Anterior cingulate cortex and ventral tegmental area activity during cost-benefit decision-making following maternal immune activation

Eloise Croy1,2, Thomas W. Elston3,4, David K. Bilkey1,2

1Dept of Psychology, University of Otago, Dunedin, New Zealand
2Brain Health Research Centre, University of Otago, Dunedin, New Zealand
3Animal Physiology Unit, Institute for Neurobiology, University of Tuebingen, Tuebingen, Germany
4Dept of Psychology, Helen Willis Neuroscience Institute, University of California at Berkeley, Berkeley, USA

Corresponding author: Eloise Croy
University of Otago, Department of Psychology, William James Building 275 Leith Walk, Dunedin, 9016, New Zealand
eloise.croy.09@aberdeen.ac.uk

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Abstract

Schizophrenia is associated with deficits in memory, behavioural flexibility, and motivation, which can result in difficulties in decision-making. The anterior cingulate cortex (ACC) and ventral tegmental area (VTA) are two brain regions that are involved in decision-making, and display dysfunction in schizophrenia. We investigated ACC and VTA activity in the maternal immune activation (MIA) model of a schizophrenia risk factor. Control and MIA rats completed a cost-benefit decision-making task in a continuous T-maze, choosing between a high cost and high reward (HCHR), and a low cost and low reward (LCLR), option. A choice reversal occurred halfway through each session. Single unit activity in the ACC and local field potentials (LFPs) in the VTA were monitored. Overall, MIA and control rats made a similar proportion of HCHR and LCLR choices across the whole recording session, suggesting similar levels of motivation. However, MIA rats made different decisions than controls during periods of increased uncertainty. This appeared to reflect memory deficits and behavioural inflexibility. MIA animals displayed an increase in ACC activity associated with cost, an increase in synchrony of ACC neurons to the VTA theta oscillation, and a decrease in coherence in the delta frequency between the ACC and VTA. These changes suggest that MIA animals may be biased towards focussing on the cost rather than the benefits of the task, a change also seen in schizophrenia. Here, however, the MIA animals may be able to increase motivation to maintain behaviour despite this change.

Motivation, Behavioural flexibility, Memory, Schizophrenia, Electrophysiology, Single unit
Introduction

Schizophrenia is associated with difficulties in working memory\textsuperscript{1, 2}, and long-term memory\textsuperscript{3, 4}. Patients with schizophrenia may also have difficulties in adapting to changes in the environment as quickly as healthy controls resulting in behavioural inflexibility\textsuperscript{5, 6}. These deficits can result in difficulties in decision-making either through a failure to effectively use previous knowledge to inform a decision, or because of perseveration with a default behaviour (or choice in decision-making), even when it is no longer beneficial\textsuperscript{7}. Patients with schizophrenia can also suffer from reduced motivation, and in decision-making this reduced motivation has been observed as a reduced willingness to expend effort even when the benefit is greater\textsuperscript{8-14}.

Two brain regions that are involved in decision-making are the anterior cingulate cortex (ACC) and the ventral tegmental area (VTA). The ACC appears to access prior knowledge\textsuperscript{15} of similar situations and their potential outcomes, including the cost required to achieve them\textsuperscript{16}, and uses this information to drive a decision towards the optimal choice\textsuperscript{17}. The ACC may provide this direction by modulating the motivational signal associated with the optimal choice, possibly via its connection with the VTA, a region known to signal motivational value\textsuperscript{18-20}.

Activity in both the ACC and VTA regions is altered in patients with schizophrenia. Within the ACC, reduced activity has been linked to deficits in various aspects of decision-making including cognitive control\textsuperscript{21, 22}, memory\textsuperscript{23, 24}, reward anticipation\textsuperscript{25, 26} and error processing\textsuperscript{27-29}. While within the VTA, reduced activity is observed in patients in response to rewarding stimuli\textsuperscript{30, 31} and increased working memory load\textsuperscript{24}. Avolition in schizophrenia has also been linked to reduced activation in the ACC\textsuperscript{32}, and the VTA\textsuperscript{33, 34}.

Here we investigate ACC and VTA activity during a cost-benefit decision task using the maternal immune activation (MIA) model. This model is based on the increased risk of schizophrenia (as well as autism spectrum disorders and bipolar disorder) as a result of immune activation in the
mother during pregnancy\textsuperscript{35-37}. Previous research has demonstrated that this animal model generates several behavioural changes that are similar to those observed in patients with schizophrenia. Deficits in memory have been observed in the MIA model\textsuperscript{38, 39}, although some studies have reported intact memory\textsuperscript{40} and the effects of the intervention are somewhat dependent on species and day of manipulation\textsuperscript{41}. Additionally, MIA rats show increased perseveration errors potentially due to decreased behavioural flexibility\textsuperscript{40, 42, 43}. However, a deficit in motivation has not been observed within the MIA model\textsuperscript{43, 44}, although no previous experiment has investigated motivation in the MIA model in a cost-benefit decision-making task.

In the current experiment both control and MIA rats completed a cost-benefit decision-making task in a continuous T-maze where they chose between a high cost and high reward (HCHR), and a low cost and low reward (LCLR) option. Halfway through each session, and between sessions, the side of each choice reversed. The behaviour of MIA and control animals was monitored, as was single unit activity in the ACC and local field potentials (LFPs) in the VTA. We hypothesised that decision-making performance would vary between the two groups, especially during periods of the session where there was greater uncertainty. In particular we predicted that behaviour at the beginning of each session will be impacted by memory deficits in the MIA rats, and that MIA rats would be less flexible in their response to the reversal procedure. We also hypothesised that ACC neuron activity and ACC-VTA coherence would be affected by the MIA manipulation, reflecting changes in the underlying neural processes.
Method

All experimental procedures were approved by the University of Otago Animal Ethics Committee.

Subjects

Pregnant Sprague-Dawley dams, were administered with either a single dose of Poly I:C (4mg/kg; MIA) dissolved in saline (1ml/kg) or with saline (1ml/kg; control) on gestation day 15 (GD 15; with GD 1 as the day after copulation), whilst anaesthetised with 5% isoflurane in oxygen. Both pregnant dams and resulting litters (until weaning) were kept in open cages. Litters were culled to a maximum of six males and the twenty-two rats used for this experiment were taken from these litters, up to a maximum of two rats per litter (12 control and 10 MIA rats, from eight control and six MIA litters). Rats were housed separately in individually ventilated cages (IVCs) in a room that operated a 12-hour light/ dark cycle and all training and experimentation occurred during the light cycle. Rats were at least 3 months old when training started, and at least 5 months old when they underwent surgery. Food deprivation was used to motivate the rats to complete the task and receive the reward and rats were food deprived to no less than 85% of their free-feeding weight. Water was always available ad libitum in their IVC.

Apparatus

The apparatus consisted of a figure-eight maze containing a touchscreen, pneumatic ram-operated doors, sensors, two 30cm high barriers which could be withdrawn via motors, and two reward bowls fed by peristaltic pumps (Figure 1). The system was operated by five Arduino microcontrollers, which fed touchscreen and sensor information into the computer. A video camera was mounted on the ceiling to track animals’ position. A tether with a head stage (that included LEDs for tracking) was affixed to the animal and connected the electrodes contained in a microdrive to the recording system.
Preoperative Training

Initially rats were habituated to the maze for five x 15 minute sessions. During this time condensed milk was available in the reward bowls and coco pops were scattered throughout the maze.

Following habituation, rats were trained to run in a unidirectional loop. The experimenter started each trial by pressing a button opening the first door (Figure 1; D1). Doors closed behind the rat as they tripped sensors to prevent reversing, and at the end of each loop they were rewarded with 0.5ml of condensed milk. The rat was forced to turn right for three trials followed by left for three trials, which repeated until the session was completed. Rats were allowed to run continuous trials for 20 minutes and were considered trained when they were running at least two trials/minute (40+ trials over the session) for three consecutive sessions (24-hour delay between sessions).

After unidirectional training rats were then trained to press the touchscreen to start the trial themselves. Initially a coco pop was held by the touchscreen to encourage the rat to press the screen, before slowly being phased out until the rat was running the entire session without encouragement. Trial configuration was the same as experienced during unidirectional training. These sessions (including training of touchscreen press and any trials completed) only lasted 15 minutes, and again rats were considered trained when they were running about two trials/minute (30+ trials) for three consecutive sessions.

Next choice and barriers were introduced to the rats. For this configuration, the rats could choose which direction they turned (both DL2 and DR2 were open) for 48 out 64 trials and had 16 forced trials spaced throughout (eight left, eight right, pseudorandom but approximately every four trials). There were no barriers in either direction for the first 32 trials and they received 0.1ml condensed milk at the reward site. For the final 32 trials both barriers were in place and rats had to climb over them to obtain the reward of 0.3ml condensed milk. The rats moved onto the next stage when they were running the full 64 laps in 32 minutes or less for three consecutive sessions.
Rats were then trained to discriminate between choices. There were two choices; one with a barrier present (high cost; HC) and 0.3ml condensed milk (high reward; HR), while the other choice was the absence of the barrier (low cost; LC) and 0.1ml condensed milk (low reward; LR). The rats were assigned to one of two groups, one experienced HCHR on the left arm and LCLR on the right, the other had HCHR on the right and LCLR on the left (counterbalancing side between rats). As before, the rats had 64 trials to run with 48 choice trials and 16 forced trials spaced pseudorandomly (eight left, eight right). At this stage of training the rat had to reach 70% HCHR choices as well as completing the entire session within 32 minutes for three consecutive sessions to progress to the final training stage.

The final stage added a reversal so that, those rats that had experienced the HCHR on the left in the previous stage continued to have the first 32 trials with the HCHR option on the left and LCLR option on the right, but for the final 32 trials this swapped so that the HCHR was now on the right and LCLR on the left (counterbalancing side within rats). The opposite was true for the rats that were trained with HCHR on the right. Therefore, there was an additional reversal between sessions (24 hours apart) as rats always experienced the same configuration each session. Rats were trained and ready for surgery when they were choosing 70% HCHR choices over the whole session and were completing the session in 32 minutes or less.

Surgery

Once rats were considered trained, they underwent surgery. A 3D-printed adjustable microdrive with seven electrode wires (one tetrode and tritrode; 25µm Formvar coated nichrome wire; California Fine Wire) was stereotaxically implanted into the ACC (AP: 2.7mm, ML: 0.4mm, DV: -1.8mm). The VTA (AP: -5.3mm, ML: 1.0mm, DV: -8.2mm) was also stereotaxically implanted with a non-movable electrode (127µm nickel chromium coated wire).
Postoperative training

Once rats had recovered, they were placed back in the maze with their head plugs attached to the recording system. Rats were retrained on the last stage of preoperative training until the rat was choosing 70% HCHR choices and running the 64 laps within an hour for three consecutive sessions. The time limit was increased compared to preoperative training as we were concerned that time involved in extended retraining post-surgery could impact the quality of recording due to scar tissue formation. During this training stage, single unit data was recorded and analysed to check for any potential issues with the recordings and to locate single cells. Electrodes were lowered slowly (40µm at a time) over the course of the experiment up to -2.2mm dorsoventral of dura.

Task Protocol and Open Field

The protocol during the recording sessions was the same as for the last stage of training. In addition we also recorded the animals in an open field so we might ascertain if any differences observed in the main experiment were task specific. For this procedure, eight control and eight MIA rats were recorded as they explored an open field (65cm x 65cm x 50cm) for 10 minutes per day, for three consecutive days.

Data acquisition

Animal movement, single unit neuronal data, and local field potential (LFP) data were acquired through the DacqUSB system (Axona, Ltd., St Albans, UK). Animal movement and location data was acquired via a camera recording the position of infrared LEDs affixed to the head stage. The system sampled the tracking data at 50Hz and converted this to x and y coordinates. Single unit data was monitored each session and each tetrode and tritrode was referenced to the quietest electrode. An action potential sampling threshold was also set between 66-90µV. Single unit data was sampled
at 48kHz and bandpass filtered between 360-7000Hz. LFP data was sampled at 4800Hz and low-pass filtered at 500Hz.

Histology

Once rats had completed the experiment they were anaesthetised with 5% isoflurane in oxygen and 2mA of direct current was passed through each electrode for approximately one second to mark the site with a lesion. Rats were then euthanised via an overdose of isoflurane before being transcardially perfused, initially with 120ml of 0.9% saline and then 120ml of 10% formalin of saline. The brain was then removed and placed in 10% formalin of saline for storage. Before sectioning brains were moved to a 10% formalin/30% sucrose solution. 60µm thick coronal sections were cut around the site of the ACC electrode and VTA wire, mounted, and then stained with thionine acetate for Nissl. Sections were then imaged using a digital microscope and electrode placement confirmed.

Data analysis

Behavioural data

Behavioural data was analysed using only the choice trials after removing the forced trials. Averages were taken for each rat across sessions, before averaging across each treatment (control or MIA).

To investigate avolition, the overall percentage of HCHR choices made was calculated by averaging across all trials. Additionally, to determine whether choices changed at different periods during the session when uncertainty may have varied, we divided the data into four quartiles (1st-16th, 17th-32nd, 33rd-48th, and 49th-64th trials). We averaged percentage of HCHR choices across trials within each of these quartiles. To assess memory deficits a percentage of HCHR choices was also calculated for the first trial.

For measuring the slope of LCLR choices, we took the percentage of LCLR choices for each trial in sequence within a quartile, then ran this through the MATLAB function polyfit. This function
returns the coefficient of a polynomial that is the best fit for the percentage LCLR choices across trials. This coefficient was then used as the gradient of the percentage of LCLR choices made.

We further analysed behaviour within the maze, including investigating time spent in each maze region calculated using the timestamps from the sensors (Figure 1). The latency was the time between the rat entering and exiting the maze region. Running speed used tracking data to determine the amount of movement within each maze region (cm) and the amount of time in the region (s) to produce a measure of speed (cm/s). For analysis of latency and running speed we averaged within each quartile and choice.

Path tortuosity was analysed by using the vessel tortuosity index function in MATLAB. This function measures the degree of tortuosity of a 2D curvilinear shape by determining, for each point on the path, the angle change from the local straight path and then calculating the standard deviation of these digressions. The measure generates a value of zero for an ideal straight path and increases as the path becomes more tortuous. Path data was taken from the choice region of the maze (the vertex; Figure 1). Path tortuosity data was normalised using natural logarithm before further analysis. Correlation between path tortuosity, and speed, to latency used data from each individual trial in the midstem region. Additional analysis examined the average tortuosity within each quartile and choice.

The total time taken to complete the session was calculated (in minutes) from the time the rat started the first trial (initial touchscreen press) until the time the rat reached the reward on the last (64th) trial (sensor SR4/SL4 was tripped; Figure 1).

Local field potential power and coherence

Power and coherence of the LFP data was analysed using the mtspecgramc and cohgramc functions respectively, from the Chronux toolbox. These functions use the multi-taper method, with a moving window of one second, three tapers, and 80% overlap. Initially we produced power
spectral density (PSD) graphs for both the ACC and VTA, for the decision-making task, and for the open field. For further analysis we normalised power using common logarithm. To determine frequency bands to perform further analysis on we compared the PSD’s between the two tasks for any potential differences. Subsequently, we averaged the LFP power and coherence for each frequency band of interest across all sessions for each task. Additional analysis of power and coherence in the decision-making task either took the average for each maze region and choice for the entire session, or the average within each quartile and choice for the entire session. To control for the multiple comparisons alpha was set to \( p < 0.01 \).

Single unit analyses

Single-units were manually identified using peak-to-valley distance and principal component analysis of the waveforms on the software Offline Sorter V3 (Version 3; Plexon). The spiking data of the single-units was then exported to MATLAB for further analysis. Firing rate (Hz) was calculated by counting the total number of spikes within particular time periods. Event timestamps where obtained from the maze touchscreen and sensors. Specifically, firing rate was calculated for each maze region of each trial and transformed using common logarithm as it was not normally distributed (D’Agostino & Pearson test, \( \alpha = 0.05 \)). Mean firing rate across the entire session was the average firing rate of a cell across all maze regions and trials. Additional analysis either examined the mean firing rate within each quartile and choice, or each maze region and choice.

Cell selectivity was determined by looking at mean firing rates. For choice (HCHR or LCLR, right or left) a two-sample t-test was performed to determine whether the cell was significantly firing in one choice or the other. For maze region a one-way ANOVA was performed followed by pairwise comparisons. The results from the pairwise comparisons were then taken, and a cell was considered significant for a maze region if it had fired significantly more often in that maze region compared to the other maze regions (e.g. cell was selective for midstem if pairwise comparisons revealed that it fired significantly more often in the midstem than the vertex, barrier, and reward
regions separately). For a cell to be significant for two maze regions the mean firing rate was taken across both maze regions and then, as previously, a one-way ANOVA and pairwise comparisons was calculated to see if the mean of the two regions fired significantly more often than each of the other two remaining regions (e.g. midstem/ vertex compared to barrier, and reward). For a cell to be considered co-selective for a choice (HCHR or LCLR) and a maze region it had fit the selectivity criteria for both. The number of cells selective for each choice, side, and maze region was compared between the MIA and control rats through analysis with a chi-square test of independence (with $\alpha < 0.01$ to correct for multiple comparisons).

Phase analysis

LFPs were bandpass filtered using a two-pole butterworth filter to the frequencies of interest, and then a Hilbert transform was applied to the data to generate phase information. For each spike, a phase angle was determined for each frequency of interest, with the trough of the oscillation referenced as zero degrees. Mean phase angle and vector length were then calculated for each cell that fired at least 20 times within whichever condition was being examined for each frequency of interest.

To test for phase locking of the cell to LFPs in a particular frequency band we used Rayleigh’s test for circular uniformity to determine if the cell was firing at a specific phase angle or was more evenly distributed\textsuperscript{48}. To compare phase locking across two groups we used Hotelling’s two sample test\textsuperscript{48}, which is a second-order analysis that takes into account both the mean firing phase and vector length describing overall phase locking of each cell.
Results

We observed no difference in the number of training sessions to criteria between control and MIA rats (see supplementary data).

Data were collected from a total of 12 control and 10 MIA rats which completed the experiment, with an average of 10 sessions (completion of 64 laps) per rat. Whilst we were able to use all these data for behavioural analysis, LFP data was only obtained from 9 control and 8 MIA rats due to poor signal/noise on either the ACC or VTA recordings. For single unit data, a total of 217 cells from 11 control rats and 141 cells from 9 MIA rats were recorded. For spike phase analysis, which required the LFP data, the number of cells was reduced to 153 control cells and 68 MIA cells obtained from 9 control and 8 MIA rats. Analysis of percentage of HCHR choices for each session and treatment (two-way mixed-model ANOVA) revealed no significant main effect ($p = 0.13$) or interaction ($p = 0.51$) involving session. Nor was there a significant difference in time taken to complete the therefore we averaged across sessions in future analyses.

Control and MIA rats make different choices during different sections of the task.

Overall both groups of animals preferred the HCHR over the LCLR option (Control, $t (11) = 18.87, p < 0.0001$; MIA, $t (9) = 16.50, p < 0.0001$; paired t-tests), as trained. Furthermore, the percentage of HCHR options chosen (%HCHR) was not significantly different ($t (20) = 0.71, p = 0.49$; unpaired t-test) between control (M = 82.74, SE = 1.74) and MIA rats (M = 80.93, SE = 1.88). To determine whether choices changed at different periods during the session, we divided the data into four quartiles. An ANOVA on %HCHR revealed a significant interaction between treatment and quartile ($F (3, 60) = 4.31, p = 0.008$; two-way mixed-model ANOVA). Bonferroni post hoc tests revealed that the difference between MIA and control animals in the 3rd quartile was approaching significance ($p = 0.07$; Figure 2).
We also examined if there was any difference in behaviour on the first trial of each session where there had been a delay of around 24 hours since the previous trial\textsuperscript{38, 39, 41}. On the first trial control animals were significantly more likely to make LCLR choices ($M = 32.31$, SE = 5.06) than MIA rats ($M = 58.56$, SE = 7.55; $t (20) = 2.97$, $p = 0.008$; unpaired t-test; Figure 2). Additionally, we observed that control rats ($M = 32.31$, SE = 5.06) were more likely than chance to select the LCLR option ($t (11) = 3.50$, $p = 0.005$; one-sample t-test). By contrast, MIA rats performed at chance ($t (9) = 1.13$, $p = 0.29$; one-sample t-test). This difference between control and MIA rats was not observed during initial reversal training (see supplementary data).

Previous data from our lab, obtained in a similar task, indicates that control rats increase their percentage of LCLR choices as the reversal approaches, in apparent anticipation of the LCLR arm becoming the HCHR arm\textsuperscript{45}. In order to determine if this pattern occurred in the data, the gradient of %LCLR responses was measured in the 2\textsuperscript{nd} and 4\textsuperscript{th} quartiles. A two-way ANOVA revealed a main effect of quartile ($F (1, 20) = 8.98$, $p = 0.007$), and a significant interaction between treatment and quartile ($F (1, 20) = 5.17$, $p = 0.03$). This indicated that control rats showed a greater increase in LCLR options chosen as they approached the reversal compared to MIA rats, an effect which was not observed as they approached the end of the session in the 4\textsuperscript{th} quartile.

MIA rats spent more time in the midstem and vertex region prior to LCLR choices

A potential indication of decision-making difficulties is increased time spent immediately prior to and at the decision-point. In the current experiment, this corresponds to time spent in the midstem and vertex regions. For the midstem region a three-way mixed-model ANOVA of treatment, choice and quartile revealed a significant interaction between these three factors ($F (3, 56) = 5.51$, $p = 0.002$). Tukey’s post hoc tests revealed that the time that MIA animals spent in this region prior to LCLR choices in the 4\textsuperscript{th} quartile was significantly different to all other groups and choices ($p < 0.0001$; Figure 3a). In the vertex region a significant interaction between treatment, choice, and quartile
was also observed \( F (3, 56) = 5.05, p = 0.004 \); three-way ANOVA). In the 4\(^{th}\) quartile the MIA rats show a significantly greater increase in latencies for LCLR choices compared to all other results \( (p < 0.0001; \) Tukey’s post hoc; Figure 3b). Additionally, a post-hoc test revealed there was no significant difference \( t (20) = 0.90, p = 0.38 \); unpaired t-test) in total time taken to complete the session between control \((M = 26.58, SE = 1.52)\) and MIA rats \((M = 28.76, SE = 1.93)\). The increase in latency we observed in MIA animals was not due to differences in running speed, but instead was associated with changes in path tortuosity in the vertex, a behaviour that has previously been associated with decision uncertainty\(^{49}\) (see supplementary data).

**Increased ACC power and decreased ACC-VTA coherence in the MIA rats**

To determine whether changes in LFP power were associated with any aspect of the decision-making task or were more general we compared the power and coherence recorded during the task to that recorded in an open field environment (Figure 4). We observed a 2-5Hz (delta) peak and a smaller 15-22Hz (beta) peak during the decision task for both the ACC and VTA power, and greater coherence between the two regions, compared to that which was observed in the open field. In particular there appeared to be a greater degree of ACC-VTA coherence in the control rats in the delta and theta bands than in the MIA rats during the task. An additional peak in power of the VTA and ACC-VTA coherence in the 5-12Hz (theta) frequency band was observed in both the task and the open field, although the peak frequency within the band appeared to shift to a higher frequency during the task. Further analysis revealed that this peak was only significantly higher for ACC-VTA coherence, with a main effect of task \( F (1, 27) = 6.68, p = 0.016 \); two-way ANOVA). We subsequently performed two-way ANOVAs on each of these frequency bands (delta, theta, and beta) comparing treatments (control versus MIA) and task (decision-making task versus open field). There was no significant main effect or interaction involving treatment, although for the coherence in the delta frequency an interaction between treatment and task approached significance \( (p = 0.058)\).
Since it had previously been observed that LFP power can vary between maze region, and we observed behaviour varying depending on the trial within the session, we wondered if there may be a difference between treatments when analysing the delta, theta, and beta frequency bands for choice and quartile, or choice and maze region (three-way mixed-model ANOVAs). There was a significant interaction between treatment and maze region in the ACC in the delta frequency band (F(3, 45) = 4.41, p = 0.008; Figure 5a). Specifically, in MIA rats an increase in ACC power in the barrier region relative to controls and the inverse for the reward region (decreased power relative to controls). For delta coherence between the two brain regions there was a significant main effect of treatment when comparing quartile (F(1, 39) = 11.64, p = 0.002; Figure 5b), and maze regions (F(1, 15) = 10.79, p = 0.005; Figure 5c), with control rats showing a greater degree of coherence than MIA rats throughout the experiment.

Mean firing rate of ACC cells was greater for MIA than control cells

The mean firing rate of MIA cells (2.21 ± 2.47 Hz) was significantly greater than control (1.78 ± 2.55 Hz) cells (t(356) = 2.87, p = 0.004; unpaired t-test; Figure 6).

MIA rats had an increased number of ACC cells selective for cost (barrier) and value (barrier/reward) compared to controls

An analysis of the cell selectivity for different aspects of the task revealed that significantly more MIA cells (88%) showed selectivity than control cells (69%) (χ²(1) = 17.55, p = 0.00003; chi-square test of independence; Figure a).

The number of cells selective for each choice, side, and maze region was compared between the MIA and control rats. For choice, a trend was observed for more MIA cells to be selective for the LCLR arm (χ²(1) = 17.02, p = 0.03; Figure 7b), than control cells. For the maze regions significantly more MIA cells were selective for the barrier region (χ²(1) = 29.79, p = 0.0006) and barrier/reward region (χ²(1) = 22.73, p < 0.00001), whilst a trend was found for more MIA cells to be selective for
the midstem/barrier region ($\chi^2 (1) = 4.04, p = 0.04$; Figure 7c). Additionally, there was a trend for more MIA cells to be selective for the LCLR barrier region ($\chi^2 (1) = 6.42, p = 0.01$; Figure 7c) than control cells. There was no significant difference in the number of cells selective for side.

To investigate any differences in the firing behaviour of the choice-selective cells we analysed the mean firing rate across treatment, maze region, and choice with a three-way ANOVA. For the HCHR selective cells a significant interaction between maze region and treatment was found ($F (3, 137) = 2.85, p = 0.04$; Figure 8a). MIA HCHR selective cells had increased firing rate in the barrier region relative to control HCHR selective cells. For LCLR selective cells there was a significant main effect of treatment ($F (1, 124) = 5.25, p = 0.02$; Figure 8b), with an increased firing rate in LCLR selective cells.

We next analysed the mean firing rate of choice selective cells with a three-way ANOVA for quarter, choice, and treatment. For HCHR selective cells a significant interaction was observed between quarter, choice, and treatment ($F (3, 85) = 2.95, p = 0.04$; Figure 8c). In the first two quarters (pre-reversal quarters) MIA HCHR selective cells have increased firing during HCHR choices compared to LCLR choices, but a similar firing rate for both choices in the last two quarters (post-reversal quarters). Control HCHR selective cells, however, have a similar firing rate for both choices in the first two quarters, but increased firing rate for HCHR choices compared to LCLR choices in the last two quarters. For the LCLR selective cells a significant interaction was found between quarter and treatment ($F (3, 67) = 3.02, p = 0.04$; Figure 8d). MIA LCLR selective cells have increased firing rate in the first quarter compared to control LCLR selective cells.

Mean phase angle differed between MIA and control rats of ACC neurons to both the ACC delta frequency and VTA theta frequency

We then analysed the mean phase angle at which the cells fired for each frequency band (delta, 2-5Hz; theta, 5-12Hz; beta, 15-22Hz) in the ACC and VTA, and compared the control and MIA
cells. We found that there was a significant difference between control and MIA cells when we analysed phase for the delta frequency in the ACC (F = 5.03, p = 0.007; Figure a,b). This difference largely reflects the difference in variance of phase angle with MIA cells more likely to fire at a similar phase angle (mean phase = 235.38°, r = 0.018) than controls (mean phase = 237.86°, r = 0.005). Additionally, we observed that control cells fired at a significantly different theta phase angle in the VTA compared to the MIA rats (F = 3.56, p = 0.03; Figure c,d).

Discussion

Our analysis of behaviour in the cost-benefit task revealed three periods where there appeared to be increased uncertainty in decision-making; at the beginning of each session, as the rats approached the reversal, and immediately post-reversal. Decision-making in these periods of uncertainty appeared to be due to memory deficits or behavioural inflexibility, in the MIA rats.

At the beginning of each session MIA rats showed increased uncertainty as to the location of the two choices, compared to controls. On the first trial of each session control rats selected the arm that had previously contained the preferred choice more often than chance possibly because they had recalled the configuration from the previous recording session 24 hours earlier. MIA rats, however, selected the arms at chance levels, suggesting that they failed to recall the previous configuration. As this behaviour was only observed after the increased delay that occurred between sessions, and not on other trials where the intertrial delay was short, it suggests reduced memory capacity over long delays. Data from initial training on the reversal (supplementary material) is consistent with this interpretation as it indicates that during training control animals shifted from uncertainty to the LCLR preference. By the second trial of each session, however, both groups were selecting the preferred choice more often, suggesting that the trial one results were not due to perseveration in the control rats. These results are consistent with previous research into memory in the MIA model where reduced capacity was observed at increased delay.38, 39, 41
Memory deficits in the MIA model may also have impacted decision-making on trials immediately pre-reversal. An increase in uncertainty was observed in the control rats as the reversal approached, with the rats increasingly choosing the less preferred choice in apparent anticipation of that arm soon containing the preferred choice, as has been previously observed in this task\(^45\). The increase in uncertainty and anticipatory choices was not observed in the MIA rats, which suggests that they failed to recall that the reversal occurred mid-session. Alternatively, however, MIA rats may have been unable to flexibly modify their behaviour or they may have had deficits in predictive mechanisms\(^50\). Unfortunately we were unable to clearly differentiate between these explanations.

Following the reversal, both control and MIA rats demonstrated increased uncertainty in their decision-making. The degree of uncertainty was, however, greater in the MIA model, with rats showing increased responding to the arm that had previously contained the preferred choice. This change may have been due to perseveration on the previous response. This sort of behavioural flexibility has previously been observed in both schizophrenia and the MIA model\(^40, 42, 43, 51-54\).

Compared to control rats, MIA animals also displayed a marked increase in the time spent in the decision-making part of the maze towards the end of each session. This increase was not due to a decrease in running speed, as might be expected if the rats were fatigued. Rather it was shown to correlate with path tortuosity, a behaviour previously linked to indecision\(^49\), suggesting that MIA rats had to engage in greater cognitive effort\(^55\) at this point of the session. This again suggests that they have a problem with memory\(^49\) or utilising memory to drive decisions. Despite these deficits, the motivation to perform the task appears to be similar across the two groups, as assessed by running speed, and the overall similarly in performance of the two groups across the whole session. The electrophysiological data we recorded suggested, however, that there was a change in task representation in the MIA model. Within the ACC of the MIA rats we observed an increase in delta activity while the animal was in the cost region, and we found a greater number of neurons encoding the cost, value (cost and benefit), and less costly choice, compared to controls. Increased ACC
activity (both LFP and single cell) has previously been associated with attention \(^{56, 57}\), therefore the increased activity in the MIA rats may indicate an increase in attention to the cost.

In addition to the increase in ACC activity in the MIA rats we also observed an increase in ensemble synchrony of ACC neurons to both the ACC delta oscillations and VTA theta oscillations. An increase in synchrony in the MIA animals is contrary to what has previously been observed for associations between medial prefrontal cortex neurons and hippocampal LFP in an open field environment \(^{58}\), however this may reflect a task and/or region-specific increase. VTA theta oscillations have previously been associated with rewarding stimuli \(^{59}\), the VTA is thought to signal motivational value \(^{18, 19, 60, 61}\), and the ACC has been implicated in action selection and motivation in effortful tasks \(^{62-65}\). The increase in synchrony of ACC neurons to the VTA might, therefore, reflect an increased motivational signal. As we have proposed that the MIA rats have an increased perception of cost, this group may require a greater degree of motivation to overcome the cost, resulting in an increased motivational signal via increased synchrony of ACC neurons to the VTA, which then allows the expression of behaviour that is similar to that of controls. Whilst we observed an increase in synchrony of ACC neurons to the VTA theta oscillations in the MIA model, we also observed decreased coherence between the ACC and VTA in the delta frequency band throughout the task. In a previous study, increases in ACC delta coherence to the VTA have been linked to the absence of effort \(^{19}\). Therefore, in the current experiment the decrease in coherence in the MIA rats may indicate that they perceive the task as more effortful. Thus the differential change in ACC-VTA synchrony between delta and theta bands may result from the MIA rats experiencing the task as more effortful (costly) which again requires a compensatory increase in motivational signal.

Our findings of reduced memory capacity and decreased behavioural flexibility in MIA animals are consistent with the deficits observed in schizophrenia \(^7, 66\). In contrast, there was no specific behavioural evidence to indicate decreased motivation in the MIA rats. While this is consistent with previous findings in the MIA model \(^{43, 44, 67}\), it does not align with the schizophrenia
Electrophysiological data from the MIA rats suggests, however, that there may be an increase in the perception of cost, and in patients with schizophrenia an increased perception of cost is associated with avolition\textsuperscript{8-14, 68}. Additionally, in studies investigating schizophrenia, a difference in motivation between patients and controls was only observed when there was a greater cost\textsuperscript{11-13}. Although it is possible that we may not have reached this cost threshold in the current task, our observations of neural activity suggest that there may be underlying changes in task representation that may have triggered changes in motivational systems that to a large degree moderated any potential behavioural differences.

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References

1. Gold JM, Barch DM, Feuerstaehler LM, et al. Working memory impairment across psychotic disorders. *Schizophrenia Bulletin*. 2018;45(4):804-812. doi:10.1093/schbul/sby134
2. Gold JM, Robinson B, Leonard CJ, et al. Selective attention, working memory, and executive function as potential independent sources of cognitive dysfunction in schizophrenia. *Schizophrenia Bulletin*. Nov 2018;44(6):1227-1234. doi:10.1093/schbul/sbx155
3. Ragland JD, Blumenfeld RS, Ramsay IS, et al. Neural correlates of relational and item-specific encoding during working and long-term memory in schizophrenia. *NeuroImage*. 2012;59(2):1719-1726.
4. Barch DM, Csernansky JG, Conturo T, Snyder AZ. Working and long-term memory deficits in schizophrenia: is there a common prefrontal mechanism? *Journal of abnormal psychology*. 2002;111(3):478.
5. Hutton SB, Puri BK, Duncan LJ, Robbins TW, Barnes TRE, Joyce EM. Executive function in first-episode schizophrenia. *Psychological Medicine*. Mar 1998;28(2):463-473. doi:10.1017/s0033291797006041
6. Pantelis C, Barber FZ, Barnes TRE, Nelson HE, Owen AM, Robbins TW. Comparison of set-shifting ability in patients with chronic schizophrenia and frontal lobe damage. *Schizophrenia Research*. Jun 1999;37(3):251-270. doi:10.1016/s0920-9964(98)00156-x
7. Dridan BA, Ong B, Lloyd S, Evans L, Crowe SF. The simple copy task: detecting higher order visual processing deficits in schizophrenia, dementia, and movement disorder groups. *Australian Psychologist*. Apr 2013;48(2):98-109. doi:10.1111/j.1742-9544.2012.00067.x
8. Reddy LF, Horan WP, Barch DM, et al. Effort-based decision-making paradigms for clinical trials in schizophrenia: part 1-psychometric characteristics of 5 paradigms. *Schizophrenia Bulletin*. Sep 2015;41(5):1045-1054. doi:10.1093/schbul/sbv089
9. Barch DM, Treadway MT, Schoen N. Effort, anhedonia, and function in schizophrenia: reduced effort allocation predicts amotivation and functional impairment. *Journal of Abnormal Psychology*. May 2014;123(2):387-397. doi:10.1037/a0036299
10. Fervaha G, Graff-Guerrero A, Zakzanis KK, Foussias G, Agid O, Remington G. Incentive motivation deficits in schizophrenia reflect effort computation impairments during cost-benefit decision-making. *Journal of Psychiatric Research*. Nov 2013;47(11):1590-1596. doi:10.1016/j.jpsychires.2013.08.003
11. Treadway MT, Peterman JS, Zald DH, Park S. Impaired effort allocation in patients with schizophrenia. *Schizophrenia Research*. Feb 2015;161(2-3):382-385. doi:10.1016/j.schres.2014.11.024
12. McCarthy JM, Treadway MT, Bennett ME, Blanchard JJ. Inefficient effort allocation and negative symptoms in individuals with schizophrenia. *Schizophrenia Research*. Feb 2016;170(2-3):278-284. doi:10.1016/j.schres.2015.12.017
13. Gold JM, Strauss GP, Waltz JA, Robinson BM, Brown JK, Frank MJ. Negative symptoms of schizophrenia are associated with abnormal effort-cost computations. *Biological Psychiatry*. Jul 2013;74(2):130-136. doi:10.1016/j.biopsych.2012.12.022
14. Park IH, Lee BC, Kim JJ, Kim JJ, Koo MS. Effort-based reinforcement processing and functional connectivity underlying amotivation in medicated patients with depression and schizophrenia. *Journal of Neuroscience*. Apr 2017;37(16):4370-4380. doi:10.1523/jneurosci.2524-16.2017
15. Blanchard TC, Hayden BY. Neurons in dorsal anterior cingulate cortex signal postdecisional variables in a foraging task. Journal of Neuroscience. Jan 2014;34(2):646-655. doi:10.1523/jneurosci.3151-13.2014

16. Kolling N, Behrens TEJ, Mars RB, Rushworth MFS. Neural mechanisms of foraging. Science. Apr 2012;336(6077):95-98. doi:10.1126/science.1216930

17. Hillman KL, Bilkey DK. Neurons in the rat anterior cingulate cortex dynamically encode cost-benefit in a spatial decision-making task. Journal of Neuroscience. Jun 2010;30(22):7705-7713. doi:10.1523/jneurosci.1273-10.2010

18. Bromberg-Martin ES, Matsumoto M, Hikosaka O. Dopamine in motivational control: rewarding, aversive, and alerting. Neuron. Dec 2010;68(5):815-834. doi:10.1016/j.neuron.2010.11.022

19. Elston TW, Bilkey DK. Anterior cingulate cortex modulation of the ventral tegmental area in an effort task. Cell Reports. Jun 2017;19(11):2220-2230. doi:10.1016/j.celrep.2017.05.062

20. Mohebi A, Pettibone JR, Hamid AA, et al. Dissociable dopamine dynamics for learning and motivation. Nature. Jun 2019;570(7759):65+. doi:10.1038/s41586-019-1235-4

21. Alústiza I, Radua J, Pla M, Martin R, Ortuño F. Meta-analysis of functional magnetic resonance imaging studies of timing and cognitive control in schizophrenia and bipolar disorder: evidence of a primary time deficit. Schizophrenia research. 2017;188:21-32.

22. Minzenberg MJ, Laird AR, Thelen S, Carter CS, Glahn DC. Meta-analysis of 41 functional neuroimaging studies of executive function in schizophrenia. Archives of General Psychiatry. Aug 2009;66(8):811-822.

23. Jalbrzikowski M, Murty VP, Stan PL, et al. Differentiating between clinical and behavioral phenotypes in first-episode psychosis during maintenance of visuospatial working memory. Schizophrenia Research. Jul 2018;197:357-364. doi:10.1016/j.schres.2017.11.012

24. Dauvermann MR, Moorhead TWJ, Watson AR, et al. Verbal working memory and functional large-scale networks in schizophrenia. Psychiatry Research-Neuroimaging. Dec 2017;270:86-96. doi:10.1016/j.pscychresns.2017.10.004

25. Walter H, Kammerer H, Frasch K, Spitzer M, Abler B. Altered reward functions in patients on atypical antipsychotic medication in line with the revised dopamine hypothesis of schizophrenia. Psychopharmacology. Sep 2009;206(1):121-132. doi:10.1007/s00213-009-1586-4

26. Gilleen J, Shergill SS, Kapur S. Impaired subjective well-being in schizophrenia is associated with reduced anterior cingulate activity during reward processing. Psychological Medicine. Feb 2015;45(3):589-600. doi:10.1017/s0033291714001718

27. Laurens KR, Ngan ETC, Bates AT, Kiehl KA, Liddle PF. Rostral anterior cingulate cortex dysfunction during error processing in schizophrenia. Brain. Mar 2003;126:610-622. doi:10.1093/brain/awg056

28. Minzenberg MJ, Gomes GC, Yoon JH, Swaab TY, Carter CS. Disrupted action monitoring in recent-onset psychosis patients with schizophrenia and bipolar disorder. Psychiatry Research-Neuroimaging. Jan 2014;221(1):114-121. doi:10.1016/j.pscychresns.2013.11.003

29. Carter CS, MacDonald AW, Ross LL, Stenger VA. Anterior cingulate cortex activity and impaired self-monitoring of performance in patients with schizophrenia: An event-related fMRI study. American Journal of Psychiatry. Sep 2001;158(9):1423-1428. doi:10.1176/appi.ajp.158.9.1423

30. Rausch F, Mier D, Eifler S, et al. Reduced activation in ventral striatum and ventral tegmental area during probabilistic decision-making in schizophrenia. Schizophrenia Research. Jul 2014;156(2-3):143-149. doi:10.1016/j.schres.2014.04.020
31. Murray GK, Corlett PR, Clark L, et al. Substantia nigra/ventral tegmental reward prediction error disruption in psychosis. *Molecular Psychiatry*. Mar 2008;13(3):267-276. doi:10.1038/sj.mp.4002058

32. Park IH, Chun JW, Park HJ, et al. Altered cingulo-striatal function underlies reward drive deficits in schizophrenia. *Schizophrenia Research*. Feb 2015;161(2-3):229-236. doi:10.1016/j.schres.2014.11.005

33. Knolle F, Ermakova AO, Justicia A, et al. Brain responses to different types of salience in antipsychotic naïve first episode psychosis: An fMRI study. *Translational Psychiatry*. 2018/09/21 2018;8(1):196. doi:10.1038/s41398-018-0250-3

34. Köhler S, Wagner G, Bär K-J. Activation of brainstem and midbrain nuclei during cognitive control in medicated patients with schizophrenia. *Human Brain Mapping*. 2019;40(1):202-213. doi:10.1002/hbm.24365

35. Sorensen HJ, Mortensen EL, Reinisch JM, Mednick SA. Association between prenatal exposure to bacterial infection and risk of schizophrenia. *Schizophrenia Bulletin*. May 2009;35(3):631-637. doi:10.1093/schbul/sbn121

36. Boksa P. Effects of prenatal infection on brain development and behavior: A review of findings from animal models. *Brain Behavior and Immunity*. Aug 2010;24(6):881-897. doi:10.1016/j.bbi.2010.03.005

37. Brown AS, Meyer U. Maternal Immune Activation and Neuropsychiatric Illness: A Translational Research Perspective. *American Journal of Psychiatry*. Nov 2018;175(11):1073-1083. doi:10.1176/appi.ajp.2018.17121311

38. Zhang Z, van Praag H. Maternal immune activation differentially impacts mature and adult-born hippocampal neurons in male mice. *Brain Behavior and Immunity*. Mar 2015;45:60-70. doi:10.1016/j.bbi.2014.10.010

39. Wolff AR, Cheyne KR, Bilkey DK. Behavioural deficits associated with maternal immune activation in the rat model of schizophrenia. *Behavioural Brain Research*. Nov 2011;225(1):382-387. doi:10.1016/j.bbr.2011.07.033

40. Savanthrapadian S, Wolff AR, Logan BJ, Eckert MJ, Bilkey DK, Abraham WC. Enhanced hippocampal neuronal excitability and LTP persistence associated with reduced behavioral flexibility in the maternal immune activation model of schizophrenia. *Hippocampus*. Dec 2013;23(12):1395-1409. doi:10.1002/hipo.22193

41. Meyer U, Nyffeler M, Yee BK, Knuesel I, Feldon J. Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice. *Brain Behavior and Immunity*. May 2008;22(4):469-486. doi:10.1016/j.bbi.2007.09.012

42. Kleinmans M, Bilkey DK. Reversal learning impairments in the maternal immune activation rat model of schizophrenia. *Behavioral Neuroscience*. Dec 2018;132(6):520-525. doi:10.1037/bne0000275

43. Millar J, Bilkey DK, Ward RD. Maternal immune activation alters sensitivity to action-outcome contingency in adult rat offspring. *Brain, Behavior, and Immunity*. 7/1 2017;63:81-87. doi:https://doi.org/10.1016/j.bbi.2016.08.020

44. Bates V, Maharjan A, Millar J, Bilkey DK, Ward RD. Spared motivational modulation of cognitive effort in a maternal immune activation model of schizophrenia risk. *Behavioral Neuroscience*. Feb 2018;132(1):66-74. doi:10.1037/bne0000230

45. Elston TW, Croy E, Bilkey DK. Communication between the anterior cingulate cortex and ventral tegmental area during a cost-benefit reversal task. *Cell Reports*. Feb 2019;26(9):2353–. doi:10.1016/j.celrep.2019.01.113

46. Khansari MM, O'Neill W, Penn R, Chau F, Blair NP, Shahidi M. Automated fine structure image analysis method for discrimination of diabetic retinopathy stage using
conjunctival microvasculature images. *Biomed Opt Express*. 2016;7(7):2597-2606. doi:10.1364/BOE.7.002597

47. Bokil H, Andrews P, Kulkarni JE, Mehta S, Mitra PP. Chronux: A platform for analyzing neural signals. *Journal of Neuroscience Methods*. 2010/09/30/ 2010;192(1):146-151. doi:[https://doi.org/10.1016/j.jneumeth.2010.06.020](https://doi.org/10.1016/j.jneumeth.2010.06.020)

48. Zar JH. *Biostatistical analysis*. Pearson Education India; 1999.

49. Bokil H, Andrews P, Kulkarni JE, Mehta S, Mitra PP. Chronux: A platform for analyzing neural signals. *Journal of Neuroscience Methods*. 2010/09/30/ 2010;192(1):146-151. doi:[https://doi.org/10.1016/j.jneumeth.2010.06.020](https://doi.org/10.1016/j.jneumeth.2010.06.020)

50. Schacter DL, Benoit RG, Szpunar KK. Episodic future thinking: mechanisms and functions. *Current Opinion in Behavioral Sciences*. Oct 2017;17:41-50. doi:10.1016/j.cobeha.2017.06.002

51. Waller J, Marston HM, McQuade R, Gartside SE. Evidence that aetiologic risk factors for psychiatric disorders cause distinct patterns of cognitive deficits. *European Neuropsychopharmacology*. 2014/06/01/ 2014;24(6):879-889. doi:[https://doi.org/10.1016/j.euroneuro.2013.12.005](https://doi.org/10.1016/j.euroneuro.2013.12.005)

52. Steiner AP, Redish AD. Behavioral and neurophysiological correlates of regret in rat decision-making on a neuroeconomic task. *Nature Neuroscience*. Jul 2014;17(7):995-1002. doi:10.1038/nn.3740

53. Dickerson DD, Wolff AR, Bilkey DK. Abnormal long-range neural synchrony in a maternal immune activation animal model of schizophrenia. *Journal of Neuroscience*. Sep 2010;30(37):12424-12431. doi:10.1523/jneurosci.3046-10.2010

54. Port BS, Hillman KL, Bilkey DK. Anterior cingulate cortex encoding of effortful behavior. *Journal of Neurophysiology*. Feb 2019;121(2):701-714. doi:10.1152/jn.00654.2018
64. Amemori K, Amemori S, Graybiel AM. Motivation and affective judgments differentially recruit neurons in the primate dorsolateral prefrontal and anterior cingulate cortex. *Journal of Neuroscience*. Feb 2015;35(5):1939-1953. doi:10.1523/jneurosci.1731-14.2015
65. Kouneiher F, Charron S, Koechlin E. Motivation and cognitive control in the human prefrontal cortex. *Nature Neuroscience*. Jul 2009;12(7):939-U167. doi:10.1038/nn.2321
66. Lee JH, Park S. Working memory impairments in schizophrenia: a meta-analysis. *Journal of Abnormal Psychology*. Nov 2005;114(4):599-611. doi:10.1037/0021-843X.114.4.599
67. Straley ME, Van Oeffelen W, Theze S, et al. Distinct alterations in motor & reward seeking behavior are dependent on the gestational age of exposure to LPS-induced maternal immune activation. *Brain Behavior and Immunity*. Jul 2017;63:21-34. doi:10.1016/j.bbi.2016.06.002
68. Xu PF, Klaasen NG, Opmeer EM, et al. Intrinsic mesocorticolimbic connectivity is negatively associated with social amotivation in people with schizophrenia. *Schizophrenia Research*. Jun 2019;208:353-359. doi:10.1016/j.schres.2019.01.023
Figure 1 Diagram of the maze. The rats could start the trial by pressing the touchscreen, which opens the first door (D1) and one or both of the second doors (DL2, DR2), depending on the trial type. Once the first sensor (S1) is passed the first door (D1) shuts. Then passing either of the second sensors (SL2, SR2) shuts both second doors (DL2, DR2), and opens the corresponding third door (SL2 opens DL3, SR2 opens DR3). These sensors also cause the reward to be dispensed, SL2 dispenses reward into the reward bowl on the right (Lr) and SR2 dispenses into left reward bowl (Rr). Finally if the rat has made a right turn the left fourth sensor (SR4) closes the last door (DR3) and allows the rat to press the touchscreen to start another trial, this is the same for a left turn and the right sensor (SL4) closes the last door (DL3) and reactivates the touchscreen. For the calculating the results the maze was split into four regions. The midstem was measured from the first door (D1) to the first sensor (S1); the vertex was the region between the first sensor (S1) and the second sensors (SL2/ SR2); the barrier region was the two areas from each second sensor (SL2/ SR2) to its respective third sensor (SL3/ SR3); the reward region was the area between the third sensors (SL3/ SR3) and the first door (D1).

Figure 2 Percentage of HCHR options chosen for each trial within the session (±SEM), forced trials not shown. ‘*’ = p < 0.05; ‘+’ = 0.05 < p < 0.10.

Figure 3 Time spent in either the midstem (a) or the vertex (b) in each quartile, choice, and treatment (±SEM).

Figure 4 Power spectral density graphs for ACC power, VTA power and ACC-VTA coherence in both the cost-benefit decision-making task and an open field environment for both control and MIA rats (±SEM). Dotted lines from x-axis represent the frequency bands used for later analyses (delta 2-5Hz; theta 5-12Hz; beta 15-22Hz).

Figure 5 (a) ACC power for each maze region, choice, and treatment in the 2-5Hz (delta) frequency bands (±SEM). 2-5Hz coherence between the ACC and VTA for either (b) each quartile, choice, and treatment, or (c) each maze region, choice, and treatment (±SEM).
Figure 6 (a) The mean firing rate (Hz) of control (n=217) and MIA (n=141) cells across the entire experiment (whiskers represent 5-95 percentile, ‘+’ shows mean).

Figure 7 Percentage of cells selective for choice (HCHR or LCLR, right or left) or maze region (midstem, vertex, barrier, reward). (a) Percentage of cells selective for either choice or maze region. (b) Percentage of cells selective for choice or maze side. (c) Percentage of cells selective for a maze region(s), and for the presence (HCHR barrier) or absence (LCLR barrier) of the barrier. Numbers above bars represent number of cells. Cells categorised as ‘none’ were either not selective for any choice (b) or not selective for maze region (c), some of these cells will have been selective for neither as indicated in (a).

Figure 8 The mean firing rate (mFR) of choice selective cells. (a) HCHR selective cells mean firing rate in each maze region for each choice. (b) LCLR selective cells mean firing rate in each maze region for each choice. (c) HCHR selective cells mean firing rate in each quarter for each choice. (d) LCLR selective cells mean firing rate in each quarter for each choice.

Figure 9 Phase angle and vector length of ACC neurons against the ACC 2-5Hz (a + b) and VTA 5-12Hz (c + d) oscillations for control (a + c) and MIA (b + d) rats. Control ACC neurons had a mean phase of 237.86° and a mean vector length of 0.005 for the ACC 2-5Hz oscillations (a) compared to MIA ACC neurons which has a mean phase of 235.38° and a mean vector length of 0.018 (b). For the 5-12Hz oscillations in the VTA the control ACC neurons fired at a mean phase of 108.76° with a mean vector length of 0.005 (c) while the MIA ACC neurons fired at a mean phase of 87.87° and a mean vector length of 0.02 (d). Note one MIA cell extended beyond the axis with a mean vector length of 0.34 to VTA 5-12Hz (d). Mean phase angle and vector was calculated for each cell, with zero degrees corresponding to the trough of the oscillations (either VTA theta or ACC beta). Each grey arrow represents a single cell’s mean angle and vector. Black radial line indicates the mean angle and the error bars around the perimeter represent the 95% confidence intervals.
Figure 2

% of HCHR choices

Trial Number
Figure 7

(a) % of total cells
(b) % of total cells

Control
MIA

Choice Selectivity

(c) % of total cells

Maze Region Selectivity
