingdom Animal (Scientific Procedures) Act of 1986 and were approved by ethical review at the University of Cambridge.

METHOD DETAILS

Apparatus

Rats were trained to perform a five-step sequential nose poke task (SNT), which was adapted from Keeler, Pretsell, and Robbins (2014), however with substantial changes including absence of cues and the number and order of responses. The task was conducted in operant chambers (Campden Instruments, UK) with five nose poke apertures available within a horizontal array and a reward receptacle on the opposing wall (Robbins, 2002). Nose pokes and the reward receptacle were fitted with infra-red beams to detect head entries and a light for illumination. Reward sucrose pellets (AIN76A, 45mg; TestDiet, UK) were delivered into the receptacle by a pellet dispenser. A house light was mounted on the ceiling and the

chamber was contained within a sound attenuating box. Overhead cameras (SpyCameraCCTV, UK) were mounted above each chamber to monitor and record behaviour remotely. Whisker Server software and custom programming software was used to operate the chambers and record responses (Cardinal & Aitken, 2010; Keeler et al., 2014).

Sequential Nose poke Task (SNT) Protocol

The SNT requires rats to make a nose poke response into each of the five holes from left to right across a horizontal array to receive a food reward. Sessions ran for 30 min unless stated otherwise and all nose pokes and head entries were recorded with the duration of each nose poke calculated based on the entry and exit times. Rats were first habituated to the chambers and retrieved rewards from the receptacle that were dispensed with each head entry until 100 were collected (stage 1). Next, rats were trained to make nose poke responses into the five-hole array (stage 2). Each hole in the five-step sequence was illuminated for 1 s before moving to the next location from left to right and finishing with reward delivery (e.g. 1-2-3-4-5-Reward), which was signalled by illumination of the receptacle. Head entry into the receptacle triggered the start of the next trial. Critically, when the rat nose poked an illuminated hole, the light and sequence counter immediately moved on to the next hole, allowing the rat to achieve reward delivery faster than if they did not nose poke. If the rat made a nose poke into an alternative hole, the illuminated hole would flash for the duration of the incorrect nose poke to draw attention to the correct location. To further encourage nose poking, the illumination duration incremented by 10% of the original delay (1 s) each trial, further delaying reward delivery if nose pokes were not made. This training protocol was implemented to reduce bias for the start or end elements (inherent to training by chaining) and rapidly produced sequencing behaviour. Once rats were successfully able to complete at least 15 sequences within a session, they moved to stage 3 where the illumination sequence only advanced to the next hole, and ultimately to reward delivery, after a correct nose poke

response into an illuminated hole. Criteria for stage 3 was 50 complete sequences, which was typically achieved in a single session. Stage 4 was identical to stage 3, except that now the nose poke holes were no longer illuminated. After each of the holes had been poked in order, a reward was delivered. Incorrect nose pokes were recorded, but not punished. After reaching 50 uncued sequences, they were moved to the final level (stage 5) where incorrect nose pokes were punished with a 5 s time out period signalled by the illumination of the house light. After the time out ended, the rat was required to start the sequence again from hole 1. Responses during the timeout period were recorded but did not extend the time out duration. Testing on stage 5 was conducted for 15 sessions and rats began immediately after reaching training criteria. Key measures included trials initiated, correct sequences, incorrect sequences, nose poke durations at each location and total sequence duration.

Table 1. Summary of training stages and criteria to move to the next stage.

Stage	Summary	Criteria	Av. Sessions
Stage 1	Habituation to chamber	100 pellets x 1 session	1
Stage 2	Start nose poking 5 holes	>15 sequences x 1 session	7
Stage 3	Cued sequence – must NP	>50 sequences x 1 session	1
Stage 4	No cues	>50 sequences x 1 session	3
Stage 5	Incorrect = Time Out	Final stage	15

Table 2. Behavioural measures used to quantify action sequencing.

Trials	Total number of trials initiated
Correct	Number of completed sequences
Incorrect	Number of incorrect sequences
Sequence Duration	NP entry at NP1 to exit on NP5
NP Duration	Time from entry to exit of correct nose poke

Inter-Poke Interval (IPI)	Time from exit of previous NP to entry of next NP
Initiation Latency	Time from exit magazine to entry NP1 of next trial
Reward Latency	Time from exit NP5 to magazine entry when correct

Surgery

Prior to training rats were randomly assigned to receive either sham surgery or intracranial bilateral lesions to the region of interest under 2-3% isoflurane anaesthesia with local application of bupivacaine (2mg/kg s.c. at 0.8ml/kg; Sigma) at the incision site. Fibre-sparing lesions were induced by quinolinic acid (0.09M in PBS, Sigma Aldrich, UK) or phosphate-buffered saline (PBS) sham infusions at 0.1ml/min using the co-ordinates in Table 3 relative to bregma based on Paxinos and Watson (2005). Rats were treated with Metacam (1mg/kg; Boehringer Ingelheim) pre- and post-operatively and rehoused in groups of four after lesion surgery. After at least 7 days recovery, rats were food restricted and began operant training as described above.

Table 3. Co-ordinates and volumes used for pre-training lesion infusions of quinolinic acid. *DMS: dorsomedial striatum; DLS: dorsolateral striatum; PrL: prelimbic cortex; IL: infralimbic cortex; mOFC: medial orbitofrontal cortex; lOFC: lateral orbitofrontal cortex; ant: anterior; post: posterior.*

Region	AP	ML	DV	Vol (ml)
DMS	-0.4	+2.2	-4.5 (skull)	0.3
DLS	+0.7	+3.6	-5.0 (skull)	0.3
PrL ant	+3.5	+0.7	-2.5 (dura)	0.3
PrL post	+2.8	+0.7	-2.8 (dura)	0.3
IL ant	+2.9	+0.7	-4.0 (dura)	0.2
IL post	+2.5	+0.7	-4.0 (dura)	0.2

mOFC	+4.0	+0.6	-3.3 (dura)	0.3
lOFC	+3.5	+2.5	-3.6 (dura)	0.3

Locomotion

After completion of operant testing, rats were tested for 30 min in an open field arena to rule out gross locomotor impairments. Testing was conducted in lidded boxes (48 x 26.5 x 21cm, Techniplast, UK) in a quiet room with dim red lighting. Locomotion was recorded by infra-red beams across the arena (Photobeam Activity System, San Diego Instruments).

Histology

Rats were transcardially perfused using 0.01M PBS with 5g/L sodium nitrite followed by 4% formaldehyde. Brains were then removed for storage in 4% formaldehyde at room temperature overnight on a shaker. They were then transferred to 30% sucrose until they sank before being rapidly frozen and cut into 60mm sections on a freezing microtome (Leica). Sections were stained for NeuN to confirm lesion placement.

NeuN protocol

Sections were washed in 0.01M PBS and then placed in primary antibody (NeuN monoclonal mouse anti-neuronal nuclear protein, Millipore MAB377, 1:2000 in 0.4% Triton X-100 in 0.01M PBS) for two hours on a rotary shaker. Sections are washed three times in 0.01M PBS over 30 min, then secondary (biotinylated anti-mouse IgG, Vector Laboratories BA-2001, at 1:200 in 0.4% Triton X-100 in 0.01M PBS) applied for 90 min. Sections were washed three times in 0.01M PBS, before applying an immunoperoxidase procedure (Vectastain ABC Kit, Vector Laboratories). Sections were washed three times in 0.01M PBS before visualising in DAB (ImmPACT DAB Peroxidase (HRP) Substrate, Vector Laboratories) and stopping reaction with cold 0.01M PBS. Sections were mounted on gelatin coated slides and dried before clearing with 100% ethanol (2 min), then 50% Ethanol/50%

xylene (2 min) and 100% xylene before cover slipping with DPX mountant (Sigma). Images were captured using a NanoZoomer digital slide scanner and visualised with the NDP.view software (Hamamatsu) for histological verification of lesion placement.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical Analysis

Rats were excluded for inaccurate or insufficient lesion placement or if they failed to perform action sequences (>20 sessions of training). Final group sizes are reported in the figure legends for each group. Acquisition data was collected over 15 sessions and averaged across blocks of three sessions leading to five blocks. Sequence duration was calculated from the onset of nose poke 1 to the offset of nose poke 5, while the nose poke duration was calculated from entry to exit at each hole. The median and standard deviation for each rat on each day was calculated from individual response times. Timing data was not stored by the program for four rats in one session and therefore their times were averaged across two sessions rather than three for that block to prevent exclusion from the entire dataset. Where appropriate we applied paired t-tests, univariate or repeated measures ANOVA, with simple effects used in the case of significant interactions or post hoc comparisons for effects between treatment groups (SPSS v.25, IBM). Greenhouse-Geisser corrections were made if the sphericity assumption was violated and epsilon was <0.75.

SUPPLEMENTARY FIGURES

Supplementary Figure 1. Additional sequencing measures in DMS and DLS lesioned rats.

(A) Groups did not differ in the inter-poke interval (IPI) between holes on the first block; sham (open, n=11), DMS lesioned (blue, n=7), DLS lesioned (red, n=8).

(B) This remained the case on the last block of acquisition with IPI's becoming faster with training (Block: $F_{4, 48}$=15.62, p<0.001).

(C) There was a significant reduction in the standard deviation of sequence durations, indicating reduced variation with training in the DMS-lesioned rats but not in sham or DLS-lesioned rats (sham: t_{10}=0.06, p=0.953; DMS: t_6=3.09, p=0.021; DLS: t_7=1.57, p=0.160).

(D) The interval between leaving the magazine and nose poking into hole 1 did not differ between groups across acquisition ($F_{2,23}$=0.49, p=0.62).

(E) Nor did the interval from the fifth hole of the sequence and magazine entry (reward collection latency; Block: $F_{4,92}$=1.91, p=0.16; Lesion: $F_{2,23}$=1.46, p=0.25).

(F) The time spent with their nose in the magazine also did not significantly differ between groups (Block: $F_{4,92}$=1.23, p=0.30; Lesion: $F_{2,23}$=1.47, p=0.25).

(G) There was a main effect of time on locomotor activity, but no effect of treatment (p>0.4).

Supplementary Figure 2. Additional sequencing measures in mOFC and lOFC lesioned rats.

(A) Groups did not differ in the inter-poke interval (IPI) between holes on the first block; sham (open, n=10), mOFC (purple, n=8) or lOFC (green, n=12).

(B) This remained the case on the last block of acquisition with IPI's becoming faster with training. However, a significant Block X Lesion interaction highlighted that the lesion groups showed greater reduction in IPI times across acquisition due to the relatively slower IPI times in the first block (Block: $F_{1, 27}$=55.0, p<0.001; Lesion: F_{12} $_{27}$=1.21, p=0.31; Block X Lesion: $F_{2, 27}$=4.34, p=0.023; Hole and Hole X Block: p<0.001; Hole X Lesion p>0.5; Hole X Block X Lesion: $F_{6, 51}$=2.36, p=0.068).

(C) There was a significant reduction in the standard deviation of sequence durations, indicating reduced variation with training in the mOFC-lesioned rats but not in sham or lOFC-lesioned rats (sham: t_7=0.67, p=0.53; mOFC: t_{11}=2.53, p=0.028; lOFC: t_9=1.31, p=0.22).

(D) The interval between leaving the magazine and nose poking into hole 1 did not differ between groups across acquisition.

(E) Nor did the interval from the fifth hole of the sequence and magazine entry (reward collection latency; Block: $F_{2,57}$=2.95, p=0.06; Lesion: $F_{2,27}$=0.26, p=0.77; Block X Lesion: $F_{4,57}$=2.35, p=0.06).

(F) The time spent with their nose in the magazine also did not significantly differ between groups (Block: $F_{3,75}$=1.07, p=0.37; Lesion: $F_{2,27}$=0.46, p=0.64; Block X Lesion: $F_{6,75}$=0.66, p=0.67).

(G) There was a main effect of time on locomotor activity, but no effect of treatment (p>0.4).

Supplementary Figure 3. Additional sequencing measures in PrL and IL lesioned rats.

(A) Groups did not differ in the inter-poke interval (IPI) between holes on the first block; sham (open, n=9), PrL (cyan, n=9) or IL (orange, n=7).

(B) This remained the case on the last block of acquisition.

(C) There was a trend towards a reduction in the standard deviation of sequence durations in the IL-lesioned rats but not in sham or PrL-lesioned rats (sham: t_8=-0.66, p=0.53; PrL: t_8=1.61, p=0.15; IL: t_6=2.45, p=0.050). It was noted that two sham rats had rare but excessively long sequence durations, perhaps due to stopping and starting sequencing. Typically, rats would subsequently make an incorrect response if they paused, however here they were still able tom complete a correct sequence and that data is captured in the large error bars for sham rat within both blocks.

(D) The effect of lesion on the interval between the magazine and hole 1 neared significance with the sham rats taking longer than the lesioned groups (Block: $F_{2,52}$=3.17, p=0.043; Lesion: $F_{2,22}$=3.34, p=0.054; Block X Lesion: $F_{5,52}$=0.70, p=0.61).

(E) Nor did the interval from the fifth hole of the sequence and magazine entry (reward collection latency; Block: $F_{2,51}$=2.69, p=0.07; Lesion: $F_{2,22}$=0.73, p=0.49; Block X Lesion: $F_{5,51}$=0.96, p=0.45).

(F) The time spent with their nose in the magazine also did not significantly differ between groups.

(G) There was a main effect of time on locomotor activity, but no effect of treatment (p>0.4).

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Figure 1.

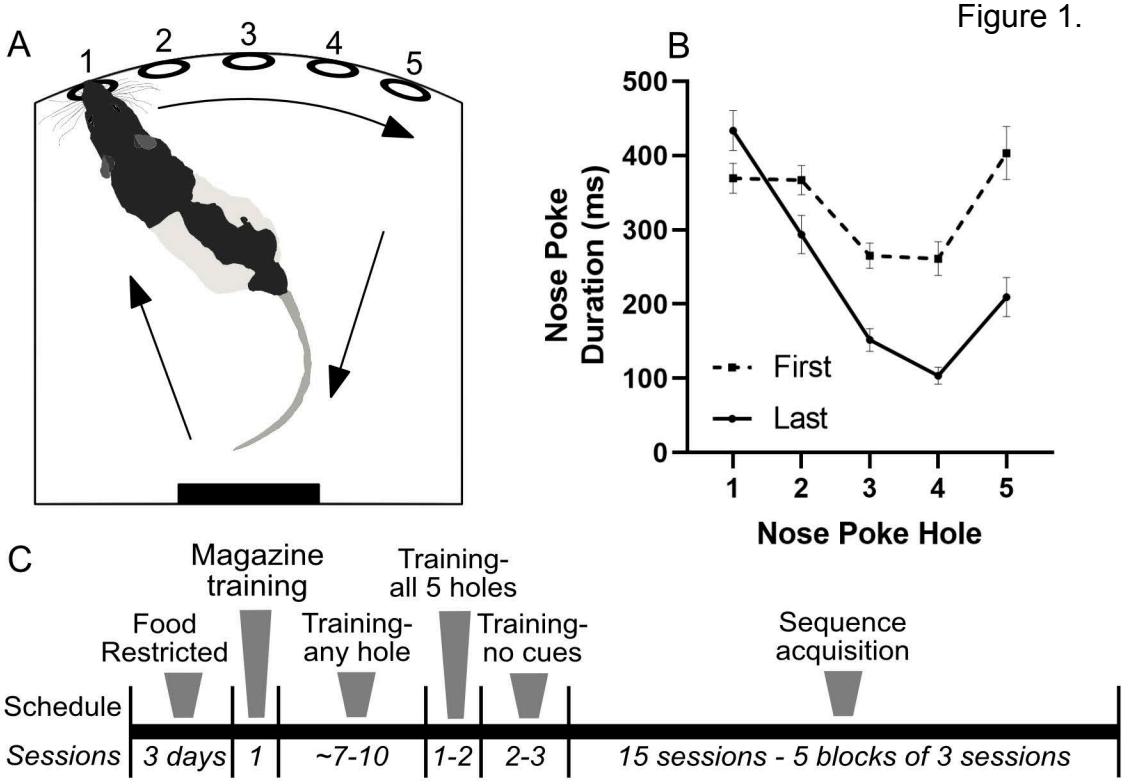

Figure 2.

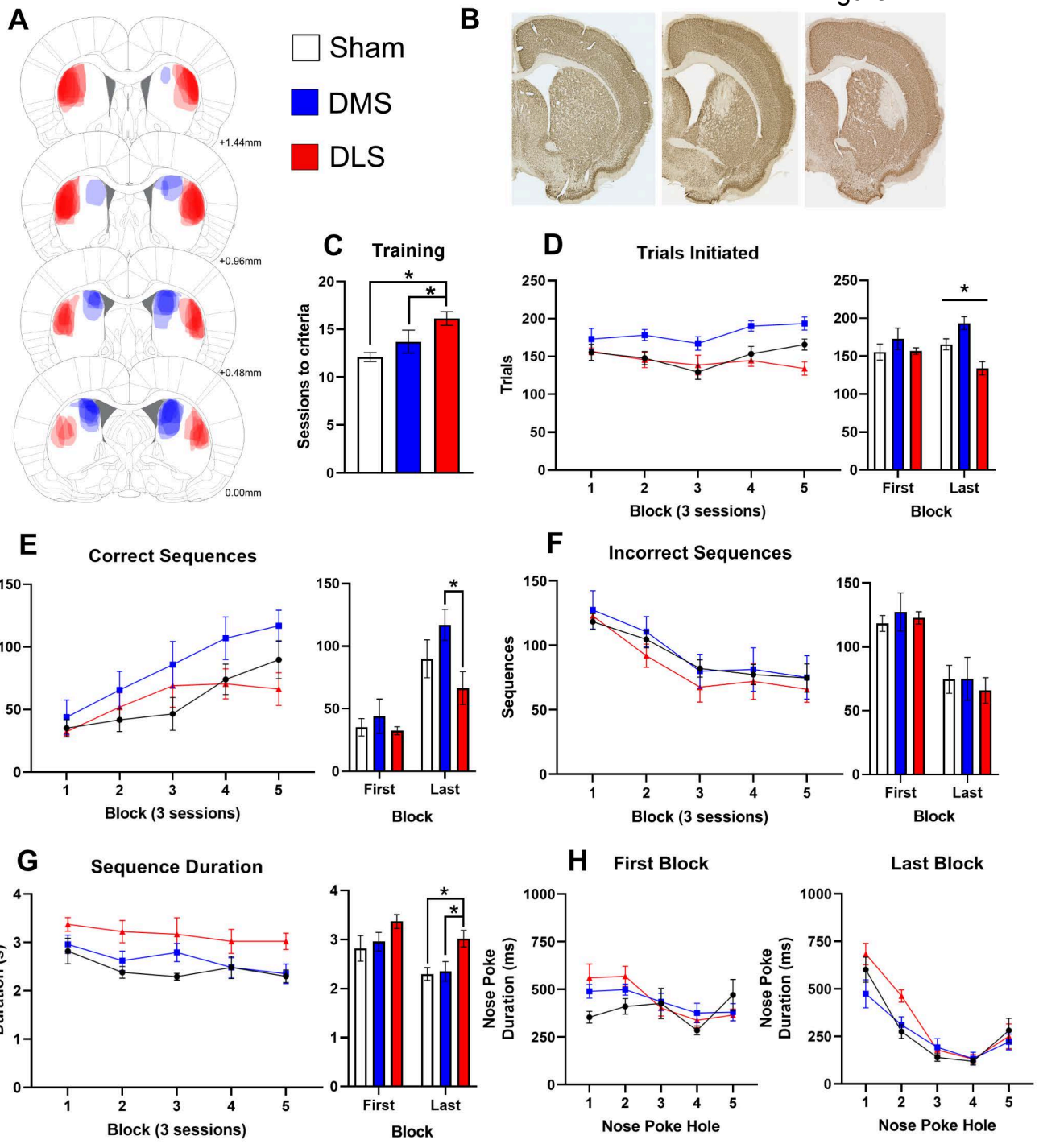

Figure 3.

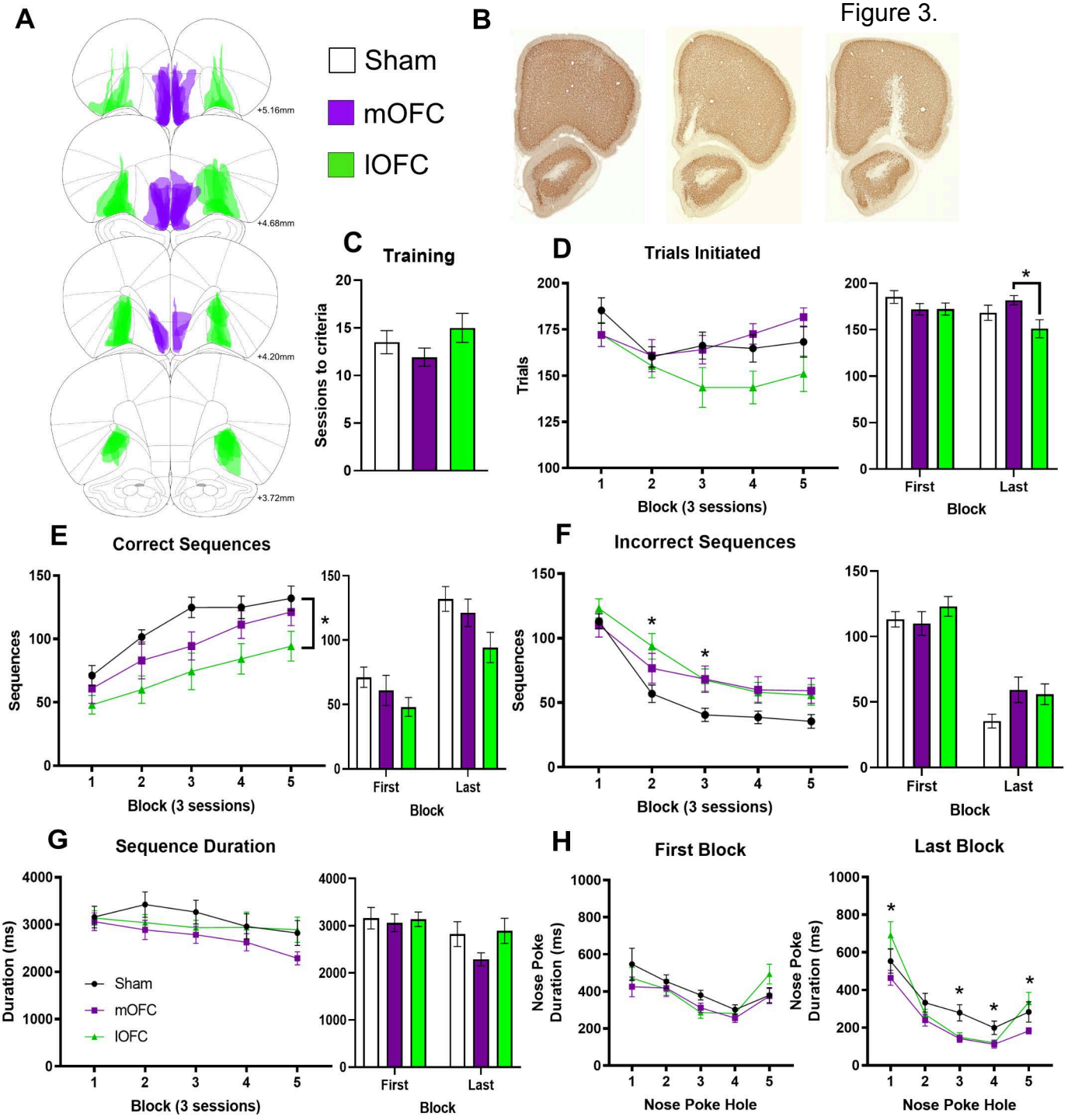

Figure 4.

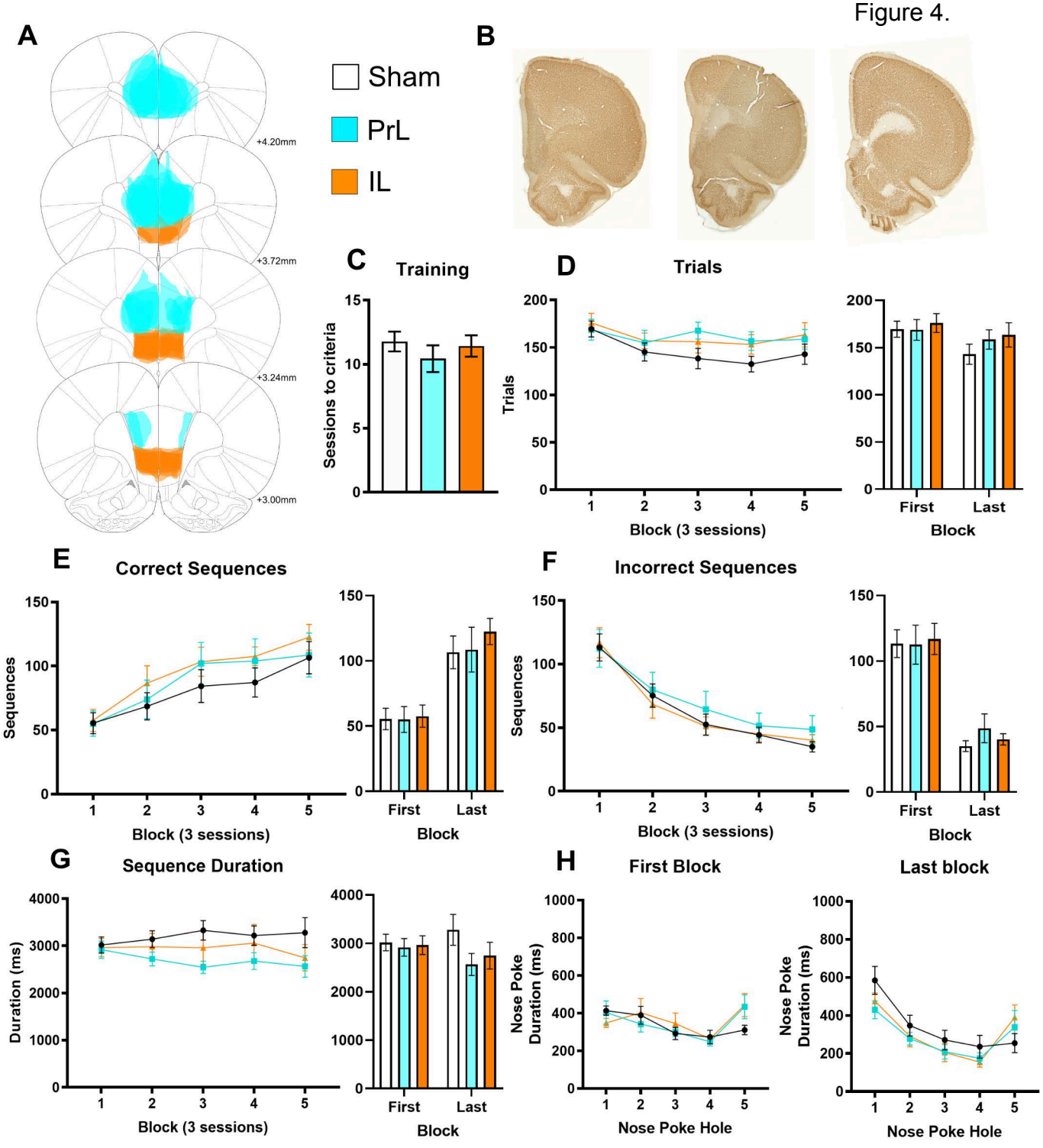

Figure 5.

A

	Adaptive flexible behaviour	Automated rigid behaviour
DMS / DLS	–	–
~~DMS~~ / **DLS**	↓	↑
DMS / ~~DLS~~	↑	↓