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Rule encoding in dorsal striatum impacts action selection

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Abstract

Cognitive flexibility is a hallmark of prefrontal cortical (PFC) function yet little is known about downstream area involvement. The medial dorsal striatum (mDS) receives major projections from the PFC and is uniquely situated to perform the integration of responses with rule information. In this study, we use a novel rule shifting task in rats that mirrors non-human primate and human studies in its temporal precision and counterbalanced responses. We record activity from single neurons in the mDS while rats switch between different rules for reward. Additionally, we pharmacologically inactivate mDS by infusion of a baclofen/muscimol cocktail. Inactivation of mDS impaired the ability to shift to a new rule and increased the number of regressive errors. While recording in mDS, we identified neurons modulated by direction whose activity reflected the conflict between competing rule information. We show that a subset of these neurons was also rule selective, and that the conflict between competing rule cues was resolved as behavioural performance improved. Other neurons were modulated by rule, but not direction. These neurons became selective before behavioural performance accurately reflected the current rule. These data provide an additional locus for investigating the mechanisms underlying behavioural flexibility. Converging lines of evidence from multiple human psychiatric disorders have implicated dorsal striatum as an important and understudied neural substrate of flexible cognition. Our data confirm the importance of mDS, and suggest a mechanism by which mDS mediates abstract cognition functions.

Keywords

attentional set-shifting; cognitive flexibility; medial dorsal striatum; rat; single unit

Introduction

Our lives are governed by rules, allowing fast and efficient behaviour without much cognitive effort. However, rules guiding behaviour do not apply equally to all situations, and we need to alter which rules we use depending on the context. The inability to adapt to changing situations is a hallmark of many human psychiatric disorders (Gold *et al.*, 2008, 2009; Strauss *et al.*, 2011).

Specifically, patients who suffer from deficits in behavioural flexibility are able to learn information and form rules to guide behaviour, but lack the ability to alter responding when the situation warrants a behavioral change (Cools *et al.*, 2000; Shamay-Tsoory *et al.*, 2004). Appropriate use of behaviour-guiding rules leads to effective behavioural flexibility, enabling animals to successfully navigate an ever changing world (Bissonette *et al.*, 2013b). Tasks testing rule flexibility require discrimination of relevant from irrelevant information. Patients with schizophrenia (Elliott *et al.*, 1995; Pantelis *et al.*, 1999), Parkinson's disease (Gauntlett-Gilbert *et al.*, 1999; Monchi *et al.*, 2004; Dirnberger & Jahanshahi, 2013) or drug addiction (Lyvers & Yakimoff, 2003) struggle with this ability.

The animal literature indicates medial prefrontal cortex (mPFC) is critical for attentional set-shifting (Dias *et al.*, 1996a,b; Birrell & Brown, 2000; Colacicco *et al.*, 2002; Bissonette *et al.*, 2008; Roy *et al.*, 2010). It is clear that activity in mPFC is modulated by context and correlated with shifts in attention (Miller & Cohen, 2001; Wallis & Miller, 2003; Durstewitz *et al.*, 2010). Yet recent literature has shown that other brain areas are also important for these functions and several models suggest a role for mDS in maintaining a novel response pattern after a rule shift (Ritchie & Noble, 2003; Stelzel *et al.*, 2010; Cools, 2011; Markett *et al.*, 2011; Collins & Frank, 2013; Baker & Ragozzino, 2014; Rodgers & DeWeese, 2014). Generally, it is thought that mPFC conveys rule information to basal ganglia so that new action policies can go into effect. It is already known that mDS encodes the direction of impending movements and the associations between response and outcomes (Ragozzino *et al.*, 2002; Pasupathy & Miller, 2005; Balleine *et al.*, 2007, 2009; Kimchi & Laubach, 2009; Balleine & O'Doherty, 2010; van der Meer *et al.*, 2010; Hilario *et al.*, 2012). However, no one has recorded from mDS during a set-shifting paradigm to uncover its role in this important function.

We hypothesise that medial dorsal striatum (mDS) is critical for rule shifting and that neural correlates of response outcomes and direction will be impacted by rule shifts. In experiment 1, we inactivate mDS and demonstrate an impairment shifting between rules. In experiment 2, we identify neural correlates in mDS of rule representation, direction and cells integrating the rule with response direction as well as a reduction in directional encoding during conflict trials. These results suggest mDS plays a more cognitive role in the shifting of rules than previously suspected and provides an additional locus for investigating the mechanisms underlying behavioural flexibility.

Methods and materials

Subjects and set-shifting task

Fourteen single housed adult male Long-Evans rats (175–200 g) obtained from Charles River Labs (Wilmington, MA, USA) were tested at the University of Maryland, College Park, in accordance with the university and National Institutes of Health guidelines and with Institutional Animal Care and Use Committee approval.

Rats were required to nosepoke and follow a cue to a well for a fluid reward (10% sucrose). Rats were first trained to follow a counterbalanced odour–response association (odour 1 = go left; odour 2 = go right). During training, rats first learned to nosepoke in a central odour

port for a 150-ms odour and then to respond to a left or right fluid well depending on the association of the odour with direction. Rats had to learn to poke and wait for a pre-cue delay (500 ms) and cue duration (500 ms) before exiting the nose poke (i.e. 500-ms pre-cue delay and 500-ms cue duration). This was accomplished by gradually increasing the delays by 50 ms on successive days.

Once rats reached 80% correct on odours and were competent with delay epochs (approximately 12 days), rats learned the light rule (i.e. left light = go left; right light = go right). The timing of the task was identical (pre-cue delay = 500 ms; cue duration = 500 ms). All trials had a 5-s inter-trial interval, which remained constant from training through the final days of the experiments. Odours were not presented during this portion of training. Rats were trained to nosepoke then respond to left or right light cues until reaching 80% correct (approximately 7 days).

After a rat had performed at 80% correct on both an odour and a light rule session, rats were given alternating days of odour-only or light-only rule training for approximately 7 days. Thus, in <28 days rats learned the temporal aspects of the task, two different rules, and were able to perform both at high rates on alternating days.

If rats were consistently performing at or above 80% correct on both rule blocks, rats were prepared for electrode or cannula implantation. After recovery, rats began set-shifting. For the set-shifting task, we combined the presentation of both odour and light cues (Fig. 1a). When a house light was illuminated, rats were required to nosepoke and hold for 500 ms to subsequently receive 500 ms of simultaneous random odour and random direction light. Rats were required to wait the entire 1 s of both the pre-cue period (500 ms) and the cue period (500 ms), then to respond. Premature responses (i.e. exit nosepoke before cue offset; 500 ms) were recorded as an 'early unpoke', and caused the houselight to go dark for a 3-s error penalty before the trial repeated.

There were two main trial types (Fig. 1b): compatible trials, where both simultaneously presented cues indicated the same direction, and incompatible trials, where both simultaneously presented cue directions were in conflict with each other. The simultaneous presentation of cues and subsequent directional responses are entirely counterbalanced between Odour and Light rule blocks. To set the rule and measure stable rule-based behaviour in the first block, rats received a day of pre-training, where they responded to only one cue (i.e. a light task, but no odours). The following day, they were presented with both simultaneous cues and the correct rule was the same as the previous day pre-training. After reaching criterion in rule 1, the correct rule was shifted to the other rule in block 2. Rules were counterbalanced daily. Performance was monitored by a running average of 20 trials. Once rats reached 80% correct in block 1, an additional 40 trials were added to their total before rules were shifted to avoid rats anticipating a rule shift and to collect enough data post-criteria to analyse. In block 2, after rats reached 80% correct, rats were run for an additional 40 trials as well. The training and testing were identical for the mDS recording and inactivation rats.

Surgical procedures

Eight rats were bilaterally cannulated in mDS (AP: -0.4 mm, ML ± 2.4 mm, DV 3.5 mm) (Fig. 2a). For the inactivation experiment, a γ -aminobutyric acid (GABA)_{A+B} agonist cocktail was made. Immediately before behaviour, rats were infused with either saline or $0.1/1.0$ mM baclofen/muscimol. Rats received a total volume of 600 nL, 300 nL per hemisphere, per infusion, and all animals had a 1-day 'washout' with no treatment between infusions. For infusions, dummy cannulae were removed and 30G injector cannulae (extending 1.0 mm beyond the guide cannula) were inserted into the guide cannula. The injector cannula was connected to a Hamilton syringe attached to an infusion pump. Saline or drug cocktail was infused at 250 nL/min, and injector cannulae remained in the rat for 5 min after infusion before the dummy cannula was reinserted (Jones *et al.*, 2012). To preserve the appropriate shift direction for each rat and to be aware of counterbalanced odours, the experimenter was not blinded to treatment or behaviour for each rat. The effects of the GABA_{A+B} agonist cocktail last for 2 – 3 h, and all rats completed their task within 2 h. The order of treatment and rule shifting was counterbalanced for all rats, where half the rats received saline infusion on the first set shift day and baclofen/muscimol cocktail on the second set shift day. Meanwhile, the other half received the baclofen/ muscimol cocktail on the first set shift day and saline infusion on the second set shift day. To start, rats received their first treatment and completed their first set shift. On the following day, rats had no infusions and completed a single-cue task to set the rule (as described above). On the following day, rats received infusion of the opposite treatment from the first day before completing the second day's set-shifting task. Thus, 48 h passed between when a rat received either saline or GABA_{A+B} agonist cocktail infusion and the next infusion, and rats completed two set shifts.

Six rats had a drivable bundle of ten, 25 - μ m-diameter FeNiCr wires chronically implanted in the left or right hemisphere dorsal to mDS (1 mm anterior to bregma, $+$ or $- 3.2$ mm laterally, and 3.5 mm ventral to the brain surface) (Fig. 3a) (Bryden *et al.*, 2012; Burton *et al.*, 2014). After testing, rats were transcardially perfused with buffered 4% paraformaldehyde with brains postfixed at 4 °C. Freezing microtome sections (50 μ m) were cut and stained with thionin. Cannula locations and electrode placements were verified under light microscopy and drawn onto plates adapted from the rat brain atlas (Paxinos & Watson, 2004). If electrodes or cannulae had been implanted into the wrong areas, rats would have been excluded from the study.

Data acquisition and analysis

Experiments were performed in a behavioural chamber previously described (Schoenbaum & Roesch, 2005). We performed daily screening of wires, and advanced the electrode assembly ~ 80 μ m [~ 4 – 5 times the average diameter (15 μ m) of a striatal medium spiny neuron] at the end of each recording session to record from a different neuronal population. Electrode assembly depth was noted during surgery, and final electrode location was recorded from histology. Total distance an electrode was driven ranged from 1000 to 2400 μ m, depending on the animal. Neural activity was recorded using Plexon Multichannel Acquisition Processor systems (Dallas, TX, USA). Signals from the electrode wires were amplified 20 times by an op-amp headstage (Plexon, HST/8o50-G20-GR), located on the

electrode array. Immediately outside the chamber, signals were passed through a differential pre-amplifier (Plexon, PBX2/16sp-r-G50/16fp-G50), where the single unit signals were amplified 50 times and filtered at 150–9000 Hz. The single unit signals were then sent to the Multichannel Acquisition Processor box, where they were further filtered at 250–8000 Hz, digitized at 40 kHz and amplified 1–32 times. Waveforms of signal-to-noise ratio >2.5:1 were extracted from active channels and recorded to disk. Neurons were sorted using OFFLINE SORTER AND NEUROEXPLORER (Burton *et al.*, 2014), and exported for analysis in MATLAB (Bissonette *et al.*, 2013a).

We used a least-squares multiple regression to determine the number of cells where firing rate was significantly correlated with either the response direction or rule block when variance for the two remaining factors was accounted for. To achieve this, we compared a base model ($k = 2$; where k = the number of parameters) with a complex model ($k = 3$) in two separate iterations:

$$Y = \beta_0 + \beta_1 \text{Direction} \quad (1)$$

$$Y = \beta_0 + \beta_1 \text{Direction} + \beta_2 \text{Rule} \quad (2)$$

$$Y = \beta_0 + \beta_1 \text{Rule} \quad (3)$$

$$Y = \beta_0 + \beta_1 \text{Rule} + \beta_2 \text{Direction} \quad (4)$$

where Y = firing rate (spikes/s) during the 500-ms cue epoch, Rule is coded as (−1 = odour rule) (1 = light rule), and Direction is coded as (−1 = right) (1 = left).

We began by grouping cells for which a single factor model led to significant change in neural activity. Following this, we calculated the number of cells that provided a significant improvement of fit (via an incremental F -test) when the second parameter in model 2 was added to the single factor model. Counts of correlated cells were compared via chi-square ($P < 0.05$). Neural data were normalized by subtracting baseline firing rates. The average firing rate from −2 to −1 s before cue onset was subtracted from neural data. When analysing neural activity during behaviour, mean neural firing rate during cue epoch for different trial-types was compared via analysis of variance (ANOVA), with *post-hoc* t -tests when appropriate. For correlations between firing rate and behavioural performance, we calculated the average cue epoch firing rate for incompatible trial-types and subtracted the average rate of activity for non-preferred direction trials from preferred direction trials. We then calculated the total behavioural performance for the session. The MATLAB function `corrcoef` was used to calculate the correlation coefficient and P values, determining if a significant correlation exists. Fisher's r -to- z transformation was used to compare correlation

coefficients within each rule block. Data were checked for normal distribution using Kolmogorov–Smirnov (KS) test and chi-square goodness of fit using the MATLAB functions `kstest` and `chi2gof`. To determine the first trial when rule neurons significantly modulated their activity in their preferred rule block, we generated an average for the first five trials, and then computed a sliding window of five trials throughout the block. We chose the first five trials as a baseline because it occurs well before rats reached criterion and provided sufficient statistical power for analysis. Behaviour results were analysed with a two-way ANOVA (Experiment 1, mDS Inactivation) or one-way ANOVA or *t*-test (Experiment 2, *in vivo* ephys) with *post-hoc t*-tests when appropriate. Mean firing rates, $\pm$ standard error of the mean (SEM), are presented as inset bar graphs along with neural data, highlighting the comparison during the pre-cue or cue epoch between different trial-types or different times within a rule block. Throughout, means ($\pm$ SEM), *t*-statistics, *P* values, Cohen's *d*, effect size and degrees freedom (d.f.) are reported for statistical comparisons.

Results

Experiment 1

Rats readily learned and responded to the appropriate rule strategy in the first block, and rats completed two full set shifts. Both Saline and Bac/Mus rats performed well in the first rule block of the day, reaching 80% correct quickly (Fig. 2b). Regardless of the dimensionality of the first block rule, both Saline and Bac/Mus rats performed similarly (Saline block 1 Light mean 37 ± 12.5 , Odor mean 33 ± 9.7 ; Bac/Mus block 1 Light mean 30.5 ± 9.2 , Odor mean 27.5 ± 6.5). A drug $\times$ block $\times$ compatibility ANOVA revealed a significant main effect of drug treatment ($F_{1,117} = 6.21$, $P < 0.05$) on trials to criterion with a significant interaction effect with block ($F_{1,117} = 4.19$, $P < 0.05$). There was also a main effect of compatibility ($F_{1,117} = 304$, $P < 0.001$) and a block by compatibility interaction ($F_{1,117} = 4.49$, $P < 0.05$). Two-way ANOVA on trials to criteria revealed a significant drug $\times$ block interaction ($F_{3,9} = 4.2$, $P < 0.01$). Bac/Mus rats took significantly more trials to reach criteria than saline control rats (planned *t*-test; $P < 0.001$, $t = -3.6$, Cohen's $d = -2.1$, effect size = -0.72 , d.f. = 7, saline trials to criterion = 50.3 ± 6.6 , Bac/Mus trials to criterion = 141.5 ± 20.9).

We determined what rule animals were following by their responses on incompatible trials. Note, this cannot be done on compatible trials because both rules apply and very few errors are made. Thus, to investigate the type and nature of errors during rule shifting, we analysed incorrect responses (errors) on incompatible trial-types. After a rule shift, consecutive incorrect choices on incompatible trial-types were coded as perseverative errors, while errors on incompatible trial-types after a correct incompatible trial were coded as regressive, representing choices regressing to the previous rule (Ragozzino *et al.*, 2002; Bissonette *et al.*, 2015). Two-way ANOVA on errors to criterion with drug treatment and error type as factors revealed a main effect of error-type ($F_{3,28} = 11.07$, $P < 0.001$) (Fig. 2c), and planned *t*-tests demonstrated a significant increase in regressive errors in mDS inactivated rats (planned *t*-test $P < 0.001$, $t = -5.13$, Cohen's $d = -2.17$, effect size = -0.74 , d.f. = 7, saline regressive error mean = 13.3 ± 1.3 , Bac/Mus regressive error mean = 41 ± 6.3), where rats would switch 'back' to the previous block-type responding on incompatible trials. To account for increased trials to criterion in Bac/Mus treated rats, we computed the percentage

of trials to criterion that were errors (Fig. 2d). Two-way ANOVA on errors as a percentage of trials to criterion with drug treatment and error type as factors revealed a main effect of error type ($F_{3,28} = 18.2$, $P < 0.001$). *Post-hoc* planned *t*-tests demonstrated a significant increase in regressive-type errors compared with perseverative errors in the Bac/Mus treated group (*t*-test $P < 0.01$, $t = -4.8$, Cohen's $d = -2.6$, effect size = -0.8 , d.f. = 7, Bac/Mus perseverative error mean = 2.9 ± 0.9 , regressive error mean = 29.4 ± 5) and compared with regressive-type errors in control rats (*t*-test $P < 0.01$, $t = -3.6$, Cohen's $d = -2.0$, effect size = -0.71 , d.f. = 7, saline regressive error mean = 7.7 ± 2 , Bac/Mus regressive error mean = 29.4 ± 5).

Because rats encountered two main trial-types and one of those trial-types (incompatible) put rule information in conflict, we calculated the per cent correct for rats both pre- and post-criterion for both compatible and incompatible trials. As performance on incompatible trials demonstrates whether an animal is following the previous or new rule after a rule shift and performance on incompatible trial-types should be sensitive to improved or impaired rule-guided behaviour, we calculated the difference in behavioural performance between the two trial-types (compatible minus incompatible per cent correct) for both pre- and post-criteria in block 1 and block 2. A two-way ANOVA on difference scores in block 1, using drug treatment and pre- or post-criterion as factors (Fig. 2e), revealed no significant main effect of drug ($F_{1,28} = 0.1$, $P = 0.8$) or pre/post-criterion ($F_{1,28} = 0.3$, $P = 0.6$), while in block 2 (Fig. 2f) a two-way ANOVA on difference scores, using drug treatment and pre- or post-criterion as factors, revealed a significant main effect of drug ($F_{1,28} = 4.19$, $P < 0.05$) and pre/post-criterion ($F_{1,28} = 23.8$, $P < 0.001$) with a significant interaction effect ($F_{1,28} = 5.7$, $P < 0.05$). *Post-hoc t*-tests revealed a significant increase in the difference score for Bac/Mus treated rats (mean = 53.1 ± 3.5) compared with pre-criterion saline treated rats (mean = 37.5 ± 3.5) (*t*-test, $P < 0.01$, $t = -4.5$, Cohen's $d = -1.6$, effect size = -0.62 , d.f. = 7). There was also a significant change in the difference score for Bac/Mus treated rats from pre-criterion (mean = 53.1 ± 3.5) to post-criterion (mean = 27.5 ± 3) (*t*-test, $P < 0.01$, $t = 4.5$, Cohen's $d = 2.8$, effect size = 0.81 , d.f. = 7). Together, these data suggest the maintenance of correct responding was diminished when mDS was inactivated and that the behavioural deficits on incompatible trial-types drive this maintenance of incorrect responding.

Experiment 2

To elucidate the mechanisms underlying the dysfunction observed after mDS inactivation we recorded single-units from mDS in six rats (Fig. 3a). Recording rats readily learned the task; six rats completed a total of 84 task-switch days where they reached 80% in both block 1 and block 2 (14 rule switches per rat), during which time we recorded a total of 334 single units. Figure 3b shows trials to criteria in block 1 (mean = 33 ± 2) and block 2 (mean = 74 ± 3.4), where rule-shifting caused a significant increase in the overall numbers of trials needed to reach criteria after a rule shift (*t*-test, $P < 0.001$, $t = -11.5$, Cohen's $d = -14.7$, effect size = -0.99 , d.f. = 83). Rats committed significantly more regressive errors (mean = 21.9 ± 1.1) than perseverative errors (mean = 2.5 ± 0.2) (*t*-test, $P < 0.001$, $t = -17.2$, Cohen's $d = -2.62$, effect size = -0.79 , d.f. = 83) (Fig. 3c). Performance in block 1 was not dependent upon which rule was the starting rule, with average trials to criteria in light rule blocks (mean 38.2 ± 5.9) being the same as odour rule blocks (mean 41.1 ± 6.4).

We recorded single unit activity during these rule-shifts and found that many neurons were modulated by response direction and rule. Breakdown of neurons by activity, as identified by regression analysis, is shown in Fig. 3d, and approximate recording location of each neuron is shown in Fig. 3e. In a simple one-factor model, we found that 17% ($n = 57$) and 46% ($n = 154$) of neurons were significantly modulated by direction or rule, respectively. In the two-factor model, 126 neurons (38%) were found to have only a rule preference, 28 neurons (8%) were found to have both a rule and a directional preference, and 29 neurons (9%) had only a directional preference while the firing of 151 neurons (45%) remained unmodulated by task events.

Thus, the regression analysis divided neurons into three different categories based on firing during the cue epoch. Activity of single neurons was correlated with either (i) the particular rule block that the animal was in, (ii) the direction indicated by the cues or (iii) an integration of the direction information and current rule block. Figure 3e illustrates the recording location of all neurons classified by the regression analysis and those found not to be significantly modulated by the task.

Response conflict in DS

Figure 4 plots activity for the directional neurons ($n = 29$). The average overall firing is plotted in Fig. 4, broken down by direction (thick vs. thin) and trial-type (black vs. grey). Preferred direction was defined as the direction that evoked the strongest firing during the cue epoch. Fifteen neurons preferred contralateral and 14 preferred ipsilateral directions, with respect to electrode location. Consistent with their categorisation based on the regression, these 'direction-only' neurons fired more for a preferred direction than a non-preferred direction ($F_{1,56} = 6.23$, $P < 0.05$), but were insensitive to the current rule block ($F_{1,56} = 0.08$, $P = 0.94$).

When broken down by trial-type, population activity for direction-only cells reflected the conflict inherent in the task (Fig. 4). A conflicted directional signal emerges because cues from the different dimensions either agree (compatible) or disagree (incompatible) with the preferred or non-preferred directional tuning for these neurons. ANOVA revealed a main effect of trial-type ($F_{3,115} = 4.1$, $P < 0.01$) and *post-hoc* *t*-tests showed significant differences between trial-types. The inset bar graph in Fig. 4 demonstrates cue-evoked means for direction neuron firing, where preferred direction compatible trial-types (mean = 1.8 ± 0.3) evoked significantly more firing than preferred direction incompatible trial-types (mean = 0.9 ± 0.3) (*t*-test, $P < 0.05$, $t = 2.1$, Cohen's $d = 0.6$, effect size = 0.3, d.f. = 28). Preferred direction incompatible trial-types evoked significantly higher firing than non-preferred direction incompatible trial-types (preferred direction mean = 0.9 ± 0.3 , non-preferred direction mean = -0.3 ± 0.5 ; *t*-test, $P < 0.05$, $t = 2.3$, Cohen's $d = 0.99$, effect size = 0.44, d.f. = 28). Thus, activity in this population of neurons reflected a directional preference and the strength of this directional preference was modulated by the conflict inherent in the task.

Integration of rule and direction in DS

In addition to encoding direction, many direction neurons also exhibited a significant improvement of fit when rule was added to the model ($n = 28$; Fig. 3d). Figure 5a shows

representative raster and peristimulus time histograms of firing for an example neuron. This neuron fired more strongly for left compared with right movements, and did so more strongly during the light rule, thus exhibiting an enhancement of directional selectivity in one block over the other (i.e. firing was an average of 70 Hz for left direction cues in the light rule, and only 57.5 Hz for left direction cues during the odour rule). Seventeen neurons preferred the first-block rule, and 11 neurons preferred the second-block rule. These rule preferences were evenly divided between light or odour rule (eight neurons preferred the light rule in block 1, nine preferred the odour rule in block 1, while six neurons preferred the light rule in block 2, and five preferred the odour rule in block 2).

To determine how rule shifts modulated these neurons, we examined early trials (first ten) and late trials (last ten) firing within each block to determine how directional signals changed as animals adjusted to the new contingencies. Figure 5 plots the average neural activity during the preferred and non-preferred rule blocks, their performance on compatible and incompatible trials, as well as the preferred direction and non-preferred direction during the first (early) and last ten (late) trials. By definition, the activity in these plots is highest in a preferred rule (*t*-test, $P < 0.01$, $t = 3.1$, Cohen's $d = 1.1$, effect size = 0.5, d.f. = 27) and for a preferred direction (*t*-test, $P < 0.001$, $t = 4.9$, Cohen's $d = 1.4$, effect size = 0.6, d.f. = 27). One-way ANOVA of firing rate between trial-types during the cue epoch revealed a significant main effect of trial-type early in the preferred rule block (Fig. 5b) ($F_{3,111} = 4.5$, $P < 0.01$). *Post hoc t*-tests revealed that the strength of the directional signal was significantly conflicted early in the preferred rule block, where activity was highest for low-conflict, preferred direction cues, followed by high-conflict, preferred direction cues (thick black vs. thick grey lines in Fig. 5b and inset, *t*-test, $P < 0.05$, $t = 2.5$, Cohen's $d = 0.8$, effect size = 0.4, d.f. = 27) in a manner similar to direction neurons observed earlier, in Fig. 4. Remarkably, by the end of the preferred rule block (last ten trials), the strength of the directional signal did not differ between compatible and incompatible trials (Fig. 5c, thick black vs. thick grey lines and inset, *t*-test, $P = 0.8$, $t = 0.2$, Cohen's $d = 0.1$, effect size = 0.03, d.f. = 27) suggesting that unlike early in the rule block, directional signals in mDS accurately represented direction on incompatible trials late in the trial block. Thus, early in the preferred rule block, directional selectivity was significantly reduced when the two rules were in conflict. However, by the end of the preferred rule block, the conflict between competing stimuli was resolved and encoding of the preferred direction on high-conflict trials (i.e. incompatible trials) was equivalent to that on low-conflict trials (i.e. compatible trials; thick black vs. thick grey lines in Fig. 5c).

In the non-preferred rule block, activity was significantly diminished in trials at the end of the block compared with early in the block (Fig. 2d and e; $F_{7,216} = 8.47$, $P < 0.001$). Early in the non-preferred rule block, neural activity for the preferred direction was similarly encoded on both high and low conflict trials, although there was a trend towards significance (Fig. 5d, thick black vs. grey line and inset, $P = 0.08$, $t = 1.8$, Cohen's $d = 0.4$, effect size = 0.2, d.f. = 27). Late in the non-preferred rule block, a conflicted directional signal existed between compatible and incompatible trial-types (Fig. 5e, thick black vs. grey lines and inset, *t*-test, $P < 0.01$, $t = 3.6$, Cohen's $d = 0.8$, effect size = 0.4, d.f. = 27). Thus, unlike neural activity in the preferred rule block, a conflicted directional signal emerged in the non-

preferred rule block, suggesting that a neuron's rule preference enabled improved encoding of the preferred direction in one rule block, but not another.

These data suggest a strong relationship between changes in neural activity and learning that occur over rule blocks. To further test this hypothesis we correlated firing during the cue epoch to performance (Fig. 5f and g, black line). Early in the preferred rule, there was a negative correlation for firing on incompatible trials for the preferred vs. non-preferred direction ($r = -0.12$, $P = 0.6$), which became a significant positive correlation by the end of the preferred rule block ($r = 0.6$, $P < 0.001$) (Fig. 2f). This change in correlation represents a significant difference in correlation coefficients ($z = -2.88$, $P < 0.01$). The same correlation with behaviour was not observed in the non-preferred rule block (early: $r = 0.04$, $P = 0.23$; late: $r = 0.23$, $P = 0.24$) (Fig. 5g) and there was no significant difference between these two correlation coefficients ($z = -0.69$, $P = 0.3$). As neural activity late in the preferred rule block was positively correlated with percentage correct for those trials, these data show that the stronger the change in firing between the two directions on incompatible trial-types, the more the behavioural performance was improved, but only in a neuron's preferred rule block and only late in that rule block. This resolution of directional conflict in a preferred rule block may represent a neural mechanism by which choice behaviour is improved and how rule information impacts action selection. These data suggest that a sub-population of direction neurons integrate rule information and that these neurons modulate their firing based on learning, consistent with loss of function after inactivation.

Rule encoding in DS

Unexpectedly, we found that many neurons in DS were not modulated by direction but rather signalled what rule block the rat was in ($n = 126$, significantly more than chance; $P < 0.001$, $\chi^2 = 485$; Fig. 3d). Among rule neurons (Fig. 3a), we identified no significant firing rate modulation between directions ($F_{1,250} = 0.06$, $P = 0.8$) or compatibility of cues ($F_{1,250} = 0$, $P = 0.98$) consistent with the regression analysis described above (i.e. adding direction did not improve fit). To investigate rule encoding at the population level, we plotted the average firing rate over all neurons that showed a significant correlation with rule. We grouped neurons by preferred and non-preferred rule based on maximal activity during the cue epoch. Rule neurons equally preferred either light or odour rules (64 neurons preferred light, and 62 neurons preferred odour rule) and rule neurons were observed equally in either block 1 or block 2 (61 rule neurons preferred block 1, 65 rule neurons preferred block 2).

Consistent with the regressions, activity was significantly higher for the preferred rule during the cue epoch (Fig. 6a, light grey bar; inset, t -test, $P < 0.01$, $t = 2.9$, Cohen's $d = 2.3$, effect size = 0.8, d.f. = 125), but rule encoding was also present before cue onset (dark grey bar), which is possible because of the block design. To quantify this effect we examined the average firing rate during the period between nose poke and cue onset (pre-cue, dark grey bar to left of light gray cue bar, Fig. 6a). Indeed, activity during the precue epoch was significantly stronger for the preferred rule (Fig. 6a, dark grey bar; inset, t -test, $P < 0.05$, $t = 2.3$, Cohen's $d = 2$, effect size = 0.7, d.f. = 125) as defined during the cue epoch, demonstrating rule-related preparatory activity in these neurons before cue presentation.

Next we determined how rule encoding developed during learning. To address this, we calculated which rule cells significantly increased their firing in the preferred block by comparing neural activity on early (first ten, Fig. 6b, dashed grey line) and late (last ten, solid black line) trials during the cue epoch. A total of 37 neurons (29% of rule cells, a significant proportion, $P < 0.001$, $\chi^2 = 107$) showed increasing activity levels over the course of the preferred rule blocks. The average over all these neurons is provided in Fig. 3b, with insets showing that rule activity late in the preferred rule is significantly elevated for both the pre-cue (t -test, $P < 0.05$, $t = -2.5$, Cohen's $d = -0.8$, effect size = -0.4 , d.f. = 36) and cue epochs (t -test, $P < 0.05$, $t = -2.6$, Cohen's $d = -2$, effect size = -0.7 , d.f. = 36).

To determine if this change in firing occurred before, after or at the same time as rats learned the correct rule for a block, we next plotted the first trial when a rule-modulated neuron's firing rate was significantly different from the start of the rule block, as well as the trial that each rat reached criteria. All behaviour sessions from the days when all 37 of these rule-modulated neurons were recorded were used and no trials or sessions were excluded. Because some rule-adapting neurons preferred a rule in block 1 and some preferred a rule in block 2, we broke data into block 1 (Fig. 6c) and block 2 (Fig. 6d) and analysed data by the neuron's preferred rule block. The block 1 rule neurons significantly increased their activity by trial 16, 18 trials before rats reached behavioural criteria (black vs. white bars, on average trial 34, t -test, $P < 0.05$, $t = -2.4$, Cohen's $d = -0.6$, effect size = -0.3 , d.f. = 36). The same effect was observed for block 2 rule neurons where activity significantly increased by trial 34, preceding behavioural criteria by 102 trials (black vs. white bars, t -test, $P < 0.05$, $t = -2.5$, Cohen's $d = -0.9$, effect size = -0.4 , d.f. = 13). These data demonstrate a relationship between rule encoding and behavioural performance consistent with the loss of function observed after inactivation.

Finally, to determine if there are differences in neural activity on correct or error trials, we computed an index of neural activity during the cue epoch for each neuron type. Figure 7 shows a comparison of the difference index between neural activity to cues for correct and error trials for incompatible trial-types. When comparing the direction cell population (Fig. 7a), a significant difference was observed (grey vs black bar, t -test, $P < 0.01$, $n = 29$, correct trial mean 0.035, error trial mean 0.07), revealing a stronger difference in direction preference on correct trials than errors. The same was not true for rule cells (Fig. 7b), where no significant difference in the relative strength of rule encoding was observed between correct or error trials (grey vs. black bar, t -test, $P = 0.7$, $n = 126$, correct trial mean 0.04, error trial mean 0.018). Interestingly, the rule and direction encoding neurons showed a rule effect, where the difference in strength of the directional signal was significant in the neuron's preferred rule block (Fig. 7c, grey vs. black bar, t -test, $P < 0.01$, $n = 28$, correct trial mean 0.04, error trial mean 0.003) but not in the neuron's non-preferred rule block (Fig. 7d, grey vs. black bar, t -test, $P = 0.2$, $n = 28$, correct trial mean 0.03, error trial mean 0.008). These data further support the correlation data in Fig. 5f and g, where enhanced firing rates on incompatible trials were correlated with behavioural performance, which takes into account errors as well as correct trials. Together, these data strengthen the claim that differences in activity in the population of neurons which integrate both direction and rules relate to behavioural success.

Discussion

Here, we have demonstrated several key components regarding the role of mDS in rule shifting. Through reversible pharmacological inactivation, we have shown that mDS is critical for the maintenance of a new rule after a shift. We have shown that the activity of direction encoding neurons in mDS reflects the conflict between response options. In addition, a subset of direction neurons also integrates rule information, and the resolution of conflict in this population correlates with improvement in behavioural performance in the task. Finally, we have shown that a large subset of neurons in mDS encodes the current rule block and that the strength of the rule signal increases over the course of this rule block, preceding the shift in behavioural strategy. Answering the question of how and where the brain maintains and integrates competing response strategies with actions is central to understanding flexible behaviour. The results provided here are the first to demonstrate that the mDS is able to abstractly represent rule information and use this information to resolve directional conflict when rules change.

Single unit studies have identified a role for the mPFC in representing abstract rules, and lesions to this region have been correlated with difficulty in shifting between available rule strategies (Dias *et al.*, 1996a,b; Birrell & Brown, 2000; Muhammad *et al.*, 2006; Bissonette *et al.*, 2008; Floresco *et al.*, 2008; Moghaddam & Homayoun, 2008; Cromer *et al.*, 2010; Durstewitz *et al.*, 2010; Roy *et al.*, 2010; Antzoulatos & Miller, 2011; Parsegian *et al.*, 2011). In our task, we specifically designed the rule modalities to be equally timed external cues, so as to directly compare neural computations when these cues represent conflicting responses during a rule shift and a stable rule. The inactivation data affirm past research (Ragozzino *et al.*, 2002), showing that mDS is critical not for the initial block responding, but for the maintenance of shifted rule-guided behaviour. Interestingly, inactivation of the nucleus accumbens core leads to a similar behavioural deficit in set-shifting (Floresco *et al.*, 2006). The error performance suggests that an unresponsive mDS may be unable to maintain the encoding of the new rule association. Without a fully operational mDS, rats were severely challenged in identifying and following the appropriate behavioural strategy after a rule shift. Importantly, these results replicate past findings and extend them, showing that rats shifting between different rules are impaired on the shift whether shifting between different competing cued rules, as in this study, or shifting between external visual cues or egocentric directions, as in previous research (Ragozzino *et al.*, 2002). Interestingly, mDS inactivation impaired shifting between two known rules while previous research has shown mDS lesions impair shifting from a known rule to a novel rule. Given this, it seems likely that mDS may be playing a similar role in both of these types of shifts, whereby mDS activity is necessary to store the updated, more appropriate rule strategy, regardless of whether this rule strategy is just learned, or well known. Indeed, the electrophysiological results demonstrated that behavioural performance is correlated with the strength of neural activity on incompatible trial-types in a neuron's preferred rule. The population of neurons which integrate both rule and direction may be the neural mechanism by which changes in behavioural performance occur.

While we expected to find some aspect of rule activity in mDS, we predicted that it would probably be a factor influencing direction, stimulus–response encoding or other aspects of

mDS function. It was therefore somewhat surprising to us to find that neural correlates of rules were recorded in mDS neurons and this activity pattern accounted for the majority of task-modulated neurons. Rule neurons encoded either rule with equal probability and adapted activity to a preferred rule, often encoding the new rule before behaviour switched from the previous rule. The fact that mDS neurons encoded rules so robustly and that it did so prior to behavioural modification reinforces the inactivation experiment data. If mDS is inactivated, presumably the encoding of rules would be slower to develop (Fig. 6b), which in turn would lead to diminished integration of direction and rules during cue presentation (also encoded by mDS, Fig. 5b and c), leading to increased errors on incompatible trials (Fig. 3c) and a difficulty switching from one behavioural strategy to a new one. Finding abstract rule representation in mDS *in vivo* may represent the confirmation of some modelling predictions, which suggest multiple brain regions structure abstract rule representations to allow for generalization of responding and, in turn, generate flexible behaviour (Collins & Frank, 2013).

The majority of our understanding of conflict signals and conflict resolution in set-shifting tasks comes from studies investigating involvement of the anterior cingulate cortex or mPFC (Pardo *et al.*, 1990; Botvinick *et al.*, 2001, 2004; Rushworth *et al.*, 2005; Brown & Braver, 2008; Mayer *et al.*, 2012). Both structures have strong projections to mDS (Gabbott *et al.*, 2005), but the presence of a conflict signal has been observed only a few times in mDS (Thorn *et al.*, 2010; Bryden *et al.*, 2012). In our task, conflict existed between response directions when cues were simultaneously instructing the rat to go either left or right. Neural activity in direction neurons reflected this directional conflict; however, in rule-direction neurons the conflict signal was resolved over the course of the preferred rule block. These data suggest that the mDS plays an important role in resolving the inherent conflict in situations where correct choices need to reflect a guiding rule for making decisions when competing information is present.

Our results have implications for computational models of decision-making. Most computational constructs leave the computation to cortical regions and posit that more downstream regions act upon signals from cortical areas (Cohen & Frank, 2009). Our results suggest that the abstract representation of rules guiding behaviour, of the associations of directions with particular cues and the integration of these two abstract representations occurs in the mDS. If this is true, it provides an additional location for the complex computations necessary for decision-making processes and puts this location in a subcortical region.

If mDS is so critical for rule shifting, encoding rules and integrating rules with responses, what is the role of mPFC? There is a large body of literature demonstrating that genetic, developmental and pharmacological manipulations to mPFC negatively impact the ability of a mammal to shift between response strategies (Dias *et al.*, 1996a, b; Birrell & Brown, 2000; Colacicco *et al.*, 2002; Stefani *et al.*, 2003; Stefani & Moghaddam, 2006; Bissonette *et al.*, 2008, 2014; Floresco *et al.*, 2008; Roy *et al.*, 2010). It may well be that mPFC is important for signalling the shift to a new rule-based response strategy (Murray *et al.*, 2000; Toni *et al.*, 2001; Wallis *et al.*, 2001; Nieder *et al.*, 2002; Durstewitz *et al.*, 2010) and that mDS maintains this new rule representation, which biases particular directional responses and

therefore flexibly alters behavioural responding to match the changed rule contingency. Alternatively, while mPFC ensembles are disordered in the early phases of a rule shift, mDS rule cell neural activity quickly reflected the appropriate rule block. It may be that successful behavioural trials, driven by the rapid encoding of mDS association, are in the service of informing mPFC of what the new correct rule should be. Furthermore, research has demonstrated that rule shifting is dependent upon PFC initially, but after many shifts, this is no longer the case (Rich & Shapiro, 2007). In conjunction with the data presented here, this suggests that mDS may be a locus where abstract rule representations are maintained, separate from those in mPFC. A critical future experiment will entail inactivation of mPFC or mPFC projections to mDS only during a set-shift to test what mPFC is actually communicating to mDS.

Further understanding the functional connectivity between prefrontal and striatal areas is especially critical in light of recent research suggesting dorsal striatum may be a source for cognitive rigidity observed in psychiatric disorders, including schizophrenia (Stelzel *et al.*, 2010) and autism spectrum disorder (Takarae *et al.*, 2007; Corbett *et al.*, 2009; Yerys *et al.*, 2009; Miller *et al.*, 2015). Additionally, D2 receptor density in striatum and decreased connectivity with frontal cortical regions are associated with deficits in cognitive flexibility (Ritchie & Noble, 2003; Stelzel *et al.*, 2010). A single nucleotide polymorphism rs1800497 determines striatal DRD2 density (Markett *et al.*, 2011). This mutation affects striatal response to dopamine, and dorsal striatum is known to be more active in mediating instrumental tasks and be modulated by prediction error signalling. In this light, dopamine-dependent flexible behaviours needed for mediating set-shifting may be affected directly by striatal dopamine and may not be a secondary consequence of impacting cortico-striatal loops themselves.

In conclusion, by developing a well-balanced task suitable for single unit investigation, the data directly support a role for mDS in the representation of abstract rule representation and integration of rule information with action selection. These results revealed a rich and robust representation of current rule contexts within the mDS as well as the integration of choices with action policies, and support recent human data suggesting a more cognitive role for mDS.

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Abbreviations

ANOVA	analysis of variance
GABA	γ -aminobutyric acid
mDS	medial dorsal striatum
mPFC	medial prefrontal cortex

SEM standard error of the mean

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Fig. 1. Behavioural schematic showing timing of the task and trial-type by rule breakdown. (a) Schematic of odour port, fluid wells and directional cues as well as flow chart of successful trial completion. (b) Example responses within different rule blocks, demonstrating compatible and incompatible trials with directional information presented by both cues and demonstrating the controlled, counterbalanced nature of the task timing and rule presentation.

Fig. 2.

Rule shifting deficits during mDS inactivation. (a) Inactivation study histology, identifying bilateral cannula tract placement. Black circles represent spread of Bac/Mus ($n = 8$). (b) Average trials to criteria for blocks 1 and 2 for saline and baclofen/muscimol infused rats. Both groups required significantly more trials to criterion in block 2 than in block 1, demonstrating difficulty in shifting behaviour from the previous rule. Bac/Mus infused rats require significantly more trials to criterion in block 2 than saline control rats. (c) Errors to criterion. Bac/Mus infused rats commit significantly more regressive errors than saline controls, but the same number of perseverative errors. (d) Errors to criterion represented as

percentage of trials to criterion. Bac/Mus infused rats commit significantly more regressive errors than saline controls. (e) Compatibility percentage correct difference score. In block 1, no differences are observed between drug or pre/post-criteria performance. (f) Compatibility percentage correct difference score, block 2. In block 2, Bac/Mus infused rats have a significantly higher difference in performance between compatible and incompatible trial types pre-criterion, compared with saline rats. This difference is reduced post-criterion. Bar graphs represent the mean $\pm$ SEM. *Significance of at least $P < 0.05$.

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Fig. 3.

Behaviour and neural analysis during mDS recordings. (a) Histology showing electrode path and recording sites ($n = 6$). (b) Recording session behaviour showing the difficulty switching between rules. (c) Rats committed significantly more regressive-type errors than perseverative errors in block 2. (d) Breakdown of single neurons showing number of neuron types as classified by two-factor regression. (e) Expanded area of electrode path, with different markers representing the approximate location a neuron was recorded and the classification of that neuron. Open diamonds represent neurons not significantly modulated by the task. Grey circles were classified as rule neurons, dark grey squares as direction neurons and black circles as rule and direction neurons. The size of markers is approximately $45 \mu\text{m}$, or roughly three times the size of a normal striatal Medium Spiny Neuron (MSN) to allow for visibility when plotted. Black horizontal lines denote the most dorsal electrode location, as noted during surgery, and the most ventral electrode location, as noted in histology. Dorsal/ ventral atlas measurements provide the recording depth from top of the skull, and demonstrate the overall drive length for each rat, with a minimum drive-length of $\sim 1000 \mu\text{m}$, and a maximum drive-length of $2400 \mu\text{m}$, showing the wide range and random sampling of dorsal striatal neurons. Bar graphs represent the mean $\pm$ SEM.

*Significance of at least $P < 0.05$.

Fig. 4.

Breakdown of neural data for direction neurons by trial-type, demonstrating conflicted directional signal in both the preferred and the non-preferred directions. Grey bar represents 500 ms cue presentation. Inset bar graphs represent the mean $\pm$ SEM for cue epoch.

*Significance of at least $P < 0.05$.

Fig. 5.

Rule-sensitive direction neurons in mDS during set-shifting. (a) Example rasters and peristimulus time histograms for a neuron which integrates preferred direction (left) with a preferred rule (Light) aligned at odour poke (time 0). Discriminative cues turned on 500 ms after poke. (b–e) Average firing for all 28 direction/rule cells during the first (b, d) and last (c, e) ten trials for all trial types in the preferred (b, c) and non-preferred (d, e) rule blocks. (b) Neural activity in the preferred rule block remains stable for compatible trials (thick black), but early conflict signals among incompatible cues (thick grey) are resolved by the end of the block (thick black and thick grey, c) Conflict signal for preferred direction incompatible trials is resolved (thick black vs. thick grey) late in the preferred rule block. (d) Neural activity shows a trend towards a conflict signal early in the non-preferred rule block (thick black vs. thick grey). (e) Neural activity degrades from early to late in the non-

preferred rule block for both preferred direction and non-preferred direction cues and conflict signal remains. (f) Neural activity is not correlated with behavioural success early in the preferred rule block, but neural activity is significantly positively correlated with behavioural performance at the end of the rule block. (g) Neural activity is not correlated with performance either early or late within the non-preferred rule block. Inset bar graphs represent the mean $\pm$ SEM for cue epoch. *Significance of at least $P < 0.05$, #trend.

Fig. 6. Neural correlates of rule encoding in mDS neurons during set-shifting. (a) Population data for rule encoding neurons ($n = 126$), demonstrating significant preference for one rule (solid line) over the non-preferred rule (dashed line). Activity is different during the cue but also from the time of nosepoke, indicative of expectation of a rule in the absence of the immediate presence of a cue. (b) Neural encoding of preferred rule increases from early (grey, dashed) to late (black, solid) in a block for a subset of rule neurons. (c) Rule cells rapidly encode the correct rule. In block 1, rule neurons significantly increased activity on average by trial 16, while behaviour on average reached criterion by trial 34. (d) Rule cells in block 2 also significantly increased encoding before behavioural change; during a rule shift rule neurons increased activity by trial 34, but behaviour lagged by 102 trials. Dark grey bar represents 500 ms from nosepoke until odour onset, which is represented by a light grey bar. Inset bar graphs represent the mean $\pm$ SEM for cue epoch. *Significance of at least $P < 0.05$.

Fig. 7.

Difference in neural signalling on correct and error trials. (a) Index of neural activity difference between preferred and non-preferred direction incompatible trials shows a stronger difference on correct trials (grey bar) than errors (black bar) for direction neurons. (b) Index of neural activity difference between incompatible trials during preferred and non-preferred rules shows no significant difference. (c) Index of neural activity difference between preferred and non-preferred direction incompatible trials shows a stronger difference on correct trials than errors for rule and direction neurons during the neuron's preferred rule block but not the non-preferred rule block (d). Bar graphs represent the mean $\pm$ SEM. *Significance of at least $P < 0.05$.