Switchmaze: automated, ongoing measurement of motivation and drive switching in mice

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Abstract

Switching between brain states underlying motivated behaviours is fundamental to survival in a dynamic environment. Inflexible repetitive motivated behaviours are a hallmark of several neuropsychiatric disorders such as anorexia nervosa and obsessive compulsive disorder. However, studies of motivated behaviours, such as feeding, drinking and socializing, seldom focus on switching between them or the underlying neural mechanisms, termed drives. In this study, we establish a behavioural assay of motivational switching in mice, using a new automated behavioural monitoring device, the Switchmaze. Motivation switching is measured as the ratio of single probe entries to continuous exploitation runs. Transition analysis is used to further dissect altered motivation switching. To study the neural underpinnings of motivation switching, we present a proof-of-concept experiment using chemogenetic inhibition of the prefrontal-hypothalamic axis. This increased the rate of motivation switching, highlighting the involvement of this pathway in drive switching.

Introduction

Motivated behaviours, like feeding, drinking and social interaction form a scaffold upon which we build our daily lives. These are generated by neural mechanisms, here termed drives, following contemporary definitions[1,2], distinct from earlier drive reduction theory[3]. In health, these drives alternate to meet internal needs, while also being controlled by availability of goal objects[4] and through conscious effort. High and low drive switching at a rapid timescale can be adaptive in different circumstances. Frequent switching is ideal when goal objects are presented in unexpected locations, such as when exploring a new or altered area, whereas infrequent switching is ideal when exploiting a familiar environment during a high need state[5,6]. Inability to switch drives rapidly could account for the behavioural rigidity seen in many neuropsychiatric disorders, and the slowness of behavioural completion in neurodegenerative disorders[7]. In order to study drive switching, we set out to measure switching between motivated behaviours in mice, i.e., motivational switching.

The fragmented nature of mouse behaviour complicates translational use of cognitive flexibility tasks like set-shifting[8–10]. Relatedly, behavioural flexibility in mice is typically studied using approach/avoid decisions[11,12] or simplified set-shifting tasks under water deprivation and after arduous training regimens[8,9]. Furthermore, most previous studies rely on teaching rodents a motor action such as lever pressing that is unlikely to be encountered in the wild. However, navigating a familiar walled environment is natural for mice[13,14]. Therefore, we set up a naturalistic sequential

foraging task which discretizes motivational switching – the Switchmaze[15]. We quantify motivational switching with a behavioural metric derived from the ratio of single probe entries to continuous exploitation runs, termed *motivation switching*. Here, we demonstrate the utility of this approach by showing that it can reveal new information about the structure of natural motivational switching and its neural control.

There is a high degree of commitment in behavioural cycles[16]. That is, once a motivated behaviour (such as feeding) is initiated, its termination likelihood is initially low and increases thereafter[17]. This is also reflected in motivated behaviours being resistant to distractor stimuli[18], and need-based meal length (rather than meal frequency) regulation[19]. We analyse the degree of commitment using run lengths (how many behavioural cycles until a run of one motivation is terminated) and likelihoods of motivational transitions.

Hypothalamic neurons are critical components of drives[20]. This has been revealed by activating or inhibiting genetically defined hypothalamic cell types resulting in initiation or termination of a given motivated behaviour[21,22]. To support these loss- and gain-of-function experiments, typically, one cell type at a time is recorded in a small chamber during interaction with simple goal objects like water and food[23], which corresponds poorly to naturalistic conditions. As a result, how naturalistic drive switching is coordinated among these networks is unknown.

Drive switching likely involves the prefrontal cortex (PFC), which is involved in goal-directed actions and densely connected with the hypothalamus[24,25]. We monitor motivation switching in discretized, repeatable behavioural cycles, while chemogenetically inhibiting the hypothalamus or PFC projections to the hypothalamus, in order to investigate their contributions to drive switching. Our results suggest that motivation switching is a useful parameter, arising particularly clearly from the Switchmaze, and that PFC→hypothalamus neurons are part of a drive network regulating motivation switching by promoting continuous feeding bouts.

Materials and methods

Switchmaze

Build instructions for the Switchmaze, a bill of materials and code are available in the accompanying manuscript[15]. The apparatus (Figure 1A,B) discretizes behavioural cycles of feeding and drinking, which animals perform one at a time in a foraging environment separated from the home cage by a single-entry module. Once inside the foraging environment, upon each entry to a goal area (a trial), food and drink were only available in a 'quantum' of either one 14 mg pellet (Bio-Serv™ Dustless Precision Pellets™ for Rodents, F05684) or one ~10-20 µl drop of water. After goal entry, the animal has one 'return' path available to the start position, which differs from the entry path. Once the animal is in the start position, the availability resets for both food and drink, and the animal also has the option to return to the home cage. Thus, switching between three fundamental motivated behaviours can be followed when the animal is in the start position. Mice live in the apparatus for several days on a 12/12 light dark cycle (lights off at 9 am). The Switchmaze records entries to the foraging environment, start point, and feeding and drinking areas, as well as the consummatory actions pellet retrieval, drinking and running wheel use. Health and welfare monitoring and maze cleaning was done at least once daily and safe operation was monitored on an overhead camera. The apparatus was in an isolated procedure room for all recordings.

Stereotaxic surgery

30 wild-type C57BL6 male mice were used in this study. All experimental procedures were approved by the Netherlands Central Committee for Animal Experiments and the Animal Ethical Care Committee of the Vrije Universiteit Amsterdam (AVD11200202114477). Of the 30 mice, 9 were controls (ctrl), 10 expressed inhibitory designer receptors exclusively activated by designer drugs (DREADDs) in the hypothalamus (H-hM4Di), and 11 expressed inhibitory DREADDs in PFC→hypothalamus projection neurons (PFC-hM4Di). Mice were anesthetized with 'sleep mix' (i.p., fentanyl 0.05 mg/kg, medetomidine 0.5 mg/kg and midazolam 5 mg/kg in saline), the scalp was injected subcutaneously with lidocaine, opened, and 0.2 mm craniotomies were drilled bilaterally at 0.9 mm lateral, 1.4 mm posterior from Bregma. For medial PFC injections of AAV8-syn-DIO-hM4Di-mCitrine, additional craniotomies were drilled bilaterally at 0.4 mm lateral, 1.8 mm anterior from Bregma, and 0.4 mm lateral, 2.3 mm anterior from Bregma. A pulled glass injection needle was used to inject the below doses of virus at a rate of 10-50 nl/min. All H-hM4Di and PFC-hM4Di animals received hypothalamic injections bilaterally 5.4 mm deep in the brain. For PFC-hM4Di animals, the hypothalamic injections contained 30 nl of AAVrg-hSyn-Cre-P2A-tdTomato ($1.5*10^{13}$ GC/ml). For 4 out of 9 H-hM4Di animals, the injections contained 30-150 nl of 1:5 mixture of AAV9-CMV-Cre-tdTomato (10^{12} GC/ml) and AAV8-hSyn-DIO-HA-hM4Di-mCitrine (10^{13} GC/ml), and the other 5 out of 9 animals received 30 nl of AAV8-hSyn-hM4Di-mCherry ($2*10^{13}$ GC/ml). All PFC-hM4Di additionally received medial PFC (mPFC) injections bilaterally. These contained three injections for each hemisphere, of 150 nl AAV8-hSyn-DIO-HA-hM4Di-mCitrine (10^{13} GC/ml). Two 150 nl doses were injected at 1.8 mm anterior from Bregma, 0.4 mm lateral at depths 2.0 mm and 1.5 mm, and one dose at 2.3 mm anterior from Bregma, 0.4 mm lateral at depth 1.75 mm. Injection needles were kept in place for 20 min in hypothalamus and 3-5 min in mPFC before withdrawing. After the injections, an RFID chip (Sparkfun SEN-09416) was implanted under the chest skin, the wounds were closed with tissue glue, anaesthesia was antagonized with wake mix (i.p., flumazenil 0.1 mg/ml and atipamezole 5 mg/ml in saline) and animals received 0.05 mg/ml carprofen in drinking water for 2-4 days as post-operative pain medication.

DREADD manipulation experiments

Animals were injected with saline on the first experiment day and 5 mg/kg C21 (agonist of hM4Di) dissolved in saline on the second day. Motivation switching was measured over a 6-h window as the effect of C21 is likely to last at least that long [26,27].

Histology

We examined viral targeting with post-experiment histology (see Supporting Files H-hM4Di_histology.pdf and PFC-hM4Di_histology.pdf for micrographs of transgene expression in target areas) in 70 μm coronal sections cut from immersion-fixed brains extracted into 4% PFA after an overdose of pentobarbital solution administered under isoflurane anaesthesia. In PFC-hM4Di specimens, the hypothalamic injection centroid was always within the lateral hypothalamic area (LH) with a minority of cases with expression elsewhere (1/11) or difficult to detect expression (1/11). In all cases the mPFC layer 5 (L5) neurons expressed hM4Di-mCitrine, with difficult to detect expression in 1 case out of 11 (same individual as the above difficult to detect hypothalamic expression) and abundant expression in all others. In H-hM4Di specimens, the injection centroids were always within

the LH with a minority of cases with stronger expression in one hemisphere (2/9). As all cases contained hM4Di-expression in the target regions, we included all cases in analysis.

Statistics

Data were analyzed using Matlab R2019a. For the chemogenetic experiments we used two-way repeated measures ANOVA with time (vehicle or C21) as the within-subjects factor, and cohort (ctrl, H-hM4Di or PFC-hM4Di) as the between-subjects factor. This was done by fitting a 'WithinDesign' repeated measures model (fitrm) in Matlab with Cohort as the predictor variable, followed by repeated measures analysis of variance (ranova). When a significant cohort-time interaction was found, three follow-up paired t-tests were used with Bonferroni-corrected significance threshold of 0.0167. Paired t-tests (Bonferroni-corrected significance thresholds: Figure 2, 0.01; Figures 3 and 5, 0.0056) and Wilcoxon rank sum tests were also performed in Matlab.

Results

Structure of discretized motivation sequences

To study the structure of the motivation sequences, C57BL6 mice were housed in the Switchmaze for up to a month in cohorts of 2-4 animals (Figure 1A,B). The mice used the foraging area purposefully for drinking and feeding: A pellet was consumed upon 93.9 ± 10.1 % of food goal entries, and drinking occurred upon 98.5 ± 2.3 % of entries into the water goal. However, running wheel use was limited to 4.0 ± 6.5 % of water goal entries, and is therefore unlikely to affect the results.

To satisfy a need such as hunger, an animal would be expected to enter the food area repeatedly, as only one quantum of consumption (see Methods) was available at each trial. These repeated entries, termed runs, occurred to both food and drink areas (Figure 1C, inset). However, the most common run length was one trial, which can be seen as a behavioural phenomenon controlled neurally by *switching the underlying drive* before and after the trial. Similar single trials are common in rodent behaviour, in particular during trained performance of simple tasks by highly skilled animals[5,9,28]. This seemingly counterproductive behavioural stochasticity may be part of a behavioural camouflage mechanism evolved to elude competitors[6]. In order to capture these prevalent 'singles' in a metric of motivational switching rate, we analysed the ratio of singles to runs (from here, we define a run as a sequence of repeated food or drink trials longer than one). This motivation switching rate was on average 1.4 ± 0.9 (Figure 1D,E) after habituation (>7 days). To test if motivation switching was different from random, we randomly shuffled the trial sequence and recalculated the switch rate 1000 times for each animal (Figure 1E,F). The arising distribution encompassed the actual switch rate in most cases (only 4/30 animals had a motivation switching rate higher than the 99th percentile of the shuffled data). This suggests that spontaneous motivational switching is optimized to appear random, which could conceivably function to decrease the predictive information available to competitors and predators.

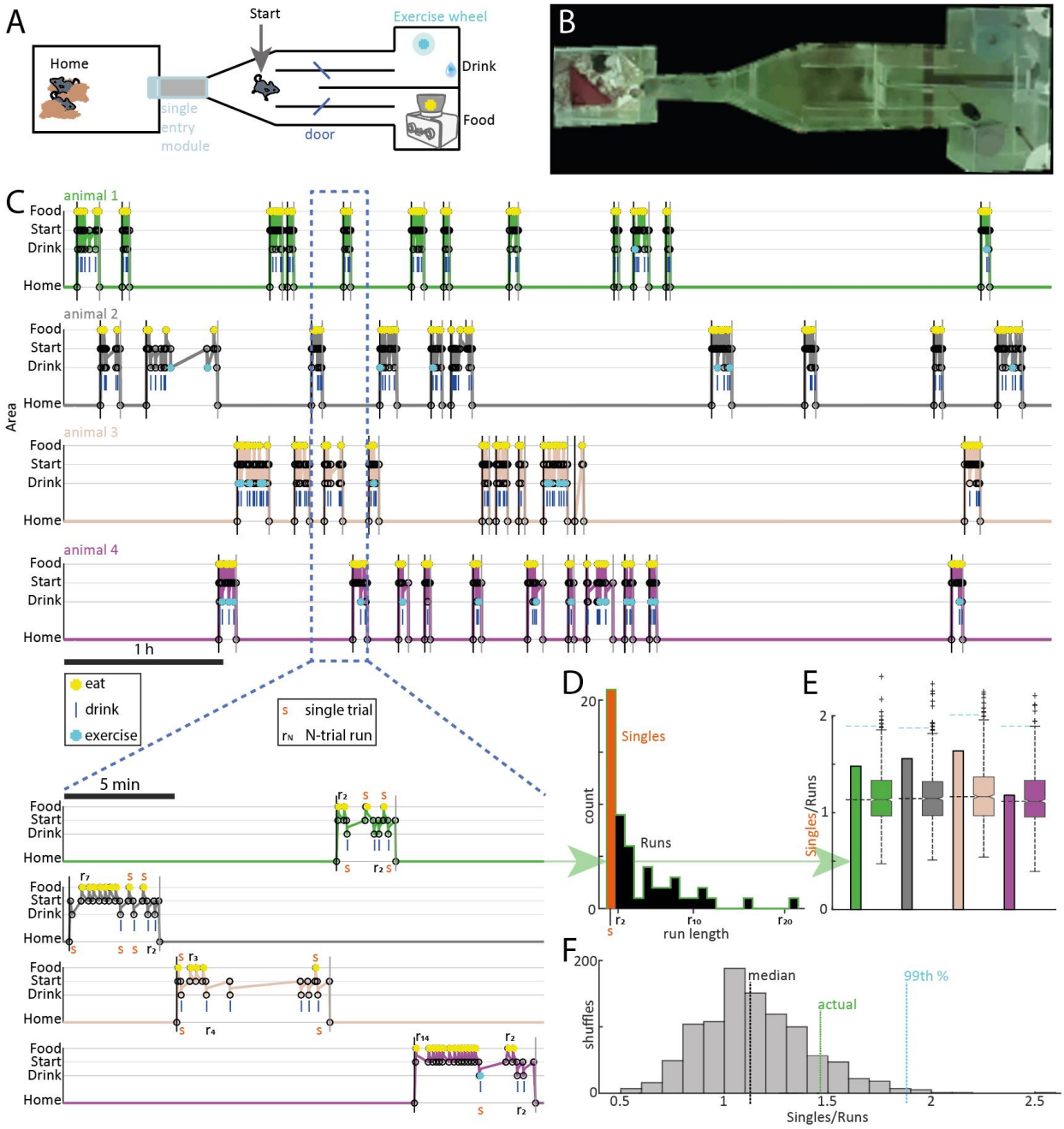

Figure 1, Example data from four mice in the Switchmaze showing discrete switching between drinking and eating. A, Schematic of the maze. B, Picture of the maze in action with an animal eating. C, Ethograms for four mice entering the foraging environment one at a time for an open-ended block. One block for each animal shown in detail in the expanded time window (dashed box). 'Singles' and runs labelled for clarity. D, For animal 1 only, distribution of singles and runs by length. E, Motivation switching for each animal (bars, number of singles divided by number of runs of any length) and motivation switching expected by random chance (box plots are the singles/runs metric from 1000 random permuted behaviour sequences for each animal; dashed horizontal lines denote median (black) and 99th percentile (cyan)). F, Distribution of switching rates from shuffled behaviour sequences for animal 1.

Habituation and environmental control of motivational switching

We next asked how does motivational switching change when the mice encounter environmental challenges. In order to measure changes in motivation switching, a steady baseline should be reached. During the first eight days in the Switchmaze, motivation switching was high in the beginning and then settled toward the steady state value around 1.4 (Figure 2A). This further suggests that while high switching rates above the random regime may be useful for exploration of a new environment, the animals optimize motivation switching toward the median of the random distribution (Figure 1F). Durations of blocks (entries into the foraging area) and trials (entries into the goal areas) also settled to a steady baseline during the first week (Figure 2B,C) and the frequency of blocks and trials settled into a steady diurnal pattern (Figure 2D,E). Blocks may be thought of as meals, because their overall number during the light and dark cycle matched previous estimates of meals in mice with *ad libitum* access to a FED3 pellet dispenser[29].

After habituation, we tested how environmental challenges affect the stabilized metrics. Removing the food dispenser (Figure 2; food deprivation, FD) rapidly increased motivation switching and trial duration, while decreasing trial number, reflecting increased food seeking exploration. These parameters returned to baseline when the food dispenser was returned, 20 hours after removal (Figure 2; re-feeding, RF). Additionally, block duration increased, as more time was spent in the foraging environment in homeostatic re-feeding.

We simulated an environmental uncertainty, similar to depleting resources in patch foraging, by swapping the food and drink areas (Figure 2; SWAP). This resulted in increased motivation switching without other parameter changes, demonstrating that the motivation switching variable is a useful indicator of behavioural structure changes that would not be evident from traditional metrics.

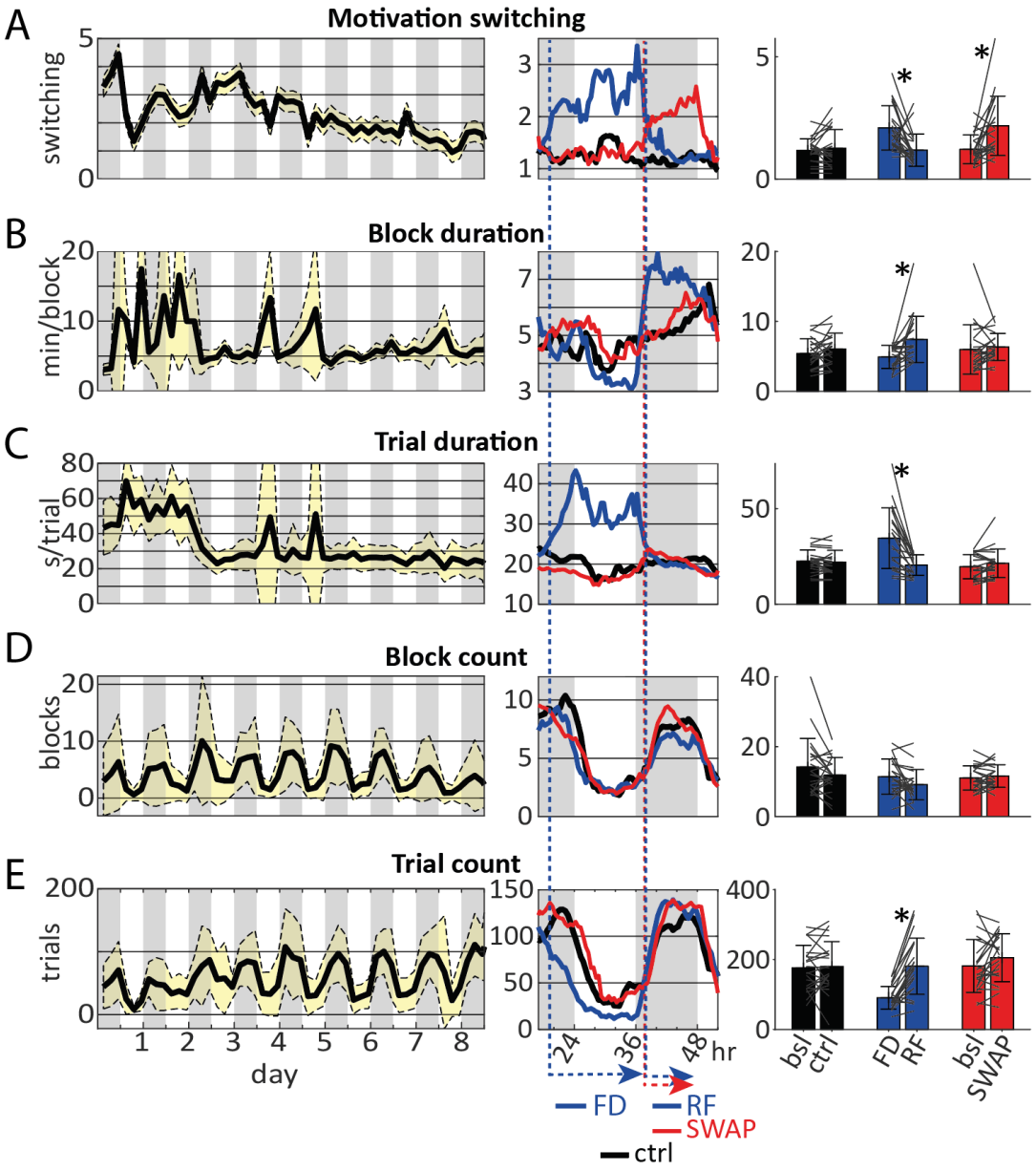

*Figure 2, Stability and dynamics of Switchmaze parameters during habituation and challenges. A, Motivation switching; B, Block duration; C, Trial duration; D, Block count; E, Trial count. Time series data (left and middle panels) have a 4 h bin width and bar graphs (right panels) are measured from the last 6 h before lights-on. N=20 for habituation plots (left), N=22 for the challenges (middle and right). FD, food deprivation; RF, re-feeding; SWAP, swap goals. *, p<0.004.*

As the increased motivation switching could arise from increased transitions between drives or decreased continuous runs of one drive, or both, we analysed the transition counts for the environmental challenges (Figure 3). In control conditions transition counts remained unchanged between the baseline and experimental day (Figure 3A). Re-feeding increased both food-to-food and drink-to-drink transitions (Figure 3B), as expected from homeostatic re-feeding[30]. Swapping goals increased food-to-drink and drink-to-food transitions, while food-to-food and drink-to-drink transitions remained unchanged, suggesting that after entering the intended goal area the animals could re-locate it. An intriguing, consistent possibility is that motivation switching was increased because erroneous expected goal outcomes are not involved in a drive switching mechanisms that functions to maintain motivation switching in the random regime. Instead, a stable territory would

be required for this. These data demonstrate the utility of identifying behavioural sequence changes by the motivation switching variable, and dissecting them further with transition analysis.

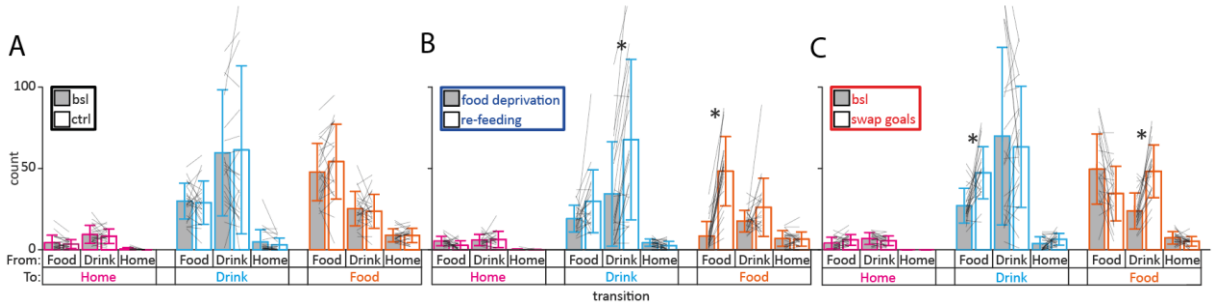

*Figure 3, Trial transitions during 6 h before lights-on. Same raw data as Figure 2 right side plots. A, Baseline and control; B, Food deprivation and re-feeding; C, Baseline and swapped goals. * p<0.0056*

Neural control of motivation switching, drive switching

To assess the potential of the Switchmaze for identifying neural underpinnings of motivational switching, we performed two chemogenetic loss-of-function experiments on neural circuits known to be involved in feeding in a non-trivial way. A broad perifornical region of the hypothalamus, containing the lateral, dorsomedial and tuberal areas, regulates feeding potentially through primary effects on arousal, locomotion and metabolism[23,31,32], and contains intermingled feeding promoting and inhibiting neural populations[21,33,34]. The medial PFC sends axonal projections to this hypothalamic area, affecting feeding in a complex manner depending on behavioural context[35,36]. To test the role of these neural populations in drive switching, we expressed the inhibitory DREADD, hM4Di in either PFC output neurons to the hypothalamus (PFC-hM4Di) or in the perifornical hypothalamus (H-hM4Di). As a control cohort, we used wild-type mice that did not express a transgene, and which were interleaved in groups of hM4Di expressing mice that lived in the Switchmaze.

Basic behavioural metrics were not changed in PFC-hM4Di or H-hM4Di mice upon activation of the inhibitory DREADDs with C21, as two-way repeated measures ANOVA tests showed no significant cohort-time interaction for food consumed ($F(2,27)=0.61$, $p=0.55$), block count ($F(2,27)=0.66$, $p=0.52$), trial count ($F(2,27)=0.50$, $p=0.61$), block duration ($F(2,27)=0.75$, $p=0.48$) or trial duration ($F(2,27)=0.69$, $p=0.51$). However, motivation switching was altered significantly (cohort-time interaction $F(2,27)=3.99$, $p=0.03$) and paired t-tests revealed the effect was a 46.5 ± 45.5% increase in the PFC-hM4Di cohort ($p=0.007$, Figure 4A). The average switch rate exceeded the 99th percentile of switch rates expected from randomly permuted trial sequences (upper dashed lines in Figure 4A). This coincided with 6 out of 11 PFC-hHM4Di animals increasing their switch rate above the 99th percentile of their distribution of random sequences (cyan circles in Figure 4A). This result supports previous findings suggesting that a behavioural role of the PFC is to regulate decision sequence stochasticity[6].

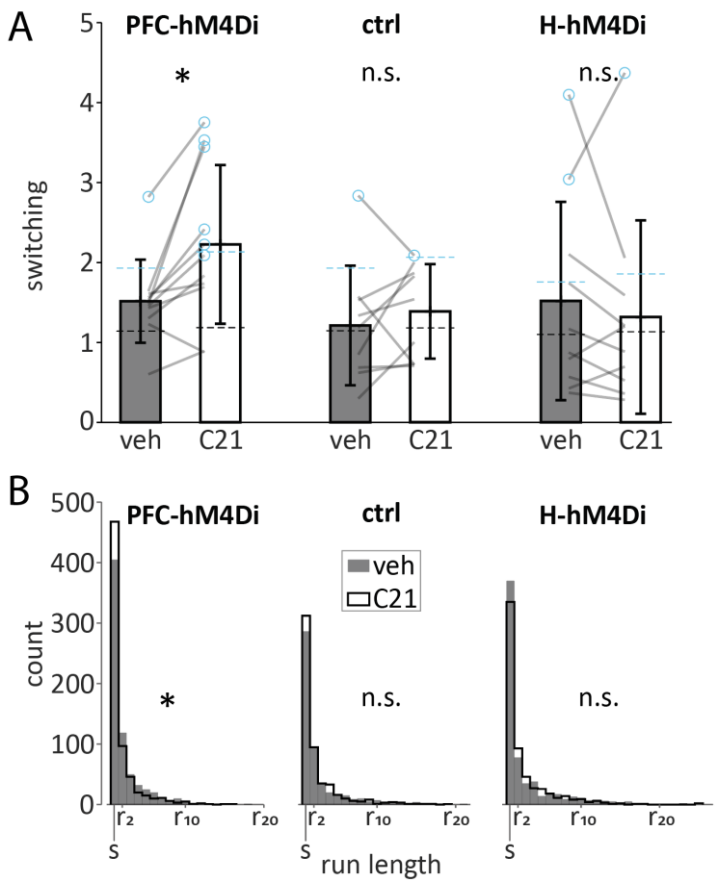

*Figure 4, Chemogenetic modulation of motivational switching. A, Motivation switching quantified as singles/runs in PFC-hM4Di (left, n=11), ctrl (middle, n=9) and H-hM4Di cohorts (right, n=10) during the 6 h after vehicle or C21 injection. Two-way repeated measures ANOVA revealed a significant cohort-time interaction F(2, 27)=3.99, p=0.03 and paired t-tests revealed the effect was in the PFC-hM4Di cohort, * p=0.007. Dashed lines denote chance level arising from the mean of medians (black dashed line) and mean of 99th percentiles (cyan dashed line) of 1000 random-permuted behavioural sequences for each animal. Additionally, for each animal, switching rate higher than the 99th percentile of its random permutations is labelled with a cyan circle. B, Run length distributions across mice in each cohort during 6 h after either vehicle (veh, saline i.p., gray) or C21 (given the following day, thick black outline and no fill). * p=0.0004 by Wilcoxon rank sum test.*

The overall motivation switching rate change in the PFC-hM4Di cohort was due to a significant change in the run-length distribution arising from increased singles and decreased runs (Figure 4B). To find out if this result was due to decreased repetitive cycles, increased transitions, or both, we analysed the trial transitions in PFC-hM4Di animals. The only significantly changed transition was Food-to-Food, which was decreased by 22.7 ± 11.5% (p=0.0009, Figure 5). This suggests that PFC→hypothalamus projections promote repetitive feeding.

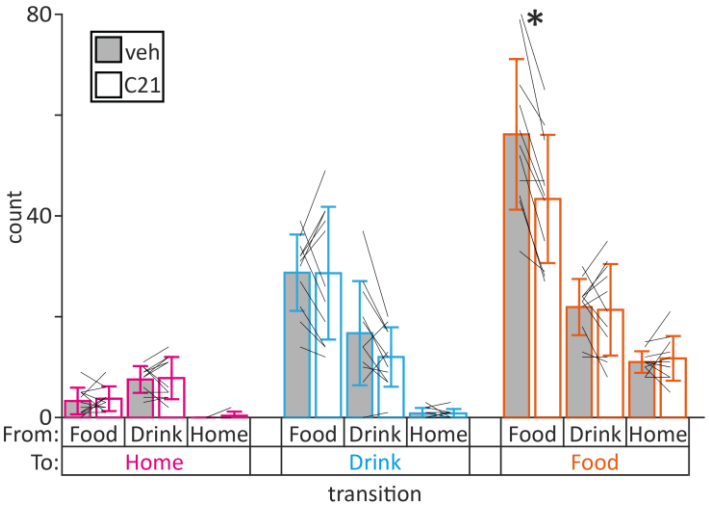

*Figure 5, Food-to-food trial transitions are decreased by PFC inhibition. Same raw data as Figure 4 PFC-hM4Di panels. * p=0.00009*

To test if this decrease in food-to-food transitions reflected shortened feeding runs or less of them or both, we analysed the structure of food runs in vehicle and C21. The mean length of runs to the food area did not change (+2.4 ± 14.9%, p=0.6), nor did the mean number of single entries to the food area (+26.2 ± 49.3%, p=0.1). However, the mean number of food runs decreased by 21.9 ± 21.5% (p=0.01, Figure 6). These results suggest that maintenance of a proportion of food runs is controlled by PFC→hypothalamus projection neurons.

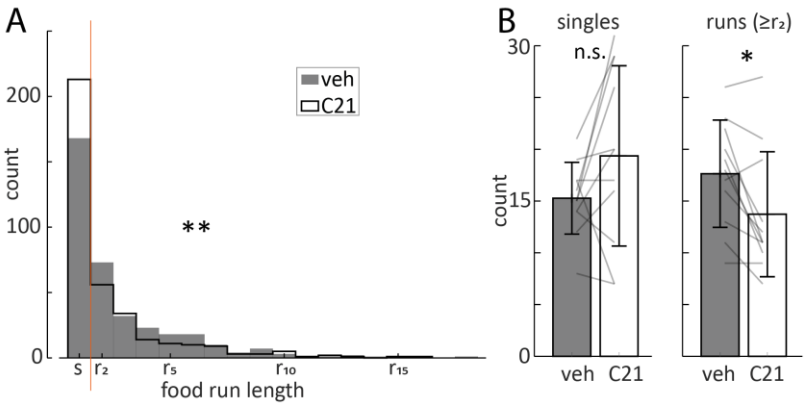

*Figure 6, Food runs are decreased by PFC inhibition. A, Run length distribution for food runs in the PFC-hM4Di cohort (n=11) from the same raw data as Figure 4 PFC-hM4Di panels. B, Food singles and runs (more than 1 cycle) analysed for each animal. ** p=0.002, * p=0.01*

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Together, these data suggest that PFC→ hypothalamus projection neurons selectively promote food runs rather than single feeding cycles, and this effect maintains motivation switching in the random regime.

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308 Discussion

We have demonstrated a behavioural measurement of motivational switching in an ethological semi-natural setting. As the switching rate index, we selected a metric that captures the probe trials (singles) which are stereotypical in rodent behaviour[5,9,28]. This metric is highly useful as it provides information that is otherwise missed by traditional metrics (Figure 2, SWAP condition). By observing distributions of random shuffled motivational sequences (Figure 1F), we found that mice optimize their motivation switching rate to near the median where the highest number of sequences would be found by chance. This behavioural pattern may be useful to hide their motivations from other animals. As this reduces their predictability, which could be exploited by competitors, it may be a form of behavioural camouflage. During habituation to the maze, i.e., exploration of a novel environment, motivation switching rates started high (predictable) and gradually came closer to the median of the random distribution (Figure 2A). During the life-threatening challenge of food deprivation, switching rates rose (predictable) and fell again during re-feeding, suggesting that behavioural camouflage through optimized drive switching (BeCODS) can be discarded when necessary and rapidly reinstated (Figure 2A). The potential survival benefits from BeCODS likely depend on a stable territory, as goal swapping could increase motivation switching. Chemogenetic inhibition of PFC→hypothalamus projection neurons increased motivation switching above the random regime (Figure 4A). As the neocortex evolved relatively recently, this suggests that BeCODS may be an evolutionarily recent refinement of ancient hypothalamic networks.

The increased switching rates due to PFC-hM4Di were accompanied by decreased food-to-food transitions (Figure 5), similar to food deprivation (Figure 3B), suggesting that PFC-hM4Di affected maintenance of the feeding drive. Of note, we did not find an overall change in food consumed,

similar to a recent study of chow consumption in the home cage[36]. The pattern of motivational transitions affected by PFC-hM4Di was distinct from that induced by swapping the goal modules (Figure 3C), which was accompanied by increased food-to-drink and drink-to-food transitions, as expected from an action-outcome mismatch. As such a mismatch would be expected to arise from amnesia, it appears likely that PFC-hM4Di did not reduce memory function. This is in line with a recent study showing improved go/no-go task performance during inhibition of PFC→LH projections[37]. In summary, the motivational pattern changed in a way that suggests a role for PFC layer 5 → hypothalamus connections in sustaining the feeding drive and, through this effect, decreasing the predictability of the animal's behavioural sequences.

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