

Neuronal dynamics of the anterior cingulate cortex during working memory and serotonergic manipulation

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Declaration of Originality

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“A smooth sea never made a skilled sailor”

- Paolo Coelho & Franklin D. Roosevelt

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Abstract

The prefrontal cortex (PFC) plays a strong role in the execution of executive tasks such as working memory. It is thought to exert top-down control over sensory regions, and integrate information from both external stimuli, and internal computation, such as choice and expectation (1-3). The anterior cingulate cortex (ACC) is a region of the PFC known to receive strong projections from serotonergic neurons of the raphe nuclei (4). In the work presented here, the influence of the hallucinogenic compound, and serotonergic agonist, psilocybin, was investigated in the neural circuits of the ACC of awake, head-fixed mice. Extracellular, multi-unit recordings were performed to examine the effects of the systemic administration of psilocybin. Psilocybin was found to transition the network into a desynchronised state, reminiscent of REM sleep. This state was characterised by an increase in activation in the network, combined with a reduction in power of low frequency LFP oscillations in the delta, theta and alpha band, coupled with a moderate increase in power in the gamma frequency band. The encoding mechanisms in the ACC in working memory were examined by conducting similar recordings in mice performing a delayed response task. The neuronal dynamics revealed strong encoding of low-level (stimulus information) and high-level (choice, reward anticipation) information on both a single cell and population level. The population code appeared to have strong predictive properties of task outcome that surpassed the speed at which the behavioural information could correctly classify the upcoming behavioural response. An effective method to test these hypotheses was provided in the optogenetics experiments, which perturbed the neuronal activity during the delay period of the task using channel-rhodopsin 2 (ChR2). This method proved effective and versatile in inducing a behavioural response and perturbing the neural response profiles of cells encoding information key to working memory.

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IN (D). THE MEAN FIRING RATE TRACE FOR EACH TRIAL TYPE IS SHOWN IN THE LOWER PANELS. WHILST THE MEAN FIRING RATE CAN DIFFERENTIATE T1 AND T2 TRIALS, A MORE PRONOUNCED DIFFERENCE CAN BE SEEN WHEN COMPARING HIT AND CR TRIALS. NOTE: FOR ALL DIAGRAMS, THE GREY VERTICAL LINE INDICATES TONE PRESENTATION, THE DARK BROWN LINE INDICATES REWARD ZONE ENTRY, AND THE GOLD LINE INDICATES REWARD ZONE EXIT.

FIGURE 28: LEARNING CURVE PROFILE AND DURATION. (A) MEAN LEARNING CURVE AND STANDARD DEVIATION FOR ALL MICE IN EACH BATCH REPRESENTED BY THE AVERAGE D' VALUE FOR EACH SESSION (NORMALISED SESSION NUMBER). THE RED LINE REPRESENTS THE D' THRESHOLD OF 1. (B) MEAN LEARNING CURVE AND STANDARD DEVIATION FOR ALL MICE, INDIVIDUAL LEARNING CURVES SHOWN IN GREY. (C) DISTRIBUTION OF LEARNING CURVE DURATION FOR ALL MICE (# MICE = 12), AND FOR EACH BATCH (BATCH 1; # MICE = 3, BATCH 2; # MICE = 3, BATCH 3; # MICE = 6). THE RED LINE SHOWS THE MEDIAN, AND THE STANDARD DEVIATION IS SHOWN IN GREY/PALE PURPLE. THE RAW DATA IS SUPERIMPOSED AS GREEN POINTS ON EACH BOXPLOT.

FIGURE 29: SEGREGATION OF TASK-RESPONSIVE AND TASK-UNRESPONSIVE NEURONS. (A) Z-SCORE PEAK LOCATION FOR ALL CELLS, ANALYSED ACROSS ALL TRIALS. THE TASK EPOCHS (CUE, DELAY, OUTCOME, AFTERMATH) WERE DEFINED APPROXIMATELY AS SHOWN IN THE DIAGRAM. (B) AS IN (A), SPLIT INTO T1 AND T2 TRIALS. (C) AS IN (A), FOR INDIVIDUAL EXPERIMENTS. FOR ALL PLOTS, THE GREY LINE INDICATES TONE ONSET, THE BROWN LINE INDICATES REWARD ZONE ENTRY, AND THE GOLD LINE INDICATES REWARD ZONE EXIT. THIS NOMENCLATURE WILL PERSIST FOR THE REMAINDER OF THE FIGURES.

FIGURE 30: SHIFT IN PEAK RESPONSE LOCATION BETWEEN T1 AND T2 TRIALS. (A) AN EXAMPLE NEURON SHOWS A SHIFT IN LOCATION OF PEAK RESPONSE BETWEEN T1 AND T2 TRIALS. (B) THIS DIFFERENCE IS EMPHASISED BY THE STRONG AUROC VALUES IN TWO LOCATIONS IN THE DELAY PERIOD.

FIGURE 31: TONE-RESPONSIVE NEURON. (A) PSPH, AND (B) CORRESPONDING AUROC PROFILE. FOR ALL AUROC PLOTS, THE PALE BLUE LINE INDICATES 95% SIGNIFICANCE FOR THE LOGISTIC REGRESSION MODEL USED TO COMPUTE THE ROC CURVE, THE DARK BLUE LINE INDICATES 99% SIGNIFICANCE, AND THE BLACK DASHED LINE INDICATES THE AUROC VALUE FOR RANDOM DISCRIMINATION. NOTE: THE SIGNIFICANCE VALUE REPRESENT HOW WELL THE LOGISTIC REGRESSION OF THE PREDICTOR (THE FIRING RATE), PREDICTS THE RESPONSE FUNCTION (TRIAL TYPE). HENCE, THE P-VALUE REFERS TO HOW WELL THE FIRING RATE PREDICTS THE TRIAL TYPE. (C) THE UPPER PANEL SHOWS THE FIRING RATE OVER 50 T1 TRIALS FOR THIS CELL, WITH THE MEAN TRACE SHOWN IN THE LOWER PANEL. NOTE: THE MEAN TRACE CORRESPONDS TO THE

PSPH OF T1 TRIALS IN (A), EXCEPT IN (A) THE MEAN IS TAKEN OVER ALL T1 TRIALS. (D) AS IN (C) FOR T2 TRIALS.

FIGURE 32: TONE-ECHO NEURONS. (A) PSPH, AND (B) CORRESPONDING AUROC PROFILE FOR T1 TRIAL TONE ECHO CELL. (C) AS IN (A) AND (D) AS IN (B) FOR A T2 TRIAL TONE ECHO CELL. FOR ALL AUROC PLOTS, THE PALE BLUE LINE INDICATES 95% SIGNIFICANCE FOR THE LOGISTIC REGRESSION MODEL USED TO COMPUTE THE ROC CURVE, THE DARK BLUE LINE INDICATES 99% SIGNIFICANCE, AND THE BLACK DASHED LINE INDICATES THE AUROC VALUE FOR RANDOM DISCRIMINATION.

FIGURE 33: DELAY PERIOD-SELECTIVE NEURONS. (A) PSPH OF SUSTAINED ACTIVITY WITH (D) CORRESPONDING AUROC CURVE. (B) AS IN (A), WITH THE CORRESPONDING AUROC TRACE (E) FOR AN EARLY DP NEURON. (C) AS IN (A), WITH THE CORRESPONDING AUROC TRACE (F) FOR A LATE DP NEURON.

FIGURE 34: REWARD ZONE-MODULATED AND AFTERMATH-MODULATED NEURONS. (A) PSPH OF REWARD ZONE-RESPONSIVE CELL WITH (C) CORRESPONDING AUROC CURVE. (B), (D) AS BEFORE FOR AN AFTERMATH-RESPONSIVE NEURON.

FIGURE 35: POPULATION DISCRIMINABILITY OF DELAY PERIOD CELLS BETWEEN T1 AND T2 TRIALS. UPPER PANEL: THE FIRING RATE FOR EACH CELL OVER ALL TRIALS WAS USED TO DETERMINE THE AUROC VALUE FOR EACH POSITION (FOR EACH CELL). AN AUROC VALUE OF 0.7 SHOWS STRONG DISCRIMINABILITY BETWEEN TRIAL TYPES. LOWER PANEL: THE FIRING RATE FOR EACH POSITION WAS SHUFFLED ACROSS TRIALS ($n_{SHUFFLE} = 500$), FOR COMPARISON. AS EXPECTED AN AUROC VALUE OF 0.5 WAS FOUND FOR ALL CELLS, FOR ALL POSITIONS, CORRESPONDING TO A CHANCE LEVEL DISCRIMINATION.

FIGURE 36: NEURONAL RESPONSE PROFILES OF TWO STIMULUS-SPECIFIC NEURONS REPRESENTING LOW-LEVEL INFORMATION ENCODING. (A) THE RESPONSE PROFILE (BIN SIZE = 20MS) BETWEEN HIT AND MISS TRIALS IS INDISCERNIBLE FOLLOWING TONE PRESENTATION. TONE T1 WAS PRESENTED IN BOTH INSTANCES. (B) SIMILAR RESPONSES WERE ALSO FOUND FOR FA AND CR TRIALS IN WHICH TONE T2 WAS PRESENTED IN BOTH INSTANCES. (C) AND (D) AS IN (A) AND (B) FOR ANOTHER STIMULUS-SPECIFIC CELL.

FIGURE 37: NEURONAL RESPONSE FOR A PREDICTION NEURON. (A) THE UPPER AND LOWER PANELS SHOW A PSTH FOR SUB-TRIAL TYPES WHEREBY A DIFFERENT TONE WAS PRESENTED IN EACH CASE. THE UPPER PANEL SHOWS THE PSTH FOR CR (T2) AND MISS (T1) TRIALS, AND THE LOWER PANEL SHOWS THE PSTH FOR HIT (T1), AND FA (T2) TRIALS. THERE WAS NO SIGNIFICANT DIFFERENCE IN FIRING RATE IN EITHER CASE (P-VALUE DETERMINED FROM ROC ANALYSIS – SEE METHODS). (B) AS IN (A) EXCEPT THE TONE PRESENTED IS THE SAME. IN THE UPPER PANEL, DESPITE TONE T1 BEING PRESENTED IN BOTH THE HIT AND

MISS TRIALS, THE FIRING RATE SHOWED A SIGNIFICANT DIFFERENCE AFTER 120MS. IN THE LOWER PANEL, DESPITE T2 BEING PRESENTED IN BOTH CASES, THE FIRING RATE SHOWED A SIGNIFICANT DIFFERENCE BETWEEN FA AND CR TRIALS AFTER 240MS. ONE ASTERISK INDICATE 95% SIGNIFICANCE, TWO ASTERISKS INDICATE 99% SIGNIFICANCE. ALL DATA IS FROM THE SAME NEURON.

FIGURE 38: REWARD-ANTICIPATION NEURON. (A) THE RESPONSE PROFILE BETWEEN T1 AND T2 TRIALS SHOWS A STRONG DECREASING RAMP RESPONSE TO THE T1 (POTENTIALLY REWARDED) TRIALS. (B) UPON CLOSER INSPECTION THIS RESPONSE IS MOST STRONGLY PRESENT IN HIT TRIALS, WITH FA TRIALS SHOWING A SIMILAR RESPONSE WITH A LONGER LATENCY. (C) THE MISS AND CR TRIALS ARE ABSENT OF THIS RESPONSE. (D-F) THE CORRESPONDING AUROC VALUES FOR EACH TRIAL TYPE IN THE UPPER PANEL. OF NOTE, DESPITE THE STRONGEST RESPONSE WITHIN HIT TRIALS, THE MISS AND CR TRIALS ARE ALSO DISTINGUISHABLE WITH THIS NEURON AS SHOWN BY THE SIGNIFICANCE VALUES IN (F). (G) A COMPARISON BETWEEN THE PEAK ROC CURVES (I.E. CHOSEN AT THE LOCATION OF THE PEAK AUROC VALUE) SHOWS THE STRONGEST DISCRIMINABILITY BETWEEN HIT AND FA TRIALS. (T1-T2, PEAK AUROC VALUE = 0.73, POSITION = 33CM, P-VALUE = 0.00, HIT-FA; PEAK AUROC VALUE = 0.87, POSITION = 77CM, P-VALUE = 0.00, MISS-CR; PEAK AUROC VALUE: 0.65, POSITION = 33CM, P-VALUE = 0.00).

FIGURE 39: LINEAR REGRESSION BETWEEN THE MEAN SPEED PROFILE IN T1 TRIALS AND THE MEAN FIRING RATE OF EACH NEURON IN T1 TRIALS. THE RAW DATA IS SHOWN IN BLUE, AND THE LINEAR REGRESSION IS SHOWN IN BY THE RED LINE. THE COEFFICIENT OF DETERMINATION (R^2) IS QUOTED IN THE TITLE OF EACH PLOT.

FIGURE 40: LINEAR REGRESSION BETWEEN THE MEAN SPEED PROFILE IN T2 TRIALS AND THE MEAN FIRING RATE OF EACH NEURON IN T2 TRIALS. THE RAW DATA IS SHOWN IN BLUE, AND THE LINEAR REGRESSION IS SHOWN IN BY THE RED LINE. THE COEFFICIENT OF DETERMINATION (R^2) IS QUOTED IN THE TITLE OF EACH PLOT.

FIGURE 41: CONTEXT-DEPENDENT SEQUENCE OF ACTIVATION IN T1 AND T2 TRIAL IN NEURONAL POPULATION. (A) NEURONS WERE SORTED ACCORDING TO LOCATION OF PEAK FIRING RATE IN T1 TRIALS (SET 1 CONTAINING 50% OF TRIALS – TRAINING SET). THE BLACK DASHED LINE SHOWS THE LINE OF BEST FIT FOR THE PEAK RESPONSE LOCATIONS. (B) THE CELL ORDER WAS MAINTAINED WHEN PLOTTING THE FIRING RATE OF CELLS IN THE SET 2 (TEST SET) OF T1 TRIALS (THE REMAINING 50% OF TRIALS). (C) THE CELL ORDER WAS MAINTAINED WHEN PLOTTING THE FIRING RATE OF CELLS IN T2 TRIALS. (D) AS IN (A) FOR T2 TRIALS SORTED ACCORDING TO THE LOCATION OF THE PEAK FIRING RATE IN T2 TRIALS (SET 1 CONTAINING 50% OF TRIALS – TRAINING SET). (E) THE CELL ORDER WAS MAINTAINED WHEN PLOTTING THE FIRING RATE OF

CELLS IN THE SET 2 (TEST SET) OF T2 TRIALS (THE REMAINING 50% OF TRIALS). (F) THE CELL ORDER WAS MAINTAINED WHEN PLOTTING THE FIRING RATE OF CELLS IN T1 TRIALS.

FIGURE 42: CONTEXT-DEPENDENT SEQUENCE OF ACTIVATION IS ROBUST IN T1 TRIALS BUT NOT T2 TRIALS. (A)

THE MEAN POSITION OF THE PEAK FIRING RATE IN THE T1 TRIAL TEST SET (OVER CROSS VALIDATED DATASET $n_{\text{CROSSVAL}} = 1000$), RELATIVE TO THE T1 TRIAL TRAINING SET. THE ROOT MEAN SQUARED ERROR IS 4.5CM. (B) THE MEAN POSITION OF THE PEAK FIRING RATE IN THE T2 TRIAL TEST SET (OVER CROSS VALIDATED DATASET $n_{\text{CROSSVAL}} = 1000$), RELATIVE TO THE T1 TRIAL TRAINING SET. THE ROOT MEAN SQUARED ERROR IS 10.3CM. (C) THE MEAN POSITION OF THE PEAK FIRING RATE IN THE T2 TRIAL TEST SET (OVER CROSS VALIDATED DATASET $n_{\text{CROSSVAL}} = 1000$), RELATIVE TO THE T2 TRIAL TRAINING SET. THE ROOT MEAN SQUARED ERROR IS 9.4CM. NOTE: THE SCATTER PLOT OF T1 TRIAL TEST SET VERSUS THE T2 TRIAL TRAIN SET WAS EXCLUDED AS THE T2 TRIAL SEQUENCE WAS NOT ROBUST AGAINST THE T2 TRIAL TEST SET. THE LOCATIONS ARE SHOWN FROM THE DELAY PERIOD (POSITION 25) TO THE END OF THE TRIAL, WITH THE DARK BROWN LINES INDICATED REWARD ZONE ENTRY AND THE GOLD LINES INDICATING REWARD ZONE EXIT.

FIGURE 43: THE NORMALISED MEAN FIRING RATE OF NEURONS WAS ORDERED ACCORDING TO THE T1 “BEST”

SEQUENCE. T1 TRIALS WERE DIVIDED INTO TWO EQUAL SETS. (A) THE NORMALISED FIRING RATE OF NEURONS ON SET 1 OF T1 TRIALS ORDERED ACCORDING TO THE T1 “BEST” SEQUENCE. (B) AS IN (A) FOR SET 2 OF T1 TRIALS. (C) THE T1 “BEST” SEQUENCE WAS TESTED ON T2 TRIALS. (D) THE T1 SEQUENCE WAS GENERATED VIA CROSS VALIDATION ($n_{\text{ITERATIONS}} = 1000$). T1 TRIALS WERE RANDOMLY SPLIT IN HALF TO GENERATE A TRAINING DATASET AND A TEST DATASET. A SEQUENCE WAS COMPUTED FROM THE TRAINING DATASET AND TESTED ON THE TEST DATASET, AND THE T2 TRIALS. THE SIMILARITY BETWEEN THE LINE OF BEST FIT IN THE TRAINING DATASET AND TEST DATASETS, AND T2 TRIALS WAS COMPARED USING SPEARMAN’S CORRELATION COEFFICIENT, ρ (P). THE BLUE DATA POINT QUANTIFIES THE SIMILARITY BETWEEN THE LINE OF BEST FIT OF PEAK RESPONSES IN TRAINING AND TEST DATASETS OF T1 TRIALS WHEN ORDERED ACCORDING TO THE T1 SEQUENCE GENERATED IN THE TRAINING DATASET ($\rho = 0.96$, $p = 0.00$). THE PURPLE DATA POINT QUANTIFIES THE SIMILARITY BETWEEN THE LINE OF BEST FIT IN THE T1 TRAINING DATASET AND THE T2 DATASET WHEN ORDERED ACCORDING TO THE T1 SEQUENCE ($\rho = 0.48$, $p = 0.14$). THE CORRESPONDING P-VALUES FOR EACH MEASUREMENT ARE SHOWN IN THE RIGHT-HAND PANEL. (E) THE PROBABILITY OF EACH CELL BEING LOCATED AT THE ALLOCATED INDEX FOR THE T1 “BEST” SEQUENCE.

FIGURE 44: THE NORMALISED MEAN FIRING RATE OF NEURONS WAS ORDERED ACCORDING TO THE T2 “BEST” SEQUENCE. T2 TRIALS WERE DIVIDED INTO TWO EQUAL SETS. (A) THE NORMALISED FIRING RATE OF NEURONS ON SET 1 OF T2 TRIALS ORDERED ACCORDING TO THE T2 “BEST” SEQUENCE. (B) AS IN (A) FOR SET 2 OF T2 TRIALS. (C) THE T2 “BEST” SEQUENCE WAS TESTED ON T1 TRIALS. (D) THE T2 SEQUENCE WAS GENERATED VIA CROSS VALIDATION (NITERATIONS = 1000). T2 TRIALS WERE RANDOMLY SPLIT IN HALF TO GENERATE A TRAINING DATASET AND A TEST DATASET. A SEQUENCE WAS COMPUTED FROM THE TRAINING DATASET AND TESTED ON THE TEST DATASET, AND THE T1 TRIALS. THE SIMILARITY BETWEEN THE LINE OF BEST FIT IN THE TRAINING DATASET AND TEST DATASETS, AND T1 TRIALS WAS COMPARED USING SPEARMAN’S CORRELATION COEFFICIENT, ρ (P). THE BLUE DATA POINT QUANTIFIES THE SIMILARITY BETWEEN THE LINE OF BEST FIT OF PEAK RESPONSES IN TRAINING DATASET OF T2 TRIALS AND THE T1 DATASET WHEN ORDERED ACCORDING TO THE T2 SEQUENCE GENERATED IN THE TRAINING DATASET ($\rho = 0.77$, $p = 0.08$). THE PURPLE DATA POINT QUANTIFIES THE SIMILARITY BETWEEN THE LINE OF BEST FIT IN THE T2 TRAINING AND TEST DATASETS WHEN ORDERED ACCORDING TO THE T2 SEQUENCE ($\rho = 0.69$, $p = 0.04$). THE CORRESPONDING P-VALUES FOR EACH MEASUREMENT IS SHOW IN THE RIGHT-HAND PANEL. (E) THE PROBABILITY OF EACH CELL BEING LOCATED AT THE ALLOCATED INDEX FOR THE T2 “BEST” SEQUENCE.

FIGURE 45: PROPORTION OF NEURAL POPULATION VARIANCE EXPLAINED BY [PC1, PC2, PC3].

FIGURE 46: PCA CLASSIFIER. (A) NEURAL TRAJECTORIES IN STATE SPACE FOR T1 AND T2 TRIALS, AND THEIR BOOTSTRAPPED ESTIMATES. THE LOCATIONS OF THE TRIAL START AND CUE ONSET ARE HIGHLIGHTED ON ALL FOUR TRAJECTORIES. (B) THE EUCLIDEAN DISTANCE BETWEEN T1 AND T2 TRIALS FOR EACH BELT POSITION. THE EUCLIDEAN DISTANCE BETWEEN THE T1 NEURAL TRAJECTORY AND THE T1 BOOTSTRAPPED ESTIMATE IS ALSO SHOWN, IN ADDITION TO THE EUCLIDEAN DISTANCE BETWEEN THE T2 NEURAL TRAJECTORY AND THE T2 BOOTSTRAPPED ESTIMATE.

FIGURE 47: THE PROFILE OF PRINCIPAL COMPONENTS 1-3 ACROSS TRIAL POSITION, FOR (A) T1 TRIALS, AND (B) T2 TRIALS.

FIGURE 48: (A) THE SCORES OF EACH NEURON FOR THE FIRST 3 PCs ARE SHOWN FOR THE T1 NEURAL TRAJECTORY IN STATE SPACE. THE SCORES REPRESENT THE CONTRIBUTION OF EACH NEURON TO A GIVEN PC. (B) AS IN (A) FOR THE T2 NEURAL TRAJECTORY IN STATE SPACE.

FIGURE 49: THE PERFORMANCE OF THE PCA CLASSIFIER IN PREDICTING ALL FOUR OUTCOMES [Hit, Miss, FA, CR] WAS COMPUTED ON A PER POSITION BASIS (PLOT TRACES) AND AN OVERALL CLASSIFICATION BASED ON

THE AVERAGE TRIAL OUTCOME PREDICTED DURING THE DELAY PERIOD (SHOWN IN FIGURE LEGEND). NOTE THE PEAK IN CLASSIFIER PERFORMANCE SOON AFTER TONE PRESENTATION.

FIGURE 50: (A) THE PERFORMANCE OF THE PCA CLASSIFIER IN MISS TRIALS WAS QUANTIFIED ON A PER POSITION BASIS (PLOT TRACES) AND AN OVERALL CLASSIFICATION BASED ON THE AVERAGE TRIAL OUTCOME PREDICTED DURING THE DELAY PERIOD (SHOWN IN FIGURE LEGEND). (B) AS IN (A) FOR CR TRIALS. (C) AS IN (A) FOR HIT TRIALS. (D) AS IN (A) FOR FA TRIALS.

FIGURE 51: (A) NEURAL TRAJECTORIES IN STATE SPACE FOR HIT AND CR TRIALS, AND THEIR BOOTSTRAPPED ESTIMATES. THE LOCATIONS OF THE TRIAL START AND CUE ONSET ARE HIGHLIGHTED ON ALL FOUR TRAJECTORIES. (B) THE EUCLIDEAN DISTANCE BETWEEN HIT AND CR TRIALS FOR EACH BELT POSITION. THE EUCLIDEAN DISTANCE BETWEEN THE HIT NEURAL TRAJECTORY AND THE HIT BOOTSTRAPPED ESTIMATE IS ALSO SHOWN, IN ADDITION TO THE EUCLIDEAN DISTANCE BETWEEN THE CR NEURAL TRAJECTORY AND THE CR BOOTSTRAPPED ESTIMATE.

FIGURE 52: (A) NEURAL TRAJECTORIES IN STATE SPACE FOR HIT AND MISS TRIALS, AND THEIR BOOTSTRAPPED ESTIMATES. THE LOCATIONS OF THE TRIAL START AND CUE ONSET ARE HIGHLIGHTED ON ALL FOUR TRAJECTORIES. (B) THE EUCLIDEAN DISTANCE BETWEEN HIT AND MISS TRIALS FOR EACH BELT POSITION. THE EUCLIDEAN DISTANCE BETWEEN THE HIT NEURAL TRAJECTORY AND THE HIT BOOTSTRAPPED ESTIMATE IS ALSO SHOWN, IN ADDITION TO THE EUCLIDEAN DISTANCE BETWEEN THE MISS NEURAL TRAJECTORY AND THE MISS BOOTSTRAPPED ESTIMATE.

FIGURE 53: (A) NEURAL TRAJECTORIES IN STATE SPACE FOR CR AND MISS TRIALS, AND THEIR BOOTSTRAPPED ESTIMATES. THE LOCATIONS OF THE TRIAL START AND CUE ONSET ARE HIGHLIGHTED ON ALL FOUR TRAJECTORIES. (B) THE EUCLIDEAN DISTANCE BETWEEN CR AND MISS TRIALS FOR EACH BELT POSITION. THE EUCLIDEAN DISTANCE BETWEEN THE CR NEURAL TRAJECTORY AND THE CR BOOTSTRAPPED ESTIMATE IS ALSO SHOWN, IN ADDITION TO THE EUCLIDEAN DISTANCE BETWEEN THE MISS NEURAL TRAJECTORY AND THE MISS BOOTSTRAPPED ESTIMATE.

FIGURE 54: (A) NEURAL TRAJECTORIES IN STATE SPACE FOR HIT AND FA TRIALS, AND THEIR BOOTSTRAPPED ESTIMATES. THE LOCATIONS OF THE TRIAL START AND CUE ONSET ARE HIGHLIGHTED ON ALL FOUR TRAJECTORIES. (B) THE EUCLIDEAN DISTANCE BETWEEN HIT AND FA TRIALS FOR EACH BELT POSITION. THE EUCLIDEAN DISTANCE BETWEEN THE HIT NEURAL TRAJECTORY AND THE HIT BOOTSTRAPPED ESTIMATE IS ALSO SHOWN, IN ADDITION TO THE EUCLIDEAN DISTANCE BETWEEN THE FA NEURAL TRAJECTORY AND THE FA BOOTSTRAPPED ESTIMATE.

FIGURE 55: CLASSIFICATION VIA THE SELECTIVITY INDEX. (A) THE SELECTIVITY INDEX (BLUE TRACE) FOR A SAMPLE HIT TRIAL CLASSED CORRECTLY AS A HIT TRIAL BY THE PCA CLASSIFIER. THE BLACK LINE REPRESENTS THE DECISION PLANE, THE MAGENTA TRACE REPRESENTS THE DECISION BOUNDARY (A POINT WITHIN THIS REGION WILL BE CLASSED AS INCONCLUSIVE). (B) AS IN (A) FOR A CORRECTLY CLASSED CR TRIAL. (C) AS IN (A) FOR A CORRECTLY CLASSED MISS TRIAL. (D) AS IN (A) FOR AN INCORRECTLY CLASSED FA TRIAL. THE CLASSIFIER CLASSED THE FA TRIAL AS “CORRECT” I.E. A HIT TRIAL. THIS CAN BE SEEN FROM THE LARGELY POSITIVE VALUES FOR THE SELECTIVITY INDEX ACROSS BELT POSITIONS FOR THIS TRIAL. OF NOTE, ALTHOUGH WRONGLY CLASSIFIED, THE SELECTIVITY INDEX OF THE FA TRIAL SHOWS THE MOST FLUCTUATIONS ACROSS THE DECISION BOUNDARY.

FIGURE 56: LOCOMOTION CLASSIFIER. (A) THE BEHAVIOURAL CLASSIFIER WAS BUILT ON THE MEAN SPEED PROFILES FOR T1 AND T2 TRIALS, (SMOOTHED USING A MOVING AVERAGE FILTER, SPAN = 6CM). THE DECISION BOUNDARY WAS DEFINED AS THE MIDPOINTS BETWEEN THE TWO SPEED TRACES AT EACH POSITION. (B) THE SELECTIVITY INDEX (BLUE TRACE) FOR A SAMPLE HIT TRIAL CLASSED CORRECTLY AS A HIT TRIAL BY THE BEHAVIOURAL CLASSIFIER. THE BLACK LINE REPRESENTS THE DECISION LINE, THE MAGENTA TRACE REPRESENTS THE DECISION BOUNDARY (A POINT WITHIN THIS REGION WILL BE CLASSED AS INCONCLUSIVE).

FIGURE 57: THE PERFORMANCE OF THE LOCOMOTION CLASSIFIER IN PREDICTING ALL FOUR OUTCOMES [HIT, MISS, FA, CR] WAS COMPUTED ON A PER POSITION BASIS (PLOT TRACES) AND AN OVERALL CLASSIFICATION BASED ON THE AVERAGE TRIAL OUTCOME PREDICTED DURING THE DELAY PERIOD (SHOWN IN FIGURE LEGEND).

FIGURE 58: (A) THE PERFORMANCE OF THE LOCOMOTION CLASSIFIER IN HIT TRIALS WAS QUANTIFIED ON A PER POSITION BASIS (PLOT TRACES) AND AN OVERALL CLASSIFICATION BASED ON THE AVERAGE TRIAL OUTCOME PREDICTED DURING THE DELAY PERIOD (SHOWN IN FIGURE LEGEND). (B) AS IN (A) FOR MISS TRIALS. (C) AS IN (A) FOR CR TRIALS. (D) AS IN (A) FOR FA TRIALS.

FIGURE 59: (A) THE DISTRIBUTION OF DELAY PERIOD DURATION FOR ALL TRIALS (BIN SIZE = 0.3s). (B) AS IN (A) FOR T1 TRIALS. (C) AS IN (A) FOR T2 TRIALS. NOTE: FOR EACH PLOT THE FINAL TIME BIN INCLUDES ALL TRIALS WITH A DELAY PERIOD GREATER THAN 2.4s.

FIGURE 60: (A-C) RASTER PLOTS FOR THREE SAMPLE CELLS ALIGNED TO TONE PRESENTATION (START OF THE DELAY PERIOD). TRIALS WERE ORDERED ACCORDING TO DELAY PERIOD LENGTH.

FIGURE 61: TEMPORALLY INDEPENDENT DELAY PERIOD RESPONSE PROFILE. (A) PSPH FOR ALL T1 AND T2 TRIALS, WITH (D) CORRESPONDING AUROC PROFILE. (B) PSPH FOR T1 TRIALS, DIVIDED INTO SHORT AND LONG DELAY PERIODS, WITH (E) CORRESPONDING AUROC PROFILE. NOTE THE LACK OF ANY SIGNIFICANT DISCRIMINATION IN THE RESPONSE PROFILE OF THE NEURON BETWEEN T1 TRIALS WITH SHORT AND LONG DELAY PERIODS. (C) AS IN (B) FOR T2 TRIALS, WITH (F) CORRESPONDING AUROC PROFILE. NOTE THE LACK OF SIGNIFICANT DIFFERENTIATION IN THE DELAY PERIOD OF THE RESPONSE PROFILE OF THE NEURON BETWEEN T2 TRIALS WITH SHORT AND LONG DELAY PERIODS. (G) THE PEAK AUROC VALUE IN (D) OCCURS AT POSITION = 32CM. THE ROC CURVE FOR THIS POSITION IS PLOTTED FOR T1-T2 TRIALS, T1 SHORT – T1 LONG TRIALS, AND T2 SHORT – T2 LONG TRIALS. NOTE THE REDUCTION IN DISCRIMINATION ABILITY AT THIS POSITION IN THE RESPONSE PROFILES OF THE NEURON BETWEEN SHORT AND LONG TRIALS.

FIGURE 62: TEMPORALLY DEPENDENT DELAY PERIOD RESPONSE PROFILE. (A) PSPH FOR ALL T1 AND T2 TRIALS, WITH (D) CORRESPONDING AUROC PROFILE. (B) PSPH FOR T1 TRIALS, DIVIDED INTO SHORT AND LONG DELAY PERIODS, WITH (E) CORRESPONDING AUROC PROFILE. NOTE THE SIGNIFICANT DISCRIMINATION IN THE RESPONSE PROFILE OF THE NEURON BETWEEN T1 TRIALS WITH SHORT AND LONG DELAY PERIODS. (C) AS IN (B) FOR T2 TRIALS, WITH (F) CORRESPONDING AUROC PROFILE. NOTE THE LACK OF SIGNIFICANT DIFFERENTIATION IN THE RESPONSE PROFILE OF THE NEURON BETWEEN T2 TRIALS WITH SHORT AND LONG DELAY PERIODS. (G) THE ROC CURVES FOR THE PEAK AUROC VALUE IN (D) ARE COMPARED BETWEEN T1-T2 TRIALS, T1 SHORT – T1 LONG TRIALS, AND T2 SHORT – T2 LONG TRIALS. NOTE: THE TEMPORAL INFLUENCE IS ONLY EVIDENT IN THE RESPONSE PROFILE DURING T1 TRIALS. THE RESPONSE PROFILE APPEARS TO BE TEMPORALLY INDEPENDENT IN T2 TRIALS.

FIGURE 63: (A) THE AUROC VALUES FOR EACH BELT POSITION SHOWING DISCRIMINABILITY BETWEEN T1 TRIALS WITH SHORT DELAY PERIODS AND LONG DELAY PERIODS. (B) AS IN (A) FOR T2 TRIALS.

FIGURE 64: RECORDINGS OF NEURONAL ACTIVITY IN THE ACC DURING A DELAYED-RESPONSE TASK USING OPTOGENETIC PERTURBATION. (A) PRE-LASER EPOCH: TRIALS 1-60 OF THE EXPERIMENT WERE PERFORMED AS IN FIGURE 27. (B) POST-LASER EPOCH: TRIALS 61+ WERE COMPOSED OF INTERLEAVED LASER TRIALS AND CATCH TRIALS. THE PROBABILITY OF THE PRESENTATION OF A LASER TRIAL WAS ~ 0.72 , AND THE PROBABILITY OF A NON-LASER TRIAL WAS ~ 0.28 . THE PROBABILITIES ARE APPROXIMATE DUE TO THE FACT THAT T1 AND T2 TRIALS ARE PRESENTED WITH A PROBABILITY OF 0.5 EACH. A LASER TRIAL WAS PRESENTED WITH THE AFOREMENTIONED PROBABILITY WITH THE INCLUSION THAT IF THE PREVIOUS LASER TRIAL WAS A T1 TRIAL, THE SUBSEQUENT LASER TRIAL WOULD BE A T2 TRIAL. HENCE, THE PROBABILITY OF

A LASER TRIAL FLUCTUATED SLIGHTLY BETWEEN EXPERIMENTS. DURING A LASER TRIAL, A 100MS PULSE OF BLUE LIGHT WAS PRESENTED 400MS AFTER THE TONE.

FIGURE 65: VERIFICATION OF PERTURBATION. (A) UPPER PANEL: A RASTER PLOT OF AN OPTO-TAGGED PV-INTERNEURONS SHOWING THE RESPONSE FOR A LASER PULSE OF DURATION = 100MS, λ = 430-490NM, INTENSITY = 2.25MW. LOWER PANEL: A PSTH OF THE SAME ACTIVITY (BINSIZE = 20MS). (B) THE RESPONSE OF AN OPTO-TAGGED PV-INTERNEURON DURING THE DR TASK. (C) AS IN (A) FOR PUTATIVE PYRAMIDAL CELLS. (D) AS IN (B) FOR A PUTATIVE PYRAMIDAL CELL.

FIGURE 66: MINOR DECREASE IN BEHAVIOURAL PERFORMANCE IN RESPONSE TO LASER STIMULATION (N = 3). (A) LEFT PANEL: THE PERCENT CORRECT FOR PRE-LASER EPOCH VERSUS LASER TRIALS. RIGHT PANEL: D' BETWEEN PRE-LASER EPOCH AND LASER TRIALS. THE RED LINE SHOWS THE D' THRESHOLD FOR PERFORMANCE. (B) THE HIT, FA AND STOPPING RATE BETWEEN THE PRE-LASER EPOCH AND LASER TRIALS. (C) THE SPEED PROFILES FOR T1 AND T2 TRIALS COMPARING THE PRE-LASER EPOCH AND LASER TRIALS.

FIGURE 67: EFFECT OF NEURONAL PERTURBATION ON MOUSE CONFIDENCE (N = 3). (A) THE HIT, FA AND STOP RATE BETWEEN THE PRE-LASER EPOCH AND NON-LASER TRIALS OF THE POST-LASER EPOCH. (B) THE SPEED PROFILES OF T1 AND T2 TRIALS BETWEEN THE PRE-LASER EPOCH AND NON-LASER TRIALS. (C) THE PERCENT CORRECT AND D' FOR THE TWO TRIAL TYPES.

FIGURE 68: THE EFFECT OF LASER STIMULATION ON NEURAL FIRING RATES. (A) THE FIRING RATE OF CELL POPULATIONS OF THREE EXPERIMENTS IN WHICH LASER STIMULATION WAS PERFORMED DURING THE DELAY PERIOD OF A WORKING MEMORY TASK IS SHOWN WHEREBY THE PRE-LASER FIRING RATE IS COMPARED WITH THE FIRING RATE OF THE CELL DURING LASER STIMULATION. MOST CELLS APPEAR TO BE INHIBITED BY THE STIMULATION (PUTATIVE PYRAMIDAL CELLS). OF NOTE, NOT ALL CELLS SHOWED A CHANGE IN FIRING RATE, AS SHOWN BY THE NEURONS THAT LAY CLOSE TO THE UNITY LINE IN THE PLOT. (B) AS IN (A) FOR ALL CELLS IN ALL EXPERIMENTS.

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FIGURE 74: A TREND IN FUNCTIONAL ROLES BETWEEN PUTATIVE PYRAMIDAL CELLS AND INTERNEURONS CAN BE SEEN WHEN EXAMINING WHERE THE PEAK RESPONSE OF EACH NEURAL SUB-POPULATION FALLS IN RELATION TO BELT POSITION. THE PYRAMIDAL CELLS AND INTERNEURONS FROM THE SEQUENCE MAP IN THE FIRST PANEL, ARE DISPLAYED SEPARATELY IN THE SECOND PANEL. THE LOCATION OF THE PEAK RESPONSES OF EACH CELL TYPE ARE SHOWN IN THE THIRD PANEL. THERE IS A MINOR TENDENCY FOR PYRAMIDAL CELLS TO BE ACTIVE DURING THE DELAY PERIOD, WHILE THE INTERNEURONS APPEAR TO PREFERENTIALLY ENCODE THE TONE AND THE REWARD AREA.

FIGURE 75: LINEAR REGRESSION BETWEEN THE MEAN SPEED PROFILE IN T1 TRIALS AND THE MEAN FIRING RATE OF EACH NEURON IN T1 TRIALS FOR ANOTHER NEURONAL POPULATION. THE RAW DATA IS SHOWN IN BLUE, AND THE LINEAR REGRESSION IS SHOWN IN BY THE RED LINE. THE COEFFICIENT OF DETERMINATION (R^2) IS QUOTED IN THE TITLE OF EACH PLOT.

FIGURE 76: LINEAR REGRESSION BETWEEN THE MEAN SPEED PROFILE IN T2 TRIALS AND THE MEAN FIRING RATE OF EACH NEURON IN T2 TRIALS FOR ANOTHER NEURONAL POPULATION. THE RAW DATA IS SHOWN IN BLUE, AND THE LINEAR REGRESSION IS SHOWN IN BY THE RED LINE. THE COEFFICIENT OF DETERMINATION (R^2) IS QUOTED IN THE TITLE OF EACH PLOT.

FIGURE 77: LINEAR REGRESSION BETWEEN THE MEAN SPEED PROFILE IN T1 TRIALS AND THE MEAN FIRING RATE OF EACH NEURON IN T1 TRIALS FOR ANOTHER NEURONAL POPULATION. THE RAW DATA IS SHOWN IN BLUE,

AND THE LINEAR REGRESSION IS SHOWN IN BY THE RED LINE. THE COEFFICIENT OF DETERMINATION (R^2) IS QUOTED IN THE TITLE OF EACH PLOT.

FIGURE 78: LINEAR REGRESSION BETWEEN THE MEAN SPEED PROFILE IN T2 TRIALS AND THE MEAN FIRING RATE OF EACH NEURON IN T2 TRIALS FOR ANOTHER NEURONAL POPULATION. THE RAW DATA IS SHOWN IN BLUE, AND THE LINEAR REGRESSION IS SHOWN IN BY THE RED LINE. THE COEFFICIENT OF DETERMINATION (R^2) IS QUOTED IN THE TITLE OF EACH PLOT.

List of Abbreviations

i. v.	intravenous
i. p.	intraperitoneal
i. c.	intracortical
LFP	local field potential
ROB	recurrent oscillatory bursting
DMS	delayed match to sample
DNMS	delayed non-match to sample
CV	coefficient of variation
SD	standard deviation
SNR	signal-to-noise ratio
5-HT	5-hydroxytryptamine (serotonin)
EEG	electroencephalogram
PCC	posterior cingulate cortex
HC	hippocampus
ACC	anterior cingulate cortex
PFC	prefrontal cortex
mPFC	medial prefrontal cortex
PCA	principal component analysis
FA	factor analysis
EFA	exploratory factor analysis
CFA	confirmatory factor analysis
ChR2	channelrhodopsin-2
AAV	adeno-associated virus
PV	parvalbumin positive
AOI	area of interest
DP	delay period
s.e.m	standard error of the mean
DR	delayed response
PBS	phosphate buffered saline

PSD	power spectral density
PFA	paraformaldehyde
WM	working memory
ROC	receiver operating characteristic
AUROC	area under the ROC curve
PSPH	peri/post-stimulus position histogram
AP	anterior-posterior
ML	medial-lateral
D	depth
NRMSE	normalised root mean square error

Chapter 1: Introduction

Prefrontal cortex; Structure and function

The prefrontal cortex (PFC) is thought to be the one of most influential evolutionary factors in the distinction between humans and their primate cousins (5). This selective region of neural tissue lies in the forebrain and can often become the chief protagonist in neural cognition studies of complex tasks. However, the impervious nature of the PFC arises when the task does not demand a sufficiently high level of cognition. Many types of memory are too simplistic for the PFC. It is not sufficient to encode a stimulus long after that information has ceased to be available, and then recall that information on demand. Rather, the PFC prefers the challenge of a particular type of memory; working memory. This mnemonic feat requires not only the memory function, but also resilience to noise whilst sustaining sufficient adaptability to regularly update to new relevant inputs. This provides ample complexity to allow the PFC to harness its cognitive prowess and to execute such goal-directed tasks.

Neural cognition within the PFC, particularly concerning working memory, is performed in an analogous form to many other mechanisms in the cortex, by completing a sensory-motor loop. It integrates sensory information (directly or indirectly via other primary sensory regions of the cortex), and produces an output function that is relayed through the nervous system to produce an appropriate response. The high-level structure of this information loop is remarkably similar across different regions of the cortex. This is likely to be partially due to the architectural similarity across different brain regions, despite diverse functionality (6). The laminar structure is composed of 6 layers, although layer IV is not a distinct region in the rodent PFC, hence the laminar structure remains restricted to layers I, II, III, V, VI. Layer I can be broadly termed as the input layer. It mostly contains neuropil from long-range projections from both cortical and subcortical regions, and the apical dendritic tufts of cortical pyramidal cells. It also contains the cell bodies of slow-spiking GABAergic interneurons, which are thought to provide feed-forward inhibition to the dendrites of pyramidal cells in deeper layers. Layers II and III are populated by the somata of small pyramidal cells, and slow and fast spiking GABAergic interneurons. Layers V and VI form the output layer, containing the soma of larger pyramidal cells, and a diverse population of interneurons, although fast-spiking interneurons appear to be the most prominent interneuron type in this region (7). Anatomically, the PFC shares similar

neuronal heterogeneity with many other brain areas, with multiple types of pyramidal cells and interneurons present to integrate information (7). These cell families have predominantly excitatory and inhibitory effects respectively on the neural circuit when activated. Moreover, there appears to be a functional dichotomy in their roles for integrating information (pyramidal cells) versus gating excitatory input and modulating pyramidal input (interneurons) (8,9). A distinguishing characteristic of the PFC when compared with a primary sensory region of cortex such as V1, is the multi-modal nature of the external stimuli to which it responds (10,11). PFC neurons show a large heterogeneity in the information they encode, from low-level information regarding stimuli characteristics, to high-level information regarding choice or relevance. In addition, there has been evidence for feed-forward and feedback connections between primary sensory areas and the PFC (12). This facilitates the role of the PFC as an integration centre that can perform more complex tasks involving both external information, and internally pre-processed information from other brain areas.

Functional role of the local field potential in the prefrontal cortex

A key player in the selection of information within the PFC and the coordination with distant brain areas is the local field potential (LFP). The LFP is the sum of the activity of a local population of neurons, measured on an extracellular level. For the purposes of the work presented here, the LFP was divided into the following neural frequency bands: delta (1-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (13-30Hz), and gamma (30-120Hz). It is worth noting that there is a slight discrepancy in the field regarding the exact bandwidths of each band, for instance, some papers refer to theta as 4-10Hz (13). However, these discrepancies are minor, and furthermore are relatively unimportant once the reference for each band definition is provided in each study. It is thought that the LFP coherence between distant neuronal populations, represents the transfer of information between regions (14,15). Of note, it is thought that much of this communication is performed in low frequency bands, given that they provide a wider temporal window for distant regions to synchronise their activity. An example of this type of communication is seen in the strong connection between the PFC and the hippocampus through LFP synchrony. Theta oscillations have been shown to provide a key role in the communication pathway between the PFC and the hippocampus (16). In particular, interneurons of the PFC have shown a strong entrainment with theta oscillations, which is thought to be due to the direct projections that interneurons of the PFC receive from the hippocampus (17). Furthermore, in spatial working memory

tasks, there has been clear evidence for coherence between theta oscillations in the PFC and the hippocampus. Moreover, neurons of the PFC were found to be phase locked to the theta frequency of the hippocampus during mnemonic and decision periods of the task (18). It also has a role in neural selection within the PFC. As will be explained in further detail later, neurons of the PFC are highly versatile in their ability to represent different stimuli in different contexts (19,20). It has been shown that when there is overlap within neuronal ensembles in the representation of information e.g. different task rules, the ensemble can be activated in a context dependent manner via increases in synchrony in specific neural frequency bands (21). Of note, the term synchrony in this paper refers to the coherence in LFP between different recording sites within the PFC. Namely that beta frequency synchronisation selects the relevant neuronal ensemble and that alpha deselects the newly irrelevant ensemble. The functional relevance of this synchrony was shown by the correlation between synchrony strength and task reaction times in a working memory task. Thus, in the PFC, and most likely across the cortex, LFP has a strong role in both information transmission globally, and information selection locally.

Serotonin within the prefrontal cortex

Whilst neuronal circuits are heavily influenced by the firing patterns of nodes within the circuit itself, they are also strongly impacted by neuromodulators emanating from the brainstem. Neuromodulators, including serotonin, dopamine, and acetylcholine, are prolific across the cortex. Their functions in each region vary vastly depending on their expression level, and the heterogeneity of the network upon which they act. The PFC receives a particularly dense network of inputs from the serotonergic locus in the raphe nuclei (7). *In vivo* studies have shown that the activation of serotonergic projections from the raphe nuclei to the PFC induces inhibition in the majority of pyramidal cells, excitation in a small portion, and a biphasic response of excitation following by inhibition in the remainder of cells (22,23). The range of responses is thought to arise from the selective expression of serotonergic receptor subtypes (namely the depolarising 5-HT_{2A} receptor and the hyperpolarising 5-HT_{1A} receptor), on subpopulations within the PFC, and co-expression in the case of the bi-phasic response (4). The work presented here will focus on the serotonergic agonist known as psilocybin. This hallucinogenic compound causes disturbances in sensory perception in humans, and alters mood and cognition. The mechanism behind these effects is thought to rely heavily on the activation of the subset of serotonergic receptors. Hence, with the strong implication of the PFC in multiple areas of cognition, combined with

a dense population of serotonergic projections, this is a key area of interest in determining the effects of psilocybin on cortical networks that may underlie the psychological effects induced by this compound.

Psilocybin; An overview

Psilocybin is a hallucinogenic compound that can cause profound alterations in sensory perception, mood, cognition (24). The psychological effects on cognition make the PFC a strong region of interest in this field of study. It is the primary hallucinogenic compound in “magic mushrooms”, and has recently been examined in the context of ego-dissolution (as defined in this paper as the disintegration of the boundary between the self and the external environment) (25). Whilst the psychological effects of psilocybin have been explored comparatively extensively, the physiological basis is unclear, with some reports providing somewhat conflicting results. This is likely due the small number of studies conducted due to the tight regulations regarding the accessibility of the compound for scientific research. It is known that hallucinogenic compounds act universally on the serotonergic (5-HT) system, with a particular affinity for 5-HT_{2A} receptors (26-28). Although the mechanistic properties are more complex than non-specific activation of 5-HT_{2A} receptors, as some 5-HT_{2A} receptor agonists have no associated psychoactive effects (29). These receptors are particularly prolific in the PFC (30). There has been evidence of increased cortical metabolism in the PFC following psilocybin administration (31), and moreover, a reduction in cerebral blood flow to the region when dynamics were examined on a short time-span (32). There has been evidence for a change in functional connectivity between cortical brain regions in response to psilocybin. In the context of fMRI, functional connectivity equates to the simultaneous change in BOLD signal in different cortical regions. It has been hypothesized that a change in this connectivity may linked to altered states of consciousness (25). However, the underlying mechanisms and the method by which psilocybin effects cortical circuits on a neural level are still unknown.

Psilocybin and serotonergic receptors of the prefrontal cortex

Psilocybin (4-phosphoryloxy-N, N-dimethyltryptamine) is the pro-drug of psilocin, an indolealkylamine (IAA) that belongs to a group of hallucinogenic tryptamines (33). IAA drugs are chemical derivatives of 5-hydroxytryptamine (5-HT/serotonin), that act on the serotonergic system

(34), with little effect on the dopaminergic system (35). Hence, structurally, psilocin is very similar to serotonin (Figure 1).

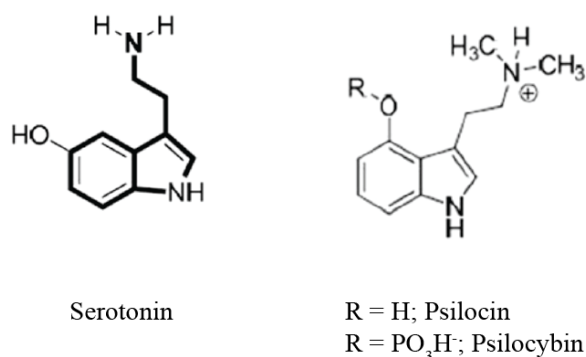


Figure 1: Chemical structure of serotonin, psilocin and psilocybin (adapted from (36)).

Psilocybin primarily acts on 5-HT_{2A}, and 5-HT_{1A} receptors (33). Although, as previously mentioned, this relationship is unidirectional as compounds such as lisuride, a 5-HT_{2A} agonist, do not have hallucinogenic effects on the brain (29). The source of serotonergic projections to the PFC cingulate, prelimbic and infralimbic regions, are the dorsal and medial raphe nuclei (37). Of the 14 subtypes of serotonergic receptors, the most prevalent in the PFC are 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{3A} (7), with a particularly strong presence of 5-HT_{1A} and 5-HT_{2A} receptors (38,39). All serotonergic receptors, with the exception of the 5-HT₃ subtype, are G-protein coupled (40). Activation of 5-HT_{1A} receptors has been reported to produce hyperpolarising effects, which is likely to be mediated by an inward rectifying potassium current (41,42), whilst 5-HT_{2A} receptors have a depolarising effect (43). Approximately 60% of pyramidal PFC neurons express 5-HT_{1A} and/or 5-HT_{2A} receptors (44), with 80% exhibiting co-expression (45). The two receptor subtypes are typically morphologically distinct, with 5-HT_{1A} receptors present on the axon initial segment of pyramidal cells (46), and 5-HT_{2A} receptors located on apical dendrites (47) (Figure 2). On the contrary, PV-interneurons have distinct populations that express 5-HT_{1A} and 5-HT_{2A} receptors (7). In layer V, ~ 30% of interneurons express one of the two types of receptors (48).

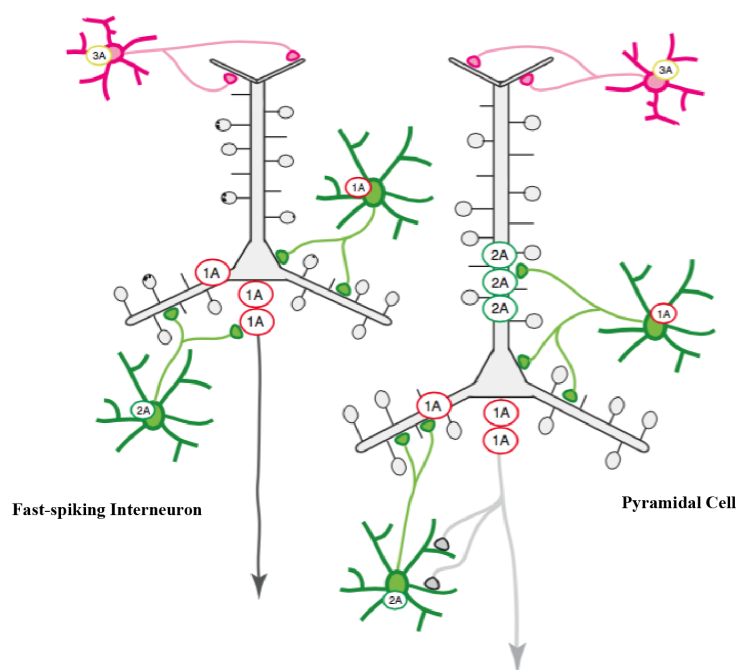


Figure 2: The distribution of 5-HT_{1A} and 5-HT_{2A} receptors on PFC interneurons and pyramidal cells (adapted from (7)).

Pharmacokinetics of psilocybin

The pharmacokinetics of psilocybin have yet to be fully characterised, however, we have some knowledge regarding the initial physiological interactions. Psilocybin is converted to psilocin within the body via dephosphorylation by hepatic metabolism using the enzyme alkaline phosphatase (49). Psilocin is considered to be the key psychoactive derivative of psilocybin, due to the largely eliminated symptoms once the dephosphorylation process was hindered using a competitive compound (beta-glycerophosphate) to block alkaline phosphatase (50). It has been shown that following oral administration of psilocybin, the distribution of psilocin around the body is non-specific (51,52). In studies of primates, the distribution of psilocin in the brain following intravenous (i. v.) administration was initially heterogeneous across brain areas, and transitioned to a uniform distribution across the brain after 30-60 minutes (52). Significant amounts have been found in the plasma within 20-40 minutes, corresponding to the electrophysiological effects seen in rhesus monkeys within a similar time period (2-4mg/kg, intraperitoneal (i. p.) injection) (53). The psychological effects were found to occur once the plasma levels had reached a concentration of 4-6µg/ml (54,55). In humans, whilst the strongest psychological effects were found to occur 70-90 minutes after administration, high levels of

psilocin were already present within 30 minutes (49). Studies of the plasma levels in humans showed that following peak concentrations, there was a plateau for ~ 50 minutes, and then a slow decline that terminated after 6 hours (56). However, the time course was found to shorten considerably when the drug was administered intravenously as opposed to via intraperitoneal injection. The half-life shortened to 74 +/-19mins in humans compared to 163+/- 64 for oral administration, with the psychedelic effects lasting only 15-30 minutes (49).

Electrophysiology

As a serotonergic agonist, psilocybin has an affinity for 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A}, and 5-HT_{2C} receptor subtypes (28,33). The strongest affinity is for 5-HT_{2A} receptors (inhibition constant (K_i) = 6nM in comparison with K_i = 190nM for 5-HT_{1A} receptors. Note: low K_i indicates high affinity) (57). These receptors are mainly postsynaptic in nature (28), with a strong concentration in the apical dendrites of some cortical neurons (58,59). Whilst non-hallucinogenic compounds can also activate these receptors, their difference lies within the activation pathway they trigger. Non-hallucinogenic compounds activate the G_{q/11} subtype protein, whereas hallucinogenic compounds additionally activate the G_{i/o} subtype protein (60). This differentiation is thought to arise from a mechanism known as agonist trafficking of receptor signalling (61), whereby agonists of the same receptor can vary in the degree to which they activate the multiple downstream signalling pathways (62). 5-HT_{2A} receptors are the only receptors with causal evidence for psychotomimetic effects (63). This study removed the psychotomimetic effects using a pre-treatment of ketanserin, a 5-HT_{2A} receptor antagonist. In contrast with other psychedelic drugs such as LSD, psilocybin does not have an affinity for D₂ dopamine receptors (64). Of note, a study that examined the effects of a pre-treatment of haloperidol, a D₂ receptor antagonist, revealed a decrease in the psychotomimetic effects induced by psilocybin (65). Although, causality may be due to the numerous functional interactions between the dopaminergic and serotonergic systems (66,67).

Psilocybin has been shown to have a medley of effects on neural tissue. An early PET study found an increase in cortical metabolism in the ACC, amongst other areas including the frontolateral cortex, temporal-medial cortex, and basal ganglia (31). However, more recent fMRI studies in both humans and rats have found somewhat conflicting results. In humans, psilocybin has been found to decrease

blood flow to the thalamus, anterior and posterior cingulate cortices (32), with the ACC demonstrating some of the largest reductions. A similar decrease in the BOLD signal was seen in the cingulate cortex of rats (68). Robin Carhart-Harris provides a potential explanation for this apparent contradiction. The half-life of the radiotracer used in PET studies, F-fluorodeoxyglucose, is 110 minutes. This timescale is markedly longer than the timespan of a typical fMRI scan. Hence, the fMRI results may reflect short term effects, whereas Vollenweider et al., (31) may have been viewing the long term effects (32).

Psilocybin appears to alter global functional connectivity patterns by increasing connectivity between regions of the PFC during resting state after injection (69), whilst decreasing connectivity between the PFC and the posterior cingulate cortex and retrosplenial cortex (32). A more recent study has found that it decreases the connectivity level in what is termed as the default mode network (defined here as a system of “spontaneous cognition” (70)) (25). With respect to the LFP, multiple lines of evidence have found a reduction in power of low frequency bands in response to psilocybin (53,71,72). EEG data in humans have shown a reduction in alpha and theta bands (0.12-0.15mg/kg oral administration) (71), which is further corroborated with data from rhesus monkeys that show a similar reduction in alpha band activity (2-4mg/kg i. p.) (53). This study also found an increase in beta band activity, all of which was found 20-40 minutes post injection. On a neural population level, the only evidence currently known is from a study whereby psilocin was applied locally to the raphe nuclei via micro-iontophoresis. This resulted in an inhibition of serotonergic neurons (73). However, as previously mentioned 5-HT_{2A} receptors are present on both interneurons and pyramidal cells, hence this modulation of the balance of excitation/inhibition is highly likely to vary largely depending on cortical area.

Behavioural effects of psilocybin in rodents

Rodents pose an interesting model for examining the effects of psilocybin. Whilst one cannot measure the hallucinogenic effects as in humans, there are behavioural phenotypes that rodents display under the influence of psilocybin that indicate that the compound is altering the physiological state of the animal. They typically manifest as muted motor function, normally within 15 minutes of injection (74-76). Rodents have been found to spend significantly longer in an immobile state, and with a flat body posture (76). Whilst low doses have been found to increase the startle reflex in rats, high doses

decreased this response (77). The canonical reaction of rodents to hallucinogenic compounds are head twitches (78,79). This reaction is normally seen after administering a dose of 2mg/kg (personal communication with Dr. Ivi Antoniadou, Sharpe Lab, Dept. of Pharmacology, Oxford University).

Neuronal effects of similar hallucinogenic substances

Due to the relatively superficial breadth of knowledge of the neuronal effects of psilocybin, it is useful to observe the effects of similar substances such as DOI (2,5-Dimethoxy-4-iodoamphetamine). As with psilocybin, the hallucinogen DOI is a structural analog of serotonin. It has an affinity for 5-HT_{2A} and 5-HT_{2C} receptors, and is known to produce cognitive deficits in rodents (63,79-81). DOI also has the behavioural by-product of head-twitches (82). A recent study of the i. p. injection of DOI in rats, has shown a decrease in population activity in the ACC (83). However, the effects appear to be very nuanced, with most neurons remaining unaltered by the administration of DOI, some becoming activated and a sub-population being inhibited. This partially echoes the findings of an earlier study in rats which found that DOI activated approximately 1/3 of the population (in particular, increased burst firing in pyramidal cells), inhibited 1/3, and had no effect on the remainder of the population (84). Contrary to Wood et al. (83), this study found an overall excitatory effect on the population. Similar, to the previously mentioned reports on the effects of psilocybin on LFP power, DOI was also found to reduce the power of low frequency oscillations (83). It also reduced the power of gamma oscillations and desynchronised the network with this frequency band (83). These findings reinforce earlier investigations in anaesthetised rats, which showed a disruption in pyramidal cell synchrony with the active phase of the LFP (84).

Overall, our knowledge of the neuronal effects of psilocybin is currently fragmented with pockets of incongruous results. Furthermore, our knowledge of the cellular effects in the PFC is a black box, with an inference window provided by the findings of the effects of DOI. This opens an intriguing field for scientific investigation, particularly on the level of neuroelectrophysiology.

Psilocybin; Research aims

The results presented here were investigated with the aim of providing some fundamental information on how psilocybin affects neuronal circuits of the ACC, in particular:

1. The effect on the LFP within the ACC.
2. The effect on single unit activity in the ACC.
3. In an effort to link and further understand (1) and (2), the influence of psilocybin on the level of synchronisation of the ACC network was examined. In particular, the change in phase correlation of single unit activity with neural frequency bands.

Working memory; A brief history

Working memory is the ability to retain information temporarily following the cessation of sensory input (85). It is a specific type of memory engaged during goal-directed tasks. It functions as a mnemonic encoder of short-term information that is resistant to distractors, and yet sufficiently adaptable to update to new relevant information when necessary. Historically, the working memory axiom resulted from a disambiguation from the more generic term, short-term memory. Research in this area began in human cognition studies. The term “working memory” (WM) was proposed by Miller, Galanter, and Pribram in 1960 as a conceptual mechanism behind the execution of goal-directed behaviour. This was further developed by Donald Norman in 1970 (86), and Kisou Kubota and Hiroaki Niki in 1971 (87), however, it remained entangled in the web of short-term memory until the first definitive disambiguation between WM and its short term memory counterpart was set forth by Alan Baddeley and Graham Hitch in 1974 (88). Early experiments examined the electrophysiology behind working memory as it is currently defined, whilst at the time it was under the umbrella term of short-term memory. Indeed the work of Jaouquin Fuster in 1971 (89) laid the foundations for many of the phenomena that would later be linked to the mechanisms by which WM is encoded. This study examined the activity of the PFC and the medial dorsal nucleus of the thalamus in a monkey during a delayed response task. They found early evidence of an elevated firing rate that was maintained throughout the delay period, which they termed short-term memory, and concluded that it was encoded, exclusively or otherwise, in a fronto-thalamic circuit. They also found neurons that exhibited inhibition in response to the delay period, and moreover a high degree of variability in the spontaneous firing rate of all PFC neurons. Interestingly, they reported that ~17% of recorded neurons displayed transient activation during the delay period, which returned to baseline levels before delay period cessation. This is some of the earliest reported evidence of nuanced response patterns of PFC neurons during the mnemonic period of a delayed response task. The concept gained further momentum throughout the 1980's (90,91), culminating in the seminal work by Goldman-Rakic in 1989 (92), which led to the first proposal of persistent activity as an underlying mechanism behind delay period memory retention.

Synopsis of working memory theories

Multicomponent model

The first prevailing theory of WM, and one which has remained the principle protagonist in the search for the mechanism behind WM in the field of human psychology for two decades, is the multicomponent model of Alan Baddeley and Graham Hitch (88). The model consists of two storage sub-systems; the phonological loop and the visuo-spatial sketch pad, which can store visual/spatial and phonological/articulatory information respectively. At the core of the model is the Central Executive, which coordinates the allocation and retrieval of memory from the two systems. Thus, it can be considered as the allocator of attention in the system. Given the electrophysiological nature of this work, the remainder of this literature review will focus on the evolution of theoretical mechanisms of WM from a physiological perspective. However, it is worth noting that one of the current schisms in the field governing whether the PFC encodes memory retention or high level processes such as attention, mirrors this model. The former advocates the PFC as a storage sub-system, whereas the latter proposes it to be the Central Executive. A full review of the multicomponent model can be found in (93).

Persistent activity

The first theories on the mechanism of WM on an electrophysiological level focused on the activity of neurons during the delay period of a task, notably, the presence of persistent activity during the mnemonic period in the absence of sensory stimuli. In brief, a WM paradigm can be divided into three phases:

- 1) Cue period – Stimulus presentation
- 2) Delay period – Time within which the memory of the stimulus is maintained
- 3) Response period – Time in which the response to the stimulus is executed

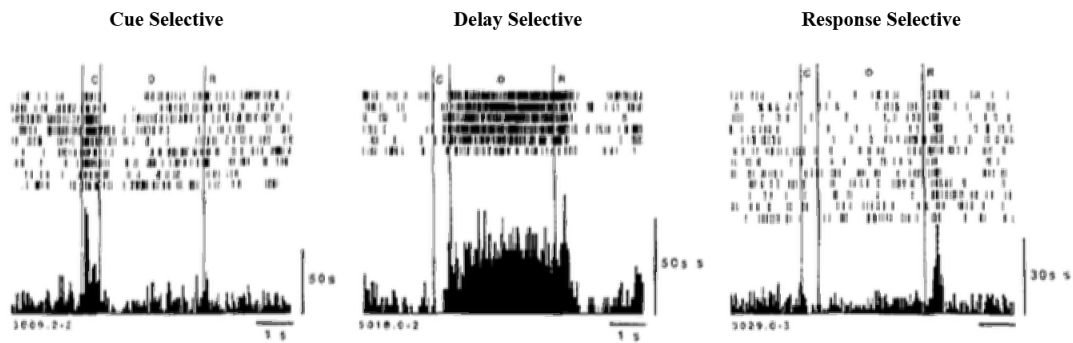


Figure 3: Early evidence of the response properties of cue-, delay-, and response-selective neurons in the PFC (adapted from Goldman-Rakic (94)).

The seminal studies in the theory were that of Fuster and Alexander (89), and Kubota and Niki in 1971 (87), both of whom identified persistent activity during the delay period in neurons of the PFC in extracellular recordings in primates. Later studies found similar results (92), and indeed a large body of work focusing on this mechanism was developed by the laboratory of Patricia Goldman-Rakic during the 1990's (Figure 3) (94).

Transient activity

However, more studies emerged showing that while neurons with persistent activity were present, there were far more that displayed transient activation/inhibition during the delay period (95,96). In fact, there has been an increasing amount of evidence to show that the representation of stimuli in delay period activity is relatively coarse, and that it is in fact high level processes such as attention and decisions that are being encoded in the PFC (97-102). The complexity of these processes are also thought to rely on population encoding rather than on single cell activity (103,104). Hence, this theory has led to the broader hypothesis that the PFC is not simply functioning as a temporary memory storage centre, but rather is the controller of where attention is allocated, and which memories/rules of the task are relevant and when they should be recollected during the task (105).

Electrophysiological mechanism

On a cellular perspective, there are two adjacent theories to the above models, which try to determine the mechanism by which a network can temporarily hold the memory of a stimulus and recall this

memory on demand. The two competing theories are that of supra-threshold memory maintenance via persistent activity, and sub-threshold memory maintenance via temporary synaptic plasticity. The supra-threshold model requires bi- or multi-stable attractor states that are representative of the stimulus (106). The synaptic plasticity model proposes that the stimulus is encoded via the temporary adjustment of synaptic weights, and so until recall, it is undetectable on an extracellular level. When the information is required, the subpopulation is stimulated and activated according to how the short-term synaptic plasticity has governed the pattern of activation (107). Both hypotheses have been centred upon electrophysiological studies in the PFC, the proposed site of WM encoding in the brain.

Working memory in the prefrontal cortex

Evidence for working memory encoding in the prefrontal cortex

The first indication that the PFC was required in a delayed response task was in a study by Jacobson in 1937 (108). They performed bilateral lesions of the frontal cortex of monkeys and showed that there was a significant decrease in their ability to locate a reward (food) that had been placed in their view a short time previously. The work regained momentum in the 1970's, and over four decades of work since has found evidence for WM encoding in the PFC (2,87,89,94,95,109-113). Whilst persistent activity in WM tasks has been found in other brain areas, the PFC has shown the strongest correlation with memory recall (114). Indeed, some of the most indicative evidence come from the performance impairment as a result of PFC lesions in monkeys (89,109,110,115,116), and humans (117-119). Though, WM is likely to be functionally localised within the PFC as both a lesion study in the ventral PFC of a monkey (120), and in the lateral PFC of humans (121), did not impair WM capacity.

Neuronal responses in the prefrontal cortex during working memory tasks

Proportion of neurons of the prefrontal cortex neurons that are working memory responsive

The reported percentage of PFC neurons that are responsive during a WM task varies wildly. Some studies report figures as low as <10% (122), whilst others report figures as high as ~76% (95), and ~84% (123). It is likely that the variability is not dependent on different WM modalities, but rather on the criteria upon which one classifies a cell as “WM responsive”. Hence, the average number of cells in

a PFC subpopulation that are WM responsive does not have a definitive answer. We do, however, have information regarding the nuances of activity of those neurons that are classed as WM responsive. Not least of which being the broad range of features represented by this population.

Time course of neuronal population responses in the prefrontal cortex during working memory

A study in the PFC of monkeys performing a delayed match-to-sample task found a strong temporal dependence in the response pattern of PFC ensembles. Cue onset initiated a large response (duration: 80-450ms, note: cue offset: 650ms), which transitioned into an intermediate elevated firing rate throughout the cue presentation period and into the early delay period (duration: 450-1150ms). The network then returned to resting state, and the ensemble of activation was re-activated late in the delay period (last 500ms of the delay period) (124). They found that this response pattern was also present on a single neuron level.

Tuning properties of neurons of the prefrontal cortex during a working memory task

The rich temporal profile of PFC population activation seen during the mnemonic period of WM tasks is thought to be shaped by inhibition, due to the fluctuating levels of inhibition found in different periods of the WM task (125). However, fast spiking interneurons of the PFC show an equal degree of tuning to task parameters as pyramidal cells during a WM task (126,127). This would indicate that these interneurons have more than a modulatory role on the pyramidal cell network. Moreover, the response properties of interneurons and pyramidal cells, are often inverted (126). A later study from the Goldman-Rakic lab also found that whilst pairs of fast-spiking interneurons and pyramidal cells with cross-directional tuning properties existed, neighbouring pairs (neighbouring defined as cells recorded on the same channel) often had isodirectional tuning properties (isodirectionality was defined as having a tuning curve within 0-60 degrees of the tuning curve of the paired neuron) (127). For many neurons, the isodirectionality was maintained during the cue and delay periods of the task, and then switched to cross-directionality during the response phase. Furthermore, the tuning properties of PFC neurons are known to rapidly adapt to changes in behavioural context (128).

Context information

PFC neurons are also thought to encode contextual information. It is known that a combination of activity between the PFC and hippocampus (HC) is important during spatial working memory (SWM) (18,129). Indeed, extracellular recordings in the PFC of rats showed that specific context could be decoded using ~50% of the recorded population (although 11% were identified as having a strong preference for one context over another) (130). Interestingly, the context response was found to have shifted when the rats re-entered a similar arena. While on initial inspection the response appeared dissimilar, the neural activity in response to re-entering a similar arena was decoded following a linear extrapolation from the neural activity from the original arena. Hence, the relative and not absolute activity of the cells encoded the context.

SNR of neuronal responses

A combination of low baseline firing rates, and high inter- and intra-trial variability in neuronal responses, predisposes PFC neurons to have a low SNR in relation to WM responses, albeit when viewed from an extracellular perspective. Though, this may not be a WM characteristic, but a consequence of PFC encoding, as spontaneous PFC activity shows a high degree of variability (89). A large number of PFC neurons that are classed as WM responsive, display a baseline firing rate of 0-5Hz (>40% in cross modal WM tasks (582 cells), and > 70% spatial delayed response tasks (149 cells) (122)). Furthermore, the relative activation/inhibition of delay period responsive cells is moderate (131). A comprehensive study of single trial statistics in cross modal WM and spatial delayed response tasks found that in both instances the relative change in firing rate between baseline and delay period activity was <4Hz for the majority of cells (~60-93% of cells) (122).

Moreover, the activation profile of cells was inconsistent across trials; delay-activated cells displayed delay period inhibition during 16-19% of trials, and delay-inhibited cells showed delay period activation during ~20% of trials. There was also significant consecutive-trial correlation in neuron responses for ~20% of the population. An examination of the coefficient of variation (CV) showed that over 37% of the cells had a large CV ($SD \gg \text{mean}$), with only 11% of cells having a reliable CV ($CV < 0.5$) across trials (122).

There is also evidence of substantial intra-trial fluctuations. An illustrative cell in Shafi et al. (122), shows the single trial responses of a delay-activated neuron. In addition to the few trials shown in Figure 4, the authors note that every trial had at least one bin whereby the firing rate was below that of the baseline firing rate. Another study found that the firing rate of 90% of neurons classed as persistent firing, varied significantly over the duration of the delay period. In fact, only 3% of cells displayed stable firing rates (96). Together, these findings illustrate the extent to which a trial averaged response masks a large amount of underlying variability

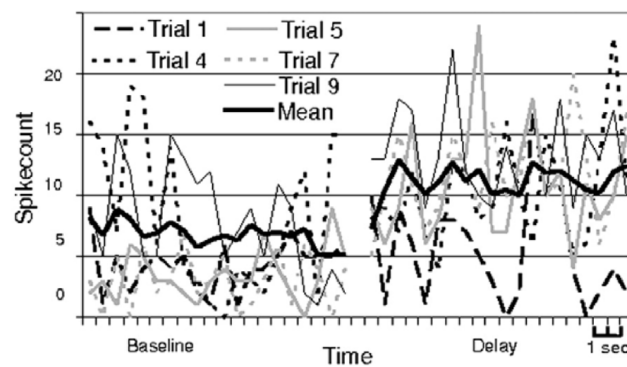


Figure 4: The response profile of five sample trials of a parietal cell that displayed a stable delay period response. The trial averaged response is given in bold. (Adapted from (122)).

A conclusion of this work was that the perceived “stable” persistent activity seen in traditional memory cells is in fact a result of averaging over substantial inter-trial variability, with intra-trial fluctuations, and that stable persistent elevation or decrease in firing was very much the exception (122).

The role of the prefrontal cortex in working memory

There are two competing (although not necessarily mutually exclusive) theories regarding the role of the PFC in WM.

1. The PFC is a temporary memory storage system for information important for the WM task (low-order information).
2. The PFC encodes strategy/decision/judgment and selectively recruits the details of a particular memory from other cortical regions (high-order information).

Both ideas were alluded to in one of the earliest electrophysiology studies by Joaquin Fuster and Garrett Alexander (89). They postulated that memory information was encoded in the PFC, but due to the inability to decode differences in stimulus information, and the disproportionately high level of activity in the PFC immediately proceeding cue cessation, that the function of PFC neurons was more likely to be related to attention allocation.

There have been a number of studies that propose that the memory required to complete a WM task is encoded in the PFC during the delay period (94,109,132). More recent studies have challenged this hypothesis of the PFC being a memory substrate for WM (133). Human neuroimaging studies have shown evidence that the delay period activity does not encode a memory of the stimulus, but rather, it encodes the target of attention allocation (99,101). A number of studies have found evidence for the preferential encoding of abstract information as opposed to stimulus specific information (97,98,100,102,134,135). The investigation by Riggall and Postle attempted to disentangle low-order (perceptual information) and high-order (decision/judgement/strategy) information in a WM task. Subjects were presented with a moving dot stimulus. Halfway through the presentation, the subjects were informed as to whether they had to indicate the speed of the dots or the direction of the motion. Analysis of BOLD signals revealed that the perceptual information such as the speed or the direction was readily decoded from the occipital cortex, whereas the categorisation of whether the trial was a speed or direction trial was encoded in the PFC (101). Another study probed this categorisation functionality further by making the decision between a perception problem and a categorisation problem as opposed to between two perception problems. The subjects were told during the task whether they had to make a decision based on the perceptual or the categorical information in the stimulus. Consistent with the previous study, the trials in which categorical information was required was encoded in the PFC, whereas perception trials were encoded in the ventral occipitotemporal cortex (136).

This theory is further supported by extracellular electrophysiology studies. A comparison of activity in the primate inferior temporal cortex and the PFC found that whilst behaviourally relevant stimuli were encoded in both regions, the PFC encoded high level information regarding whether the test stimulus matched the cue stimulus, rather than a representation of the stimulus itself (95). A recent investigation

trained monkeys on a WM task that involved remembering the colour of a block (137). Most PFC neurons did not encode the colour of the block but rather the amount of time that passed and the location of the block. This information is necessary for the task but not a memory of the stimulus itself. Furthermore, they increased task difficulty by requiring the monkeys to remember two colours. The monkeys' behaviour showed microsaccades to one or the other stimulus (potentially a mnemonic strategy with the increased WM load), and whichever colour they attended to was represented in the PFC with more fidelity. They found that PFC neurons were encoding the locus of attention. Another study which examined the effects of lateral PFC lesions in monkeys found that the WM deficit was independent of specific stimulus features e.g. motion coherence (138). The deficit was more pronounced when the cue and test stimuli were presented in different locations as opposed to the same location, leading to the postulation that the lesion disrupted the ability of the PFC to shift attention. Another study found that while stimulus information was encoded in the PFC in the initial part of the delay period, this encoding diminished over time and the information regarding whether a stimulus was a target or a distractor was more dominant (128).

Whilst WM studies have found evidence of target memory encoding in the PFC (104), PFC neurons have also been found to be tuned to many stimulus-content-independent characteristics such as task rules (105), motor responses (139), and stimulus-response mappings (140). Moreover, some neurons can represent many task-relevant signals, both high and low level information simultaneously (103). A recent study has shown that the high-dimensionality of the information encoded in the PFC is linked to behaviour, as a low-dimensionality was found in error trials (141). In conjunction with the debate over the mnemonic versus executive role of the PFC, there are further debates over how either information is encoded on a cellular basis.

Neural mechanisms of working memory

The theories governing the cellular mechanisms behind working memory focus primarily on the storage systems of Baddeley's multicompartment model, i.e. they focus on how a neural network maintains or recollects a particular memory during a WM task. Whilst one could argue that the trigger of this mechanism could be seen as part of the Central Executive, this would be removing significant functionality from the storage systems. In my opinion, the trigger of the neurophysiological mechanism

that stores a memory during a WM task is a function of the slave systems. The task of choosing which memory to trigger, and other functions such as which memories are important, are the tasks of the Central Executive. Hence, in my opinion, the current investigations on persistent activity, and the information that a particular attractor state is encoding, represent research on the slave systems. When we start to focus on how a particular attractor state is activated above another attractor state (as opposed to what the attractor state is representing), we will be getting closer to determining the mechanism underlying the central executive.

Persistent activity

As previously mentioned, the earliest proposed cellular mechanism behind working memory was persistent activity, an example of which can be seen in the delay-selective neuron of Figure 3. Whilst PFC neurons were thought to encode WM in ensembles representing different task epochs, the DC shift in firing rate that persisted for the duration of the delay period in some neurons was thought to be encoding the memory of the target stimulus (94). This has been supported by fMRI studies, which have shown a link between persistent activity and behaviour, as the strength of the BOLD signal correlated with the accuracy of the memory-guided saccade in delayed match and non-match to sample tasks (142). Furthermore, diminished persistent activity has been found to increase the error rate in WM tasks (92,143).

Transient activity

However, as early as 1980, Batuev found evidence of what was termed as a “relay-race” between neuronal populations that were sequentially, transiently active during the delay period (123). Only 5% of neurons showed sustained activation during the delay period, for which further evidence was shown in their later paper (144). However, this idea did not gain similar traction to the persistent activity hypothesis, as it wasn’t until the early 2000’s that the concept of nuanced activity in the delay period began gaining momentum (122,124,145). There is now a considerable amount of work to show that the delay period is represented by bouts of elevated activity of different latencies and durations (20,122). There is evidence for a dynamic sequential activation of neuronal populations that together encode the information required to execute the task (146). However, it is important to remember that the majority

of studies find a mixture of the two types of activities, with some neurons showing a phasic response, and others a sustained increase in firing rate (95,96,139). Indeed, Baeg et al. (147), found that ~20% of their recorded population in rats performing a delayed alternation task showed what they termed as “noncontinuous differential delay period activity”, whilst ~10% showed continuous differential delay period activity. Moreover, both neuronal responses are found to be predictive of trial outcome in WM tasks (96).

Population encoding

When studying the response of the PFC to cognitive tasks, there is a large temptation to examine the circuit from the response profiles of single neurons, due to the well characterised, repeatable findings of stimulus-response profiles in neurons in primary sensory areas (148,149). However, multiple studies (2,128,141,150) have shown that the stimulus-response profiles of PFC neurons appear to be significantly more complex. They can be multi-modal, transient, and context dependent (95,146). Studies of the PFC have found that a seemingly complex, intricate, incoherent system becomes infinitely clearer when examined as a population response (103,104). An example of this was found in a study examining the gating mechanism of relevant stimuli in a working memory task (103). Evidence of representations of all stimuli was found in the PFC before the integration stage, i.e. there was no gating of irrelevant stimuli up until this point. The relevant stimulus was more strongly represented late in the task by the same neural ensemble that encoded choice. This separation of input representations and choice were heavily entangled on a single neuron level, but readily separable on a population level. This has led to the idea that WM may be encoded by the transient activation of subpopulations that are re-activated during different stages in the delay period.

Computational models

The encoding mechanism behind WM has been investigated further in computational models, giving rise to two competing theories regarding delay period activity:

1. Supra-threshold activity; in the form of persistent or transient activation, disruption to spiking activity will cause information loss.

2. Sub-threshold activity; the information is expressed during brief attractor states, between which the network is silent. The information is stored in synaptic changes in recurrent connections, and hence it is not lost if the network spiking activity is interrupted.

Persistent activity

Persistent activity has been proposed to be generated via some of the following mechanisms; strong recurrent connections in a cell assembly (151), syn-fire chains consisting of feed-forward loops with no feedback connections between successive groups (152), or bi-stability within single neurons (153,154). The Wang laboratory have also argued that NMDA receptors are likely to have a crucial role in the generation of persistent activity as they provide the necessary slow dynamic in a recurrent network (155). This is echoed by Compte et al. (156), who found that it was crucial for recurrent excitatory synaptic connections to be mediated by NMDA receptors.

Synaptic theory

An alternative method that has been proposed is the transient reconfiguration of synaptic weights (107,157). In this system, the memory is encoded by temporarily altering the synaptic weights of a neuronal sub-population. The memory information contained in this network can thus be read by activating the network. The mechanism behind the shift in synaptic weights has been proposed to be residual calcium (107), or gluR1-dependent (159). Though neither of these postulations have been proven *in vivo* as yet. The proponents of this hypothesis argue that the persistent activity model fails to explain the phenomenon seen experimentally whereby delay period activity can cease before the end of the delay period (124). Moreover, persistent activity is metabolically expensive (158), and is more vulnerable to perturbation to the network from other irrelevant cortical activity. For example, a global increase in the excitation of the network, which could uniformly alter all synapses of the network without decreasing the SNR, but would radically decrease the SNR of the supra-threshold activity. This is an important consideration given the low SNR of the resting state PFC network.

The synaptic theory hypothesis was examined by Mongillo et al. (107), in an integrate-and-fire network. The recurrent network principally relied on calcium-mediated synaptic facilitation to facilitate memory retention. Due to the long time constants of calcium kinetics, information can be maintained in memory for 1 second without disrupting spontaneous spiking activity. Thus, this system is robust, and

metabolically efficient. Only a single stable state (spontaneous activity state) was required. A memory can be recalled via an external stimulus, a global excitatory input that re-activates the sub-network. This excitatory input can come in the form of a tonic input or an oscillatory input. This removes the need for bi- or multi-stable attractor states to be maintained simultaneously, as in the supra-threshold model.

Attractor system

The attractor system, be it bistable, multistable, or a continuous attractor is a very compelling theoretical framework for working memory (106). The principle is that the system (neural population), has a number of attractor states modelled as Lyapunov functions, visually seen as basins (see Figure 5 a), the trough of which is the stable state of the function. A characteristic of the attractor system is that the network can return to the attractor state despite altered network activity patterns. On the contrary, a chaotic system diverges in this case. As an example, in keeping with the persistent activity model, let's consider the state model to be represented by a single parameter, firing rate. Any firing rate that falls within the state space will be move into one of the basins depending on the vector forces at that point. Small perturbations will result in a minor shift in state space that will be restored into a stable state. A large perturbation will be strong enough to push the firing rate out of this basin and potentially into another basin. Hence, this model represents the balance in working memory of being robust against distractors but flexible enough to update when needed. There are two types of attractor models; discrete (Figure 5 (a)) and continuous attractor models (Figure 5 (b)). The discrete model is very robust to perturbation; however, it does not have the information capacity of a continuous attractor that is often required in cognitively demanding tasks. It can only represent a finite number of discrete items. However, a continuous attractor requires perfect symmetry. In the case of the circular attractor shown in Figure 5 (b), this system would require translational symmetry. Minor imperfections in the basin will result in local minima, which will disrupt the continuous trajectory Figure 5 (c). Even a small tilt in the basin would radically reduce the information capacity of the system (Figure 5 (d)). A proposed solution is to form a discrete approximation of the continuous attractor with a series of discrete attractors that sample the space sufficiently to provide adequate information capacity, whilst retaining the robustness level provided by discrete attractor states (106).

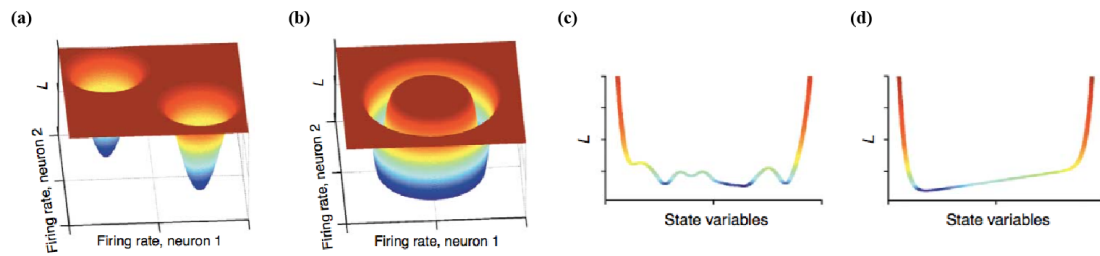


Figure 5: A model of an attractor system for working memory. (a) A discrete attractor system that may be used for binary outcomes. (b) A continuous attractor system that may be used to remember a continuous variable such as location on a track. (c) A continuous attractor is vulnerable to imperfections in the attractor basin introducing local minima. (d) A minor tilt in the attractor basin can also be detrimental to a continuous attractor system (adapted from (106)).

Searching for mechanism through perturbation

A useful approach to discerning the mechanism of a system is to determine the sensitivity of the network to different types of perturbations. Cortical phenomena have been examined using this method via micro-stimulation (159), cooling (1), current injection (160), and optogenetics (161). The earliest perturbation of a PFC network in a WM task was in 1985 by Joaquin Fuster and colleagues (1). They cooled areas of the PFC during a WM task and found that PFC neural activity diminished and the selectivity of inferotemporal cortex neurons was impaired, indicating a top-down mechanism originating in the PFC. A more recent study has found that perturbation via micro-stimulation in the PFC increased the error rate of monkeys in an attention task (159).

Unilateral versus bilateral perturbation

In the context of working memory, it is important to note the potential for redundancy across hemispheres when examining how to perturb an area of the cortex. A key finding of recent work has shown that there is likely to be a high level of compensation across hemispheres. A recent study showed that unilateral photoinhibition in the anterior lateral motor cortex during an object location discrimination task produced a minor decrease in performance, despite an increase in the volume of tissue perturbed using optogenetics. However, when a bilateral protocol was introduced, a large reduction was found, even when a relatively small area was perturbed (162). Hence, when a

manipulation is being paired with a behavioural output, it is important to consider the potential for redundancy across hemispheres.

Working memory behavioural paradigms

The first behavioural study of working memory in the rodent was in the 1970's (85). The setup was an eight arm radial maze, with one arm baited. The performance of the rat was assessed by determining the number of correct arms (i.e. previously non-visited arms) the rat entered following the baited arm. Oltman et al., showed a decrease in performance in the task with an increase in time period between the time of exit of the baited arm and the time in which the rat can freely explore the other arms of the maze. The authors argued that a distinct form of memory (WM) was being utilised as the stimulus-response associations were non-stationary, there was a temporal pattern to performance, and it was vulnerable to perturbation.

Delayed alternation task

Numerous different working memory tasks have been used in scientific endeavours to unveil the underlying neuronal dynamics. One of the earliest paradigms used in rodents was the delayed alternation task (163), which capitalised on the tendency of rodents to explore novel arms (e.g. the opposing arm to which they visited in the previous run of a T-maze (164)). However, in such a task, one cannot remove the possibility that the animal may use an odour cue as a means of discerning which arm it explored previously. Of note, an earlier implementation showed that a rat could perform the task with an interval of 5.5 hours (165), which many would consider to be beyond the temporal limits of the current definition of WM. Hence, later delayed alternation tasks assumed that the delay period was only within the central arm of the maze (146), thereby alleviating much of this potential caveat.

Delayed match and non-match to sample task

A delayed-match-to-sample (DMS) or delayed-non-match-to-sample (DNMS) paradigm attempts to overcome this odour caveat. In this case the animal must respond to the same stimulus as the cue stimulus or the opposing stimulus respectively. However, this approach is still vulnerable to postural mediation strategies. A DNMS study presented videos to independent observers, of the activity of the

rat during the delay period only, and the observers were successfully able to predict where the rat would nose-poke (166). Whilst it can be argued that this is a means of reducing the working memory load, it can also be argued that it is a means of implementing working memory. In my opinion, if working memory was not being employed, the performance of the rat should not be impaired for an increased delay period length. This however, is not the case in the work presented in this thesis.

Working memory; Research aims

The following research was conducted with the aim of:

1. Revealing further information regarding the engram of spatial working memory in the PFC.
 - a. Determine the response characteristics of the ACC population, in particular the role of persistent versus transiently activated cells during the delay period.
 - b. Determine the nature of the information encoded during the delay period; is it low-level (e.g. stimulus information), or high level (e.g. choice) information?
 - c. Examine the saliency of encoding on a single neuron and population level.
 - d. Examine whether the neural or behavioural information can be more predictive of task outcome.
2. Investigate the mechanisms underlying working memory networks by disrupting the network during delay period activity
 - a. Establish if response profiles of neurons are robust to perturbation.
 - b. Determine the degree of robustness of WM activity of the network to perturbation.
 - c. Determine the robustness of behavioural performance to unilateral optogenetic manipulation.

To achieve these aims I have performed extracellular population recordings on awake head-fixed mice performing a delayed response task. I have also used optogenetics in conjunction with a Pvalb-Cre genetic mouse line to perturb the circuit by stimulating a subpopulation of pv-interneurons during the delay period of the task.

Advantages of this experimental approach

A mouse model was used to facilitate the use of the PV-Cre mouse line for optogenetic manipulations. The delayed response task was executed on this particular setup as contrary to many implementations of spatial working memory, the spatial information between trial types is identical. Thus, one can disentangle context encoding from a simple spatial engram of a particular part of the environment. Furthermore, the head-fixed nature of the recordings radically increases the size of the recorded

population when compared to chronic implantation. This is in fact a crucial component, as with many chronic implants, the effect of glial formation around the implant can radically reduce the cell count that the probe can record (167). Given the broad spectrum of information encoded for a given WM task, a population this small is likely to provide too sparse a representation of the PFC network. Though, it must be noted that even with the population sizes quoted in this study, one can only presume to be seeing a highly compressed snapshot of the PFC network encoding WM, and one which would indeed benefit from a large degree of redundancy in the population. Optogenetics was used as a means of perturbation for the temporal speed, reversibility, cell-specific targeting of perturbation and versatility in the scope of activation. The latter refers to the ability to stimulate a small local population using an optrode or a (relatively) large population using an implanted optic fibre, uni- or bi-hemispherically.

Chapter 2: Methodology

Multi-Unit Extracellular Recordings *in vivo*

Animals

Experiments were conducted on male and female C57 BL6 mice (Charles River UK Ltd & Harlan UK Ltd), and homozygous PValb-IRES-Cre mice (JAX), aged 6-14 weeks, weighing 18-25g upon electrophysiology recording. They were housed under a 12/12 hr light-dark cycle with food and water available *ad libitum*, unless undergoing behavioural training (see section Behavioural training). Training and recordings were performed during the dark phase of the cycle. All experiments were conducted under the UK Animals (Scientific Procedures) Act 1986.

Anaesthesia, analgesia, antibiotics and surgical procedures

Mice were anaesthetised for all surgical procedures. The mouse was placed in an induction chamber, into which 5% v/v isoflurane (Harvard Apparatus Ltd) was administered via inhalation using oxygen (1.2-1.6 L/min). Once the breathing of the animal had slowed from a normal level, the depth of anaesthesia was ascertained by testing the pedal reflex. A firm pinch was administered between the toes of the mouse using the index finger and the thumb. The absence of a jerk reaction from the mouse was taken as an indication that an appropriate level of anaesthesia had been reached. This reflex was periodically tested throughout the surgery, together with the rate and depth of respiration, to ensure that the consciousness level of the animal was appropriate. The concentration of isoflurane was lowered to 1-2% v/v to maintain anaesthesia for the duration of the surgery.

Analgesia was provided via i. p. injection of carprofen (5mg/kg) into the shoulder of the mouse, approximately 10 minutes before the removal of the anaesthetic, followed by the administration of buprenorphine jelly (Vetergesic containing 0.3mg/ml buprenorphine in Hartley's Jelly, dose: 0.8mg/kg every 8-12 hours as required) orally for 2-5 days post-operation. Antibiotics (Baytril, dose: 250µL of 1mg/ml solution, 6mg enrofloxacin) were administered via i. p. injection into the alternate shoulder 10 minutes prior to surgery completion.

Headplate Implantation

The head implant surgery was performed as an aseptic surgery on a stereotaxic frame. Once the mouse was anaesthetised, and the surgical drapes were applied, the animal was prepared for surgery. The body temperature of the mouse was maintained at $37 \pm 0.5^{\circ}\text{C}$ using a homeothermic heat mat (DC Temperature Control System, FHC). A parting was created in the fur of the mouse down the centre of the skull. Iodine was applied across this section and the surrounding area to mitigate infection. Corneal drying was prevented using an ocular lubricant (Lacrilube, Allergen, UK). A sagittal incision was made to expose the skull. The periosteum vasculature was removed to facilitate a larger opening for the headplate implantation. The skull was scored to increase the surface area for adhesion. A layer of glue was applied to the skull surface, which was also scored whilst it dried. The principal sutures of the skull (incl. lambda and bregma) were marked using a permanent marker to act as a location aid in later surgeries. Two holes were drilled above the cerebellum for the insertion of ground screws. Once the ground screws were inserted, dental cement (Associated Dental Products Ltd) was applied to the perimeter of the exposed skull, upon which a custom built, in-house headplate (Figure 6) was fixed. The exposed skull in the interior of the headplate was covered with kwik-cast (World Precision Instruments).

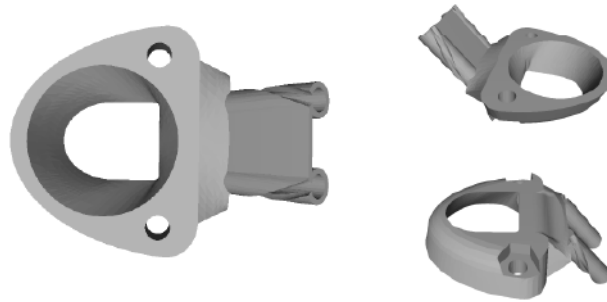


Figure 6: A 3D model of the headplate.

Experimental Setup

The experimental setup was composed of a treadmill with a headplate fixation mechanism. The treadmill belt was $80 \times 2 = 160\text{cm}$ long (trial length = 80cm), with a tactile feature signifying the reward zone. Two screws of the headplate shown in Figure 6 were used to fix the mouse into the slots shown in Figure 7 (b). Electrophysiology recordings were performed from above the mouse, via the

window exposed by the headplate (Figure 6). All hardware relating to the behaviour of the mouse was controlled using an Arduino Uno (Arduino). The code for the behaviour was written in-house using a sub-library of C/C++ functions. The electrophysiology recordings were performed using a multi-channel systems data acquisition system (ME64-FAI-MPA). For many hardware triggers, direct signal links were set up between the Arduino and the data acquisition system to ensure the highest level of synchronisation between the two systems. All neuronal signals were high-pass filtered at 0.5Hz – this parameter is inherent in the recording system.

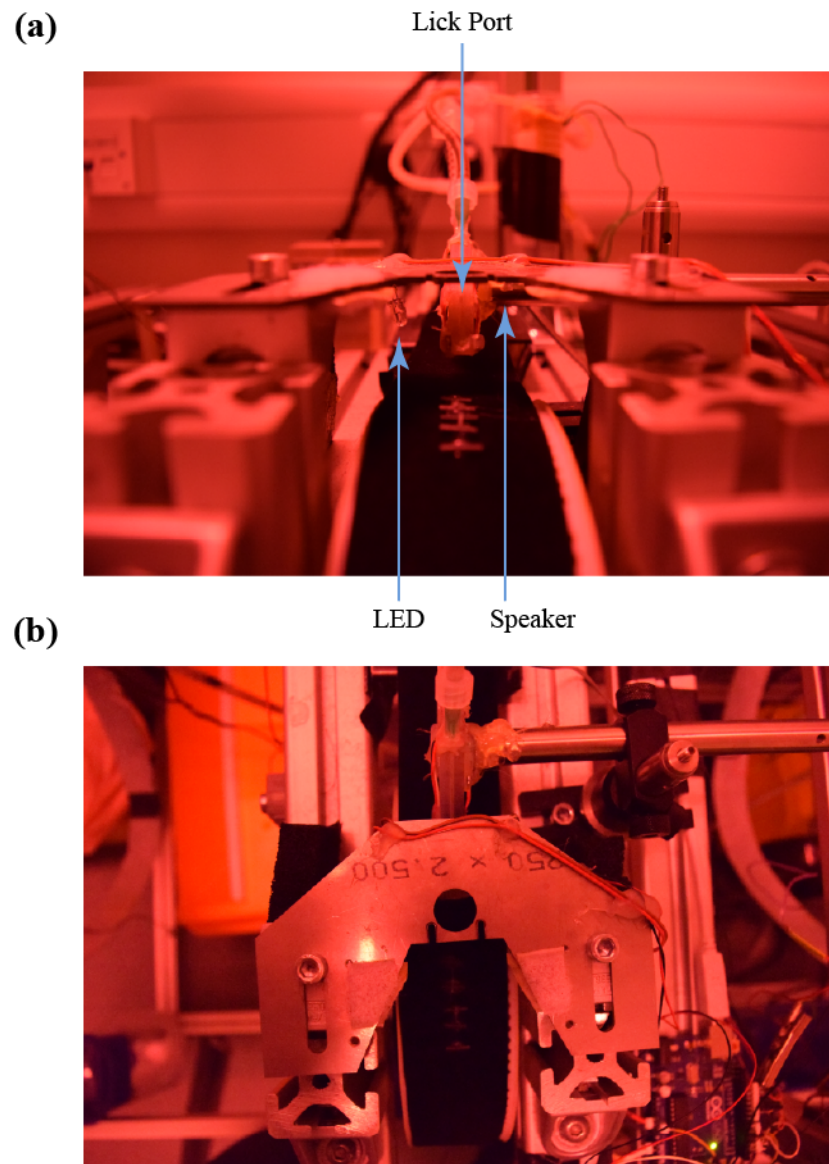


Figure 7: Electrophysiology/Behaviour Setup. (a) The feature enriched belt can be seen with the head fixation platform and the hardware utilised in the DR Task. **(b)** A plan view of the head fixation platform, highlighting the circular entry window for probe insertion. Note: The red lighting is due to the reverse light-dark cycle of mice so as to mimic their natural light cycle during training and recording sessions.

Extracellular Recordings

Electrophysiology recordings were performed on awake head-fixed mice that were engaged in locomotion and/or the delayed response task (described in section Delayed response task). The mice were anaesthetised (as described in section Anaesthesia, analgesia, antibiotics and surgical procedures) to perform the craniotomy. The cranial window was created bilaterally (or occasionally unilaterally) across hemispheres, approximately AP: 1-2mm, ML [1 -1] from the midline. A durotomy was performed in both hemispheres. The brain was lubricated throughout using PBS.

The probe (NeuroNexus A32 4x2 tetrode; shank spacing 150 μ m, site area 200 μ m², and an identical optrode with an optic fibre aligned to an inner shank, terminating 200 μ m above the upper tetrode) was manoeuvred using a micro-manipulator, with the z-axis controlled using LinLab (Scientifica, version 1.6.78), on a stereotaxic frame. The probe was inserted using the approximate coordinates: AP: 1.6mm, ML: 0.5mm, D: [0.8 1.1] mm (168). For DR task recordings the probe was inserted in the sagittal plane, for psilocybin recordings the probe was inserted in the coronal plane to span multiple neural layers. On the final recording in a brain region, the probe was coated with di-I (80mg/ml in 50/50% acetone/ethanol). Once inserted, a small well of PBS was applied in the cranial window, with the remainder filled with 1.2% agarose solution diluted in PBS to reduce signal drift. The signal was grounded by soldering a silver-chloride wire to a ground screw that had been inserted above the cerebellum during the head implantation procedure. The probe was allowed to “settle” for ~10-15 minutes before recording was initiated. If subsequent recordings were to be performed on the same mouse, the following measures were taken to preserve the condition of the brain in between recordings; once the probe was removed, a layer of PBS was applied to the cranial window, followed by a 1.2% agar solution. Once dry, Kwik Cast (World Precision Instruments) was applied, and the space between the Kwik Cast and the headplate was sealed with nail varnish.

All recordings for psilocybin experiments were single acute recordings. For the DR Task, four recordings were performed, twice in each hemisphere, over four days.

Post-Operative care and recovery

Following all procedures involving anaesthesia, mice were placed in a recovery heat chamber for approx. 1 hour, with food and water provided *ad libitum*, unless under water restriction. The mice were then returned to their home cages and monitored closely for the subsequent 24 hrs.

Perfusion and fixation

A solution of 4% paraformaldehyde (PFA) in PBS was used as a fixative in the perfusion of mice. In brief, an overdose of euthatol (20mg pentobarbital in a 100 μ L dose), was administered. Once anaesthetised but prior to respiratory arrest, the circulatory system of the mouse was perfused by introducing 4% PFA into the left ventricle of the heart. PFA was perfused for ~ 4minutes, followed by a 1 minute perfusion of PBS. The brain was extracted from the skull and placed in 4% PFA for 24 hours. The brain was then sliced (see Histology), or transferred to a PBS solution until histology was performed.

Intra-peritoneal injection of a serotonergic agonist

Preparation of the systemic psilocybin solution

A dose of 2mg/kg was prepared as a systemic dosage of psilocybin for mice, to be injected via i. p. injection to the lower right/left peritoneum. Psilocybin was dissolved in sterile saline using a sonicator for 1-2hours, to produce a solution of 0.4mg/ml. Due to light-sensitivity, the solution was stored in darkened aliquots at -20°C.

Psilocybin intra-peritoneal injection

Recordings were performed as described in Extracellular Recordings. Following the pre-injection period (20 minutes after probe insertion, for ~ 20 minutes), the mouse was briefly anaesthetised using isoflurane, to administer an i. p. injection of the psilocybin solution. The anaesthesia was necessary so as to reduce mouse movement during the injection. As the mouse was head-fixed throughout the experiment, sudden movements would almost certainly result in a loss of units between pre- and post-injection periods. Full anaesthesia lasted approximately 30 seconds (the duration of the injection), with

an approximate 2 minute recovery period of the mouse to full consciousness once the isoflurane had been removed. The post-injection period was recorded 25 minutes after injection, as psilocybin takes ~15-20 minutes to take effect (74-76). All neural data was recorded in the intervening time, however it was not included in the analysis presented here. Control experiments were performed by injecting an equal volume of sterile saline, and examining the pre- and post-injection effects.

Intra-cortical injection of a serotonergic agonist

Preparation of localised psilocybin solution

There has not yet been any published results on the localised effects of psilocybin, via intracortical injection (i. c.) or otherwise. As such the dosage was estimated by taking the plasma levels of psilocin reported by Hasler et al., and Holzmann (49,54) when the physiological effects manifested, and converting this to a molar solution to be injected into the cortex. Assuming the molar mass ($MM_{\text{psilocybin}} = 284.25\text{g/mol}$), the concentration for i. c. injection was calculated as follows:

$$\text{Plasma [psilocin]} = 4\text{-}6\mu\text{g/ml} = 4 \times 10^{-3} \text{ g/L}$$

$$\text{Molarity}_{\text{plasma psilocin}} = \frac{0.004}{284.25} = 1.407 \times 10^{-5} \text{ M} \approx 14\mu\text{M}$$

$$\text{Stock Solution [psilocybin]} = 0.04\text{mg/ml} = 4 \times 10^{-2} \text{ g/L}$$

$$\text{Molarity}_{\text{stock solution}} = 1.407 \times 10^{-4} \text{ M} \approx 140.72\mu\text{M}$$

The stock solution of psilocybin was thus diluted by a factor of 10 to give the correct concentration for the i. c. injection (volume = 1 μ L).

Psilocybin Intracortical Injection

For i. c. injections, a cannula (Bilaney Guide Cannula, $\varnothing = 0.404\text{mm}$) was implanted during the headplate implantation procedure. It was implanted superficial to the target region, with co-ordinates AP: 1.54, ML: 0.5, D = 0.7mm), at an angle of 14°. The cannula was implanted at an angle to aid the insertion of the probe in close proximity to the location of the cannula. It was secured in place with dental cement.

The pre-, and post- injection portion of the electrophysiology recordings were performed in a similar manner to that described in section Psilocybin intra-peritoneal injection. The key difference was the lack of anaesthesia during injection, due to the imperceptibility of the mouse to the i. c. injection (the experimental rig is arranged such that any movement occurring directly above the mouse is visually occluded). The solution was injected using a 1 μ L Hamilton Neuros Syringe, for 1 minute. The post analysis period was taken 20 minutes after the injection, although the effects were likely to occur more quickly than those elicited by systemic administration. At the end of the recording, the location of the probe in relation to the region injected with the drug was confirmed by injecting CNQX (6-cyano-7-nitroquinoxaline-2, 3-dione) into the cannula and observing the obliteration of neural activity. CNQX is an AMPA and kainate receptor antagonist (169), which has the temporary effect of inhibiting neural activity when present in the extracellular space.

The craniotomy was performed adjacent to the cannula (the dental cement and thread of the cannula were removed via drilling). The probe (A4x2 tetrode) was inserted at an angle of 10°, to the closest possible location to where the cannula was inserted. It should be noted that this coordination of cannula and probe was performed blind, and hence relied entirely on the accuracy of stereotaxic coordinates. Thus, the confirmation using CNQX was vital to ensure that the volume of neural tissue injected with psilocybin was precisely the neural tissue that was recorded from. Given the approximation that a 1 μ L injection permeates through ~ 1mm of tissue (personal communication with Dr. Matthew Brown, Chadderton Lab, Dept. of Bioengineering, Imperial College London), this leaves a very narrow margin for error.

Behavioural training

The mice were placed under water restriction for 3 days prior to training commencement. The mice were given 0.5mL of water each day until their weight reached 85% of their pre-training weight. During this time, the mice underwent habituation to the experimental setup. Excluding the habituation period, training sessions occurred once per day (duration = 40 minutes), and were performed at approximately the same time of day, 6 days per week. The mice were weighed after each training session to ensure that their weight remained at 85% of their normal weight to maintain motivation. Of note, no punishment was used during any aspect of training.

Habituation

The mice were habituated to the setup for 2-3 days prior to training commencement. On the first day the mice were allowed to roam around the treadmill belt, i.e. they were not head-fixed into the setup. On the second day the mice were head fixed into the setup, and on the final day of habituation the lick port was placed in front of the mouse whilst they were head-fixed.

Delayed response task

A delayed response (DR) task was implemented as a working memory paradigm. The treadmill belt was set up as single feature belt on the experimental rig described in Experimental Setup. It was configured to have the following dimensions; length = 160cm (two 80cm trials), trial start at position $p_{\text{start}} = 0, 80\text{cm}$, tactile feature (i.e. the reward zone), placed at $p_{\text{rz}} = [60\ 65]$ cm from trial start position. Two pure tones were presented ($T_1 = 1000\text{ Hz}$, $T_2 = 9000\text{ Hz}$) to distinguish between two trial types. The delay period was the time between tone presentation and entry into the reward zone. As the length of the delay period was dependent on the speed of the mouse, the characteristics of each trial in the spatial domain (i.e. per cm) were consistent across trials, whereas in the temporal domain they were inconsistent across trials. The task consisted of two trial types; Stop (T_1) and No-Stop (T_2). For a Stop trial, the mouse had to stop upon reaching the reward zone for a period of 500ms (this may be performed at any point inside the reward zone). This triggered the reward, which was administered as a droplet of water through the lick port. The reward remained available for 2s, after which it was removed. For a No-Stop trial, the mouse had to continue running through the reward zone without pausing. If the mouse stopped for more than 500ms, the trial was incorrect. This was a self-initiated task by the mouse. The following is a description of the full expression of the task:

- i. The mouse started the trial by running past p_{start} .
- ii. When the mouse had reached the tone onset position ($p_{\text{tone-onset}}$), T_1 or T_2 were triggered at random, using a pseudo-random function programmed in the Arduino.
 - a. T_1 indicated a Stop trial
 - i. Hit trial – The mouse successfully paused within the reward zone.
 - ii. Miss trial – The mouse did not pause for sufficient time in the reward zone.
 - b. T_2 indicated a No-Stop trial

- i. Correct Rejection – The mouse did not pause in the reward zone for longer than 500ms.
- ii. False Alarm – The mouse incorrectly paused in the reward zone for 500ms.
- iii. Once one of the four possible outcomes had occurred, the mouse completed the trial by running to the final position on the belt (p_{finish}).

Training Protocol

Each session from Phase II onwards began with 5 introductory runs whereby the mouse had to run 10cm to receive a reward. This was enforced to increase mouse motivation. As training progressed, the volume of water administered as reward was reduced to ~5-7 μ L.

Phase I

The initial phase had two facets; familiarisation and locomotion training. Day 1~2 (familiarisation) - Following habituation (as previously described in section Habituation), the mice were introduced to the lick port. A droplet of water was consistently available at the lick port. Once the mouse licked the water away, another droplet appeared after 2s. Day 2~5 (locomotion training) – The mouse must trigger the reward by running a set distance, $d_{\text{tr}} = 10\text{cm}$. Once the mouse successfully triggered and licked the reward away for $\text{successThres} = 30$ instances, d_{tr} increased by a 10cm increment. This continued occurring until $d_{\text{tr}} = p_{\text{rewardZoneEntry}} = 60\text{cm}$.

Phase II

The second phase introduced two further facets; Pavlovian learning and reward trigger training. Day ~6-9 (Pavlovian learning) – The mouse only received a reward in the reward zone. Once the mouse entered the reward zone, T_1 was played with probability of $p(0.9)$, whereby T_2 had a probability of $p(0.1)$. If T_1 was played, the reward was immediately available. It remained available for 2s. This began to form the association between the tone and the reward, although this association was largely not necessary for the mouse to attain water at this point. The performance of the mouse was measured by the hit rate: $\text{successThres} = 85\%$ hits. Once the mouse had reached successThres , the mouse had to stop in the reward zone for 100ms to trigger the reward (reward trigger training). The stop time was

increased by an increment of 100ms to be almost imperceptible to the mouse. If the mouse formulated a strategy in the previous phase of the task for “licking-on-the-go”, the lack of reward would naturally elicit more cautious behaviour in the mouse and hence, they would start triggering the reward. The stop time was incremented by 100ms every time successThres was reached until a stop time of 500ms was required to trigger the reward.

Phase III

The final phase introduced Pavlovian learning for the No-Stop tone (T_2), and the delay period. Day ~9-14 was the most crucial part of the training. When the mouse was 5cm before the reward zone, the tone was presented as in Phase II. Once successThres was achieved, the probability ratio of $T_1:T_2$ shifted by 0.1, hence $p(T_1) = 0.8$, $p(T_2) = 0.2$. Once the ratio had shifted to $p(T_1) = 0.7$, $p(T_2) = 0.3$, the performance of the mouse was measured in terms of d' prime (d' , see section Signal detection theory). The probability ratio continued to shift until $p(T_1) = p(T_2) = 0.5$. Due to the difficulty in forming the association between T_2 and no-stopping behaviour, the progress was restricted to only shift one probability ratio per training session. Hence, the shortest duration of this part of training was 4 days. Once complete, the delay period was increased. Once the mouse completed 30 successful hit trials, $p_{\text{tone-onset}}$ retreated 1cm on the belt. Mice would typically retreat 2-7cm per training session during this period. It was deliberately chosen as a very slow progression so as not to disrupt any prior learned associations. At the end of each session, d' was measured to ensure that the mouse was still performing the task overall. If d' dropped below threshold ($d' = 1$), the tone was brought back to the original $p_{\text{tone-onset}}$ at the start of the previous training session. The delay period was lengthened to ensure that there was a sufficiently high cognitive demand on mice during the DR task. This criterion was assessed by increasing the working memory load in the form of the delay period length, until the mouse performance began to decrease. The delay period length was then maintained until performance was restored. This typically occurred at $p_{\text{tone-onset}} = [25:30]$ cm. The correlation between delay period length (cm) and performance is shown in Figure 8. The learning curve is represented by the initial portion showing a positive correlation between the two parameters. However, once the delay period distance had increased sufficiently, the relationship reverses. Once this occurred, the increase in delay period length was tempered (note the reduction in slope of the curve), to allow performance to recover. This method was used to sustain behavioural performance, whilst ensuring sufficient cognitive demand in

the task. Note the low standard deviation seen in the latter portion of Figure 8 (a). This was a very stereotyped behaviour in mice and hence a reliable indicator of when mouse training was approaching completion. A more detailed view of how the performance of a single mouse evolved is seen in Figure 9. As before, the initial portion of both panels represent the learning curve. Upon completion this transitioned into a reduction in performance as seen in the decrease in Hit rate (Figure 9 (b)). Interestingly, the FA and Hit rate fall concurrently. This is potentially an indication that the reduction in performance is not due to the mice forgetting how to perform the task (a classic reaction of a poorly performing mouse is to stop at every trial and so have a simultaneously high Hit and FA rates, showing an inability to discern that T1 and T2 require two distinct responses), but rather that their ability to recall the stimulus has diminished, hence lowering their overall engagement in the task.

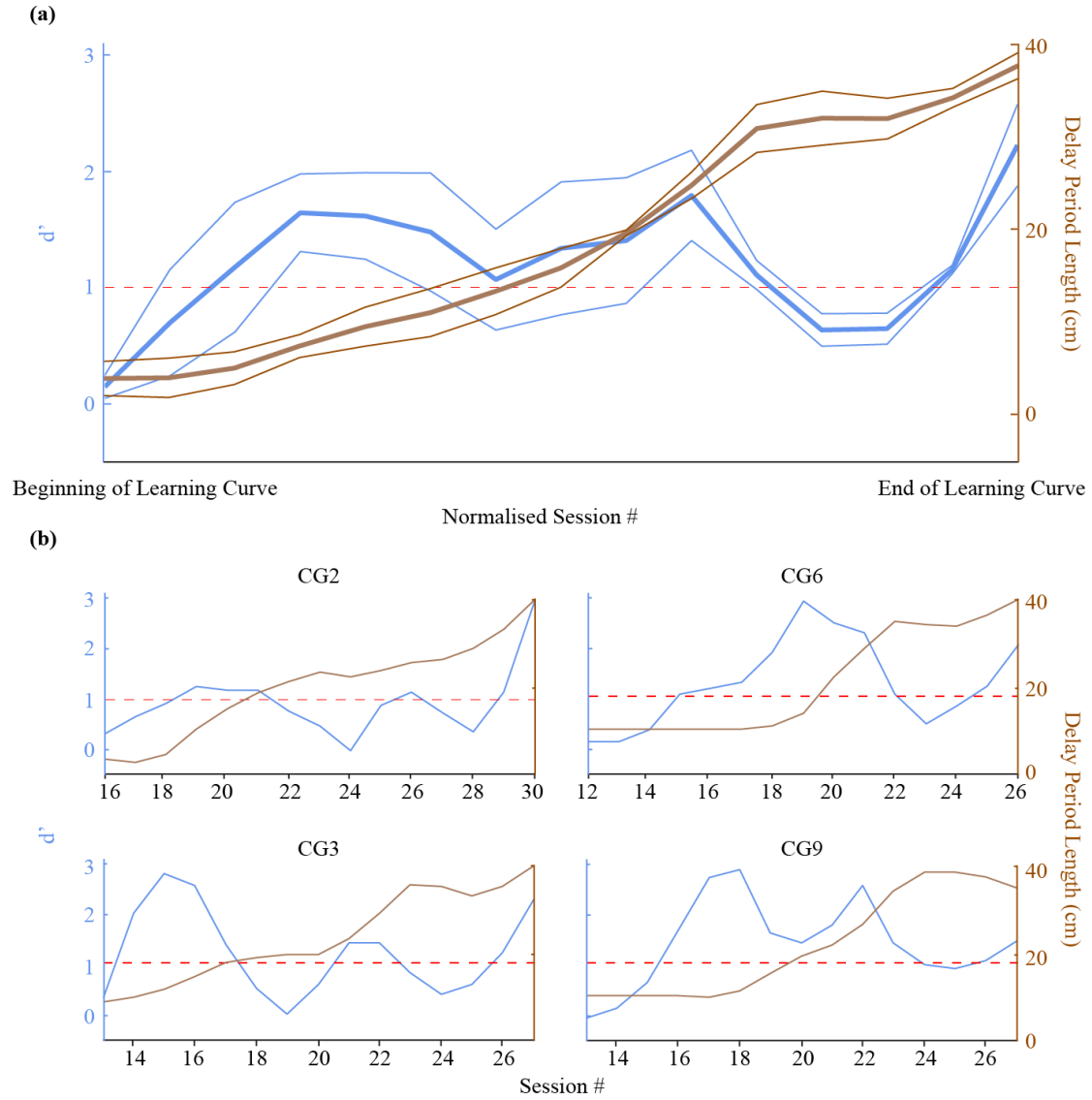


Figure 8: Delay period load influences performance during training. (a) Mean session d' and delay period distance (working memory load) for all mice in Batch 3. (b) As in (a), examples for individual mice. For all mice in question, this figure represents a subset of training sessions. Curves smoothed with a Savitsky-Golay filter (span = 5cm). Note: This data is from Batch 3 only as this batch displayed the most linear learning trajectory of all three batches.

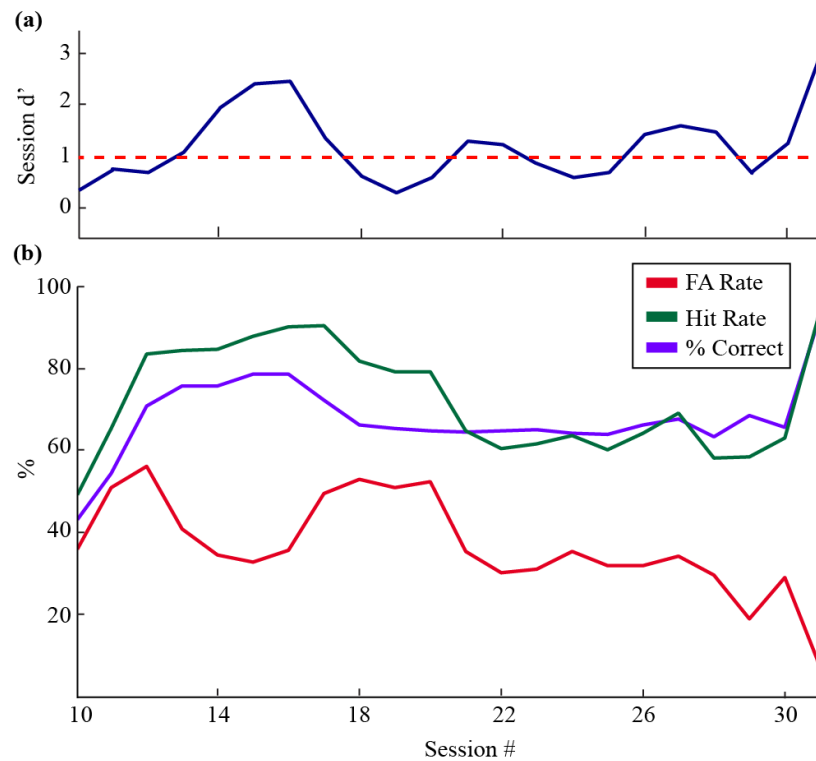


Figure 9: Example of training evolution of a mouse. (a) d' progression across sessions. (b) The concurrent progression of Hit, FA rate, and % Correct.

Behavioural training hurdles

There were two predominant criteria through which mice would be removed from behavioural training:

1. Lack of stable locomotion
2. No evidence of association between tone T_2 , and the corresponding behaviour

Criteria (1) was surpassed if the mice were running fluidly on the treadmill and transitioned to Phase III within 12 days. Criteria (2) was passed if the mice surpassed the d' threshold within 10 days from the beginning of Phase III.

A common strategy employed by mice in the early experimental batches, while the protocol was being developed/refined, was to engage in constant Hit/False Alarm behaviour. Mice would consistently, and deliberately stop at the reward zone, independent of what tone was played. This behaviour was mitigated in subsequent batches by introducing and increasing the prevalence of T_2 trials as early as possible in training. This seemed to cement the initial association between T_1 and the reward, rather than simply a tone and the reward.

If the mouse dramatically reduced the degree to which he/she licked the reward, the reward volume was increased. This could occur due to the mouse being unsure as to how to trigger the reward, hence the reward volume was generally increased soon after an increase in task difficulty. Once the mouse was performing the task, the volume of the reward was kept constant throughout the session. Hence, given that the duration of the training session and the reward volume were fixed, the satiation level of the mouse was governed by the number of trials the mouse could perform within a 40 minute session. This training strategy was successful in increasing the number of trials each mouse would perform in a task.

Once mice were trained, a large amount of care was taken to preserve their performance. This was due to the high cognitive demand inherent in the task, based on the fact that the delay period was increased to the point where their performance would drop beyond this point. As such, if the mice were sufficiently perturbed prior/during training, they would typically engage in one of the following behaviours:

1. Remain stationary on the belt.
2. Continuous locomotion, independent of any experimental stimuli e.g. the tone.

Both are examples of non-engagement in the task, which can be produced by a number of factors. While it is impossible to know the exhaustive extent of this list, the following parameters proved crucial to be aware of in order to successfully train mice in this task. Behaviour (1) would typically result from a perturbation such as training at an unusual time of day, fighting within the cage prior to the session with other cage mates, a hardware change such as a new lick port. Behaviour (2) would typically arise when the mouse was satiated, or when stimulus control was lost and the mouse would no longer know what to do to get a reward and disengage. It was necessary to refine and mitigate all of the aforementioned factors to successfully train mice in the DR task.

Signal detection theory

The performance of the mouse was quantified by measuring d' using signal detection theory. In brief, the value of d' measures the degree of overlap between the signal and noise in a given system (Figure 10 (a)). There are four outcomes in using this model; Hit (H), Miss (M), Correct Rejection (CR) and

False Alarm (FA), as shown by the confusion matrix in Figure 10 (b). The application to the DR Task is as follows; H and M are possible outcomes for a T_1 trial, CR and FA are possible outcomes for a T_2 trial. As the performance of the mouse improves, the distance between the two curves increases, and as such the ability of the mouse to respond correctly to the stimulus increases. The threshold value for d' is based on the ROC (Receiver Operating Characteristic) curve (see Figure 10 (c)). This indicates the amount of true positives (Hits) versus false positives (FAs) the experimenter is willing to accept to assign sensitivity to the subject's response. As seen in the plot, all positive d' curves are bowed upwards and will thus always have a higher Hit rate than FA rate. However, the greater the d' value, the fewer FAs are accepted. A threshold of $d' = 1$, which equates to a percent correct of 69%, was chosen as the criteria by which the mice were performing the task. There is a lot of valuable information to be gained from error trials (i.e. Miss and FA trials), hence having a d' at an accepted level of sensitivity, although not too conservative so as to include a reasonable number of FA and Miss trials was considered optimal.

To compute d' the H and FA rates were z-transformed to standardise the data. The value d' was then calculated as:

$$d' = z(H) - z(FA)$$

Equation 1

Intuitively, this can be thought of as maximising H to minimise M and minimising FA to maximise CR, and hence produce a larger d' value. In the DR task, if the mouse was stopping at random, then $H = FA$, and $d' = 0$. If the mice were learning the opposing association then d' would be negative, whereas, d' will increase as more correct associations were being performed. As the z-transform is a normal distribution based on a finite window size of trials (for example, $n_{trials} = 40$, for moving average d'), Equation 1 may be biased by high Hit rates and/or very low CR rates. As such, the probabilities of Hits and False Alarms have upper bounds given in Equation 2, to prevent saturation of either value, thus avoid producing artificially high or low d' values (170).

$$p_{min} = \frac{1}{n_{trials}} \quad p_{max} = \frac{n_{trials} - 1}{n_{trials}}$$

Equation 2

Motivation for using d' versus percent correct

D -prime was chosen as the statistical measure of performance due to the ability to measure sensitivity without bias. In DR behavioural paradigms, percent correct is often used as a measure of performance. Whilst this will measure sensitivity, it will also be influenced by bias. For example, in the case where an equal number of signal and noise trials (in the DR task described these would equate to T1 and T2 trials), the value of d' would map monotonically to the percent correct value. However, in the instance whereby the number is not equal, which due to the random nature of which trial is presented is more often the case, percent correct will be influenced by bias. For instance, if T1 trials are presented 70% of the time, and are consistently Hit trials, and the 30% of T2 trials are False Alarms, the percent correct will be 70%, but the d' value will be zero. By performing z-transformation in the d' calculation, variables with different numbers of measurements can be compared without bias. Hence, this method examines the sensitivity of the subject to a given response.

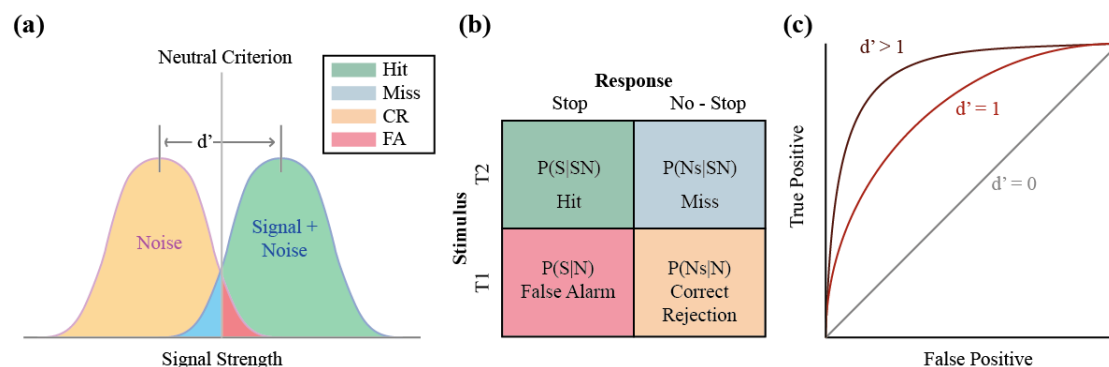


Figure 10: Signal detection theory. (a) The four trial outcomes are shown as signal and noise probability distributions. (b) The confusion matrix for the four trial outcomes. (c) ROC curves for different values of d' .

Behavioural training considerations

Developing the behavioural paradigm of the DR task on the treadmill setup was a lengthy and arduous task, thus, in an effort to share the fruits of this labour, the following points of advice are a culmination of the key hurdles that were overcome to develop the DR task paradigm described in the section Training Protocol.

1. **Transfer to recording setup:** If there are training setups and recording setups, the mouse must be transitioned to the recording setup before the difficulty of the task reaches beyond a

certain level (determined empirically) – this is crucial to prevent loss of training time later. If the mice are transferred once they have learned the task, the transfer will be too much of a perturbation that they can often forget it entirely and a week (or more) is spent re-training the mice.

2. **Increasing task difficulty:** Make each training phase as seamless as possible. This may seem obvious, but one cannot underestimate the level of pedantry required. Any seemingly superfluous steps can be removed gradually once the paradigm has been firmly established – this is a much more controlled environment in which to change the protocol.
3. **Engagement:** At any stage of training, if mice become confused, it is crucial to maintain their engagement in the task. Do this by administering a reward for a very simple action a few times, and then return to the normal stage of training. Disengagement is the coup de grace in behavioural training.
4. **Motivation:** Once mice have reached a stage whereby they can receive sufficient water in the training session, train six days per week and on the seventh day, omit water. This will stabilise weight and lead to the minimum fluctuation of motivation. Perhaps contrary to intuition, supplementing with a small amount of water on the 7th day changes motivation more than if it is omitted.
5. **Treadmill-specific:** Once tone discrimination becomes necessary to perform the task, train mice individually, unless each treadmill is in a soundproof chamber.

Optogenetic stimulation of interneurons of the anterior cingulate cortex

Channelrhodopsin (ChR2-H134R) (171) was injected via a cre-dependent floxed adeno-associated virus (AAV) construct into uni- or bi-hemispherically into the ACC of Pvalb-Cre mice. Hence, ChR2 was embedded into the membranes of pv-interneurons within the injected region of cortex. Light was delivered via an optrode (NeuroNexus, 4x2 tetrode), which consisted of an optic fibre (diameter ($\varnothing$) = 50 μ m, terminating 200 μ m above the proximal channel on the probe), with a transmission rate of 75% of light intensity from the external optic fibre tip. The external optic fibre was coupled to the optrode using a bare ferrule-to-ferrule connection. The laser stimulation protocol was calibrated using a range of laser pulse protocols varying pulse frequency, length and intensity. The following stimulation parameters proved sufficient for opsin activation: single pulse, duration = 100ms, wavelength (λ) =

430-490nm, intensity = 2.25mW at the optic fibre tip. The light intensity is within the range of the intensity of light required to activate ChR2 (172), and below that which would damage the tissue (173).

Histology

The location of the probe was confirmed post-hoc via histochemistry. Following perfusion, the brains were sliced using a vibratome (Leica Biosystems, slice width = 80 μ m), and imaged under the confocal microscope to locate the di-I that coated the probe upon insertion (di-I solution: 80mg/ml in 50/50% acetone/methanol) (Figure 11).

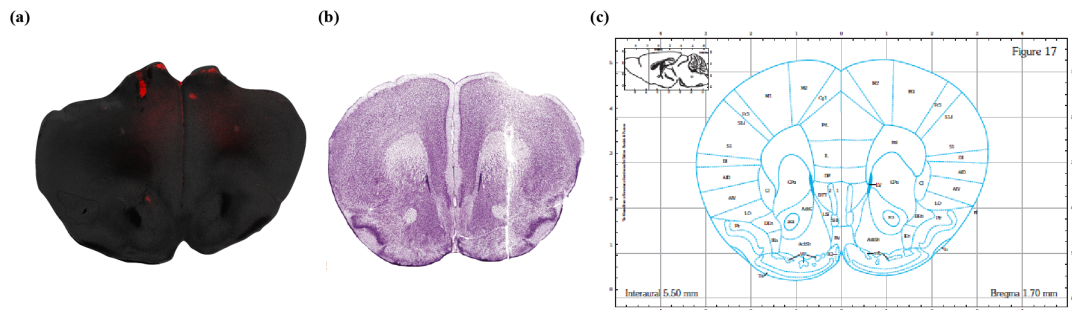


Figure 11: Identification of probe location using di-I. (a) The probe track and the di-I can be seen in the image overlay. (b) and (c) The reference slices in Paxinos Brain Atlas (168). Note the probe penetrates deeper than the di-I signal as much of the di-I will be removed with penetration into the brain.

Data analysis

All data was processed in Matlab (Mathworks). The LFP was band-pass filtered ($BW_{\text{delta}} = 1\text{-}4\text{Hz}$, $BW_{\text{theta}} = 4\text{-}8\text{Hz}$, $BW_{\text{alpha}} = 8\text{-}13\text{Hz}$, $BW_{\text{beta}} = 13\text{-}30\text{Hz}$, $BW_{\text{gamma}} = 30\text{-}120\text{Hz}$) to isolate each neural frequency band, and subsequently down-sampled from 25kHz to 500Hz. The power spectral density was computed across 10 minutes of data (2s sliding window, overlap = 0.9, $nfft = 2^{12}$). For population synchrony analysis, the method of Jones et al., was employed (18). In brief, the local maxima and minima of the band-pass filtered data were detected to assign the peaks and troughs of the oscillation. The phase was then linearly interpolated from trough (0°), to peak (180°), to trough (360°). The level of phase modulation within each frequency band was quantified using the circular correlation coefficient (κ) for each cell (18,174). This parameter was computed via the parameters of a von Mises

distribution of spiking activity in each phase of the neural frequency band. This distribution is characterised by μ , the centre of the distribution i.e. the phase to which the neuron is tuned, and $1/\kappa$, the variance. Hence, the reciprocal, κ , is a measure of the concentration of phase tuning. (Note: κ was calculated using custom scripts from CircStat {Berens:2009vb}). Single units were isolated offline using the semi-automatic clustering software Klustasuite (175). An example of some manually sorted clusters is shown in Figure 12. Clusters were rejected if they contained >1% spikes within a 1ms interspike interval (176). Only single unit data was used in analysis. The baseline firing rate for z-score calculations was taken as the mean firing rate of a cell over all spatial bins (1 cm) for all trials. The discrimination of a cell between T1 and T2 trials was computed using receiver-operating characteristics (ROC) analysis (177-180). For a given position, logistic regression was performed on the firing rates a cell across all trials for each trial type, to generate a probability of a given firing rate resulting in the trial outcome (with a corresponding p-value quoted for each belt position). This vector of probabilities was then used to generate a ROC curve and compute the area under the ROC curve (AUROC) value for each position on the belt. The AUROC value is the probability of correctly classifying a randomly drawn pair of observations, hence it was interpreted as the discrimination ability of a cell at a particular position on the belt.

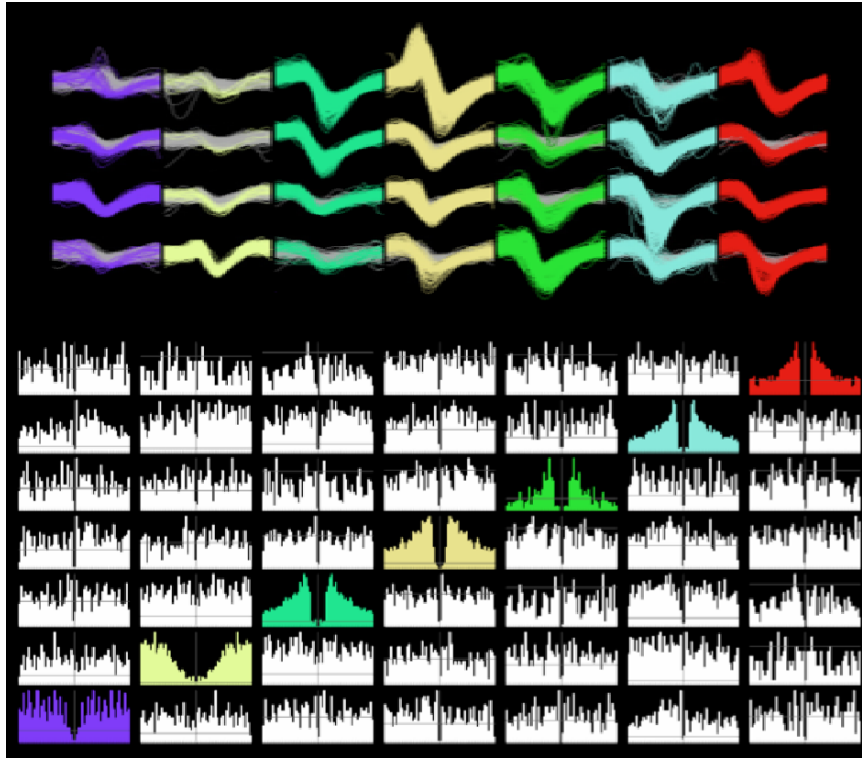


Figure 12: Manual clustering using Klustaviewa. The upper panel shows a selection of waveforms across four channels of a tetrode for each neuron. The lower panel shows the auto-correlogram for each cell in colour, with the cross-correlogram with each other cell in white.

Chapter 3: The effect of psilocybin on the mouse prefrontal cortex

Introduction

The psychoactive effects of psilocybin induce a modified state of consciousness in the brain (181). Much of neuroscience is focused on the neural mechanisms within a single state: wakefulness, with a relatively small amount of research on sleep and anaesthesia. In order to fully understand neural mechanisms within a given state (e.g. wakefulness), it would seem that a more comprehensive view would be obtained from comparisons with other states of consciousness, rather than trying to characterise a state of consciousness from observations within said state. Hence, by inducing altered states of consciousness through the use of hallucinogenic substances such as psilocybin, one can hope to more fully understand neural mechanisms, and not be restricted to a truncated snapshot restricted to those active within the most accessible brain state; wakefulness.

The current literature on the effects of psilocybin on neural dynamics has revealed some interesting results, predominantly from the fMRI domain. Analysis of the BOLD signal following psilocybin administration has shown decreased blood flow to areas of the PFC in the early onset of the drug (32), and increased blood flow during what is postulated as the late effects of psilocybin (31,32). The connectivity within regions of the PFC increased, whilst the functional connectivity between the PFC and areas such as the posterior cingulate cortex was reduced (32), in addition to an overall reduction in the functional connectivity of the default mode network in the brain (defined here as a system of “spontaneous cognition” (70)) (25). On a neurophysiological level, EEG studies have found evidence of a reduction in power of low frequency oscillations in both humans and rhesus monkeys following psilocybin administration (53,71). A change in low frequency power can be seen as characteristic of a transition from a synchronised state associated with slow-wave sleep (increased low frequency fluctuations), to a desynchronised state associated with attention (decreased low frequency fluctuations). However, synchronised and desynchronised states are also associated with a simultaneous decrease/increase in gamma power, respectively. Interestingly, stimulation of the raphe nuclei decreases low frequency power, simultaneously with gamma power (48). Hence, the decrease in low frequency power seen in psilocybin is likely to be linked to a strong stimulation of the serotonergic system, which is known to have feedback projections from the PFC to the raphe nuclei (182,183).

However, in order to more fully understand the differentiating factors between stimulation of the serotonergic system via the raphe nuclei, and hallucinogenic compounds, it is necessary to examine the activation pathway more closely. Examination of the effects of psilocybin on a cellular level may provide more knowledge into where these pathways bifurcate. However, as yet there are no reports of *in vivo* electrophysiology using psilocybin. Hence, we must look to the studies performed on a similar psychoactive substance, DOI. In the ACC, DOI has been found to inhibit approximately 1/3 of the cell population, whilst activating some cells, and has a negligible effect on the firing rate of approximately 1/3 of the population (83,84). The overall population activity level was found to decrease in response to DOI administration (83), however it is important to note that this was a nuanced effect. The decrease in power of low frequency oscillations was also shown with DOI (83). Interestingly, DOI was also found to desynchronise the network by disrupting phase locking between pyramidal cells and gamma oscillations (83,84).

Whilst DOI is also an analog of serotonin, one cannot assume linear inference of the neuro-electrophysiological effects of psilocybin. Hence, to characterise the effect of this compound on the ACC, a set of experiments were performed on awake, head-fixed mice on the previously described treadmill setup shown in Figure 13. Following dose verifications via observation in an open cage, a control period of neuronal data from the ACC was recorded, followed by an i. p. injection of 200 μ L of a 0.4mg/ml psilocybin solution in sterile saline. Following a 20 minute onset period, the neuronal data for the post-condition was recorded. The control experiments were performed identically with the exception of the injected fluid being composed of an equal volume of sterile PBS.

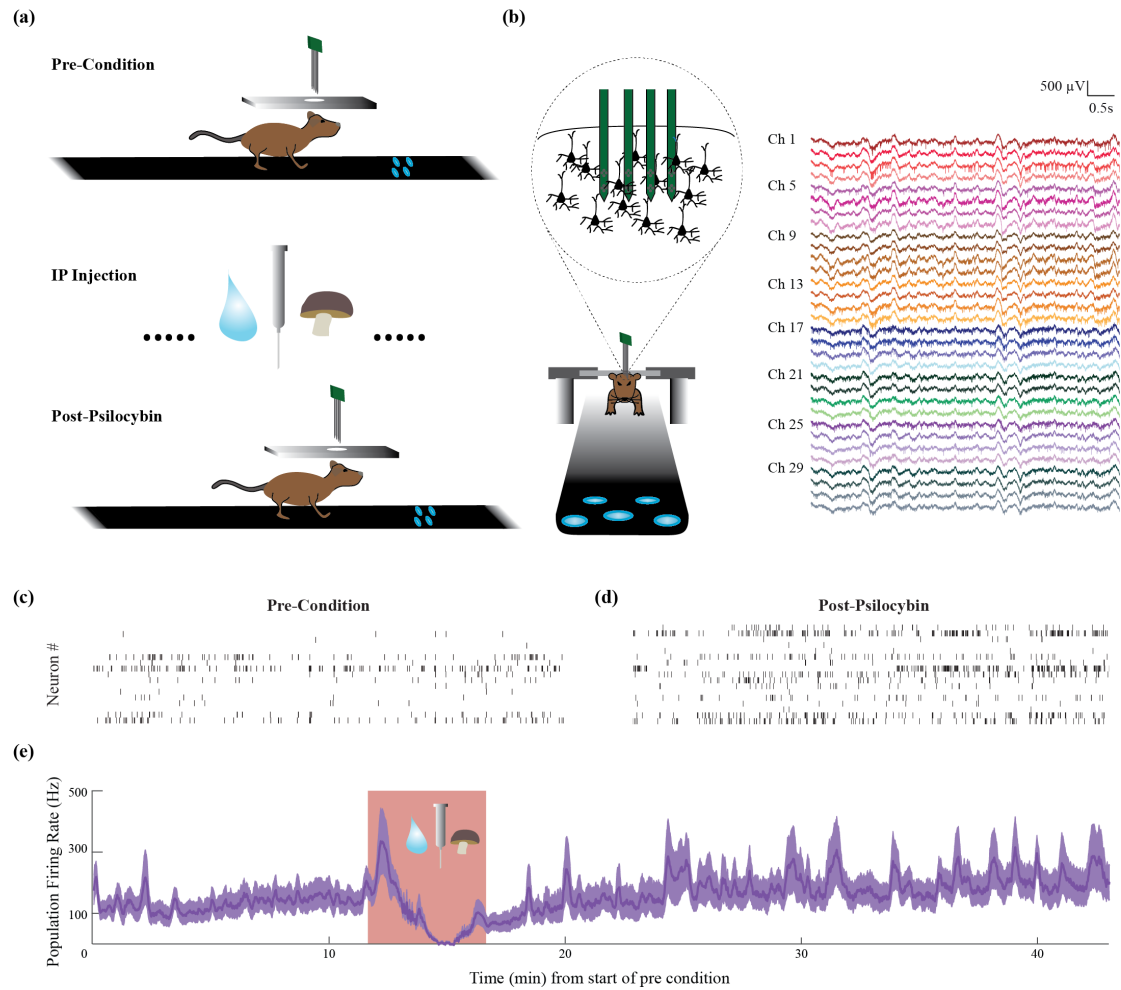


Figure 13: A schematic of the experimental setup for the systemic administration of psilocybin. (a) Awake, freely moving mice were head-fixed to the treadmill setup for the duration of the recording. The baseline activity was recorded during the pre-condition, followed by a brief sedation to facilitate the injection of either psilocybin or PBS. Following mouse recovery and a sufficient onset time for the compound, the post-injection data was recorded. **(b)** An example of the raw data that was recorded from an experiment using a 4x2 tetrode probe. Each trace represents a probe channel. Each channel is colour coded to show that they are grouped by tetrodes. The deflections represent fluctuations in the LFP, and action potentials can be seen on many of the channels. **(c)** A raster plot of a sample of neurons in the pre-condition and **(d)** in the post-psilocybin condition. **(e)** The population firing rate through the entirety of a psilocybin experiment. The point during which sedation was administered is reflected strongly in the population firing rate (highlighted in red). Note: This plot is for illustrative purposes, only data previously defined in the pre- and post-epoch was included in the analysis.

Dose Calibration

The previously reported dose for humans and monkeys was used as a starting point from which to calibrate the appropriate i. p. injection dose for mice (aged 6-8 weeks). Dose calibration was performed in freely moving mice within a cage environment, whereby mice received an i. p. injection of [100,

150, 200, 250] μL of 0.4mg/ml psilocybin solution. Blind observation was performed on mice to assess the frequency of the canonical head-twitch response, pre- and post-injection. At a volume of 200 μL , mice exhibited a notable increase in head-twitch behaviour. This was in agreement with the 2mg/kg dosage used for rats (68).

Effects of psilocybin on the local field potential

The relative strength of specific neural frequency bands of the LFP can be indicative of population wide changes in firing activity, and furthermore, provide indications of information transfer with other brain areas (15). For example, the theta band has been hypothesised to play a key role in information transfer between the PFC and the hippocampus (18). Furthermore, gamma oscillations have been proposed to be linked to local networks given that the period of gamma oscillations is closely linked to the decay time of interneuron populations (184). Given the propensity for high frequency gamma band activity to influence local circuits, and the ability of low frequency oscillations to provide a wide enough temporal window to facilitate synchronisation across long distances, a dominance of low frequency band activity such as delta or theta may be indicative of interactions with distant brain areas. Thus, shifts in patterns of LFP activity can be powerful indicators of changes in information processing/states of the local network. To investigate the presence of population effects of psilocybin on the ACC, the LFP was examined to determine any psilocybin-induced changes in each neural frequency band.

The baseline activity was defined as a 10 minute period of spontaneous activity in the ACC prior to i. p. injection (referred to throughout this chapter as the pre-condition). This is in accordance with previous studies that defined the baseline activity for assessing the effects of psilocybin in a similar manner (68). The pre-condition began at a minimum of 25 minutes following probe insertion to allow the brain to recover from probe penetration. Mice were briefly sedated using isoflurane during the injection period. This was necessary to reduce movement, which would be highly likely to result in a loss of cells and result in an unpaired pre-post recording. The injection/anaesthetic period duration was 2-5 minutes (including the time for anaesthesia recovery). The time in which the effect of the vehicle or psilocybin was analysed, referred to as the post-condition (or post-psilocybin referring to only those experiments in which psilocybin was injected), was taken as a 10 minute period, 20 minutes after the

end of the injection period, to allow time for the compound to take effect as per previous studies (74-76).

The effect of psilocybin on LFP neural frequency bands was performed in the time-frequency domain. Each neural frequency band was isolated using a band-pass filter, examples for which are shown in the upper panels of Figure 14 and Figure 15. The power spectral density of each neural band [delta: 1-4Hz, theta: 4-8Hz, alpha: 8-13Hz, beta: 13-30Hz, gamma: 30-120Hz] was computed in the pre- and post-conditions for vehicle and psilocybin experiments. In accordance with previous findings in humans and rhesus monkeys (53,71), low frequency bands showed a significant decrease in power (delta; $p = 0.01$, theta; $p = 0.01$, and alpha; $p = 0.01$, paired student t-test), whilst the beta band remained unaffected ($p = 0.96$). The power of the gamma band was moderately increased but not to a level of significance ($p = 0.11$) (Figure 16 (b)). A summary plot is shown in panels (c) and (d) of Figure 16, whereby the change in power of LFP neural frequency bands can be seen in the psilocybin condition, and the absence of any change can be seen in the vehicle condition. This effect echoes that of a transition into a desynchronised state in awake, attentive animals whereby low frequency fluctuations decrease, and gamma power increases (185). A visualisation of the neural frequency band power is shown for low frequency bands in Figure 14, and high frequency bands in Figure 15. The results for the control experiments can be seen in Figure 16 (a), where no significant change in the power of any neural frequency band was found. This would suggest that while the brain activity of an awake mouse is consistently fluctuating, the changes that we see in the post-condition of psilocybin experiments are due to the injected compound, and not simply a consequence of rudimentary fluctuations.

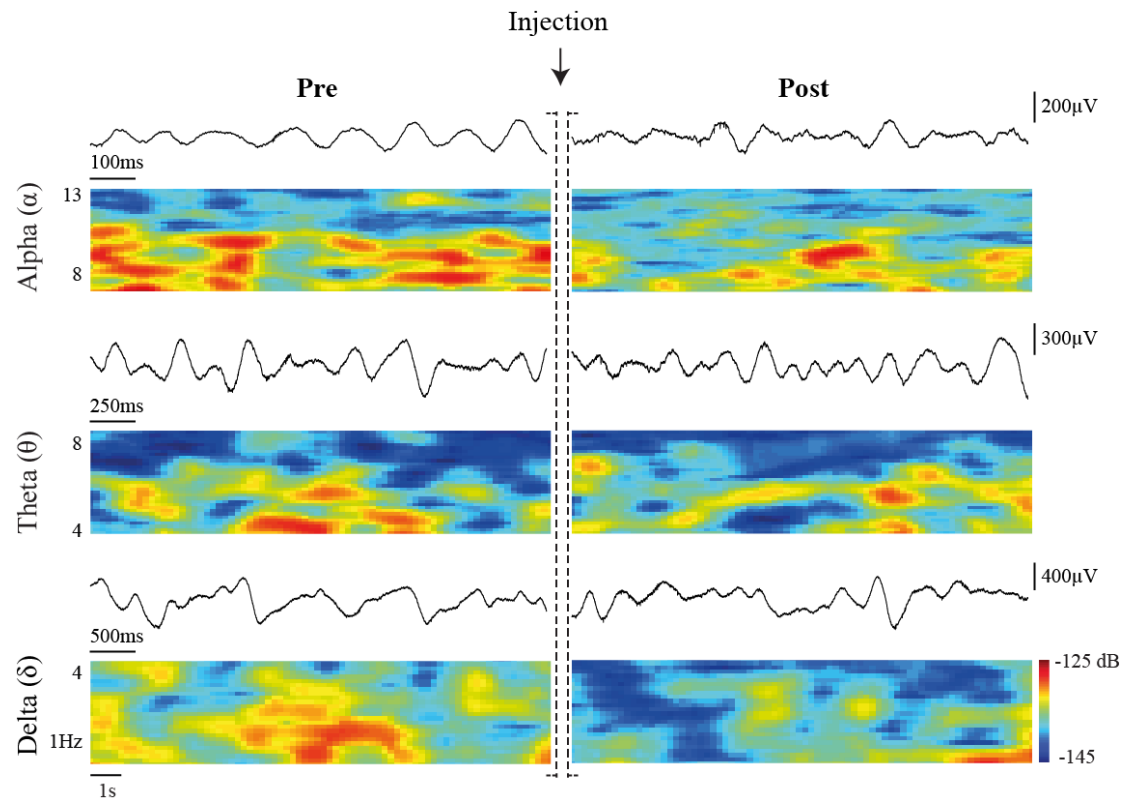


Figure 14: Example spectrograms (smoothed using a moving-average filter, span = 1.5s) of delta, theta and alpha neural frequency bands, pre- and post- psilocybin injection. The upper panels of each spectrogram show the band-pass filtered signal of the respective frequency band. Note: Both the temporal and voltage scales differ for the sample traces shown in the upper panel so as to allow the oscillatory activity to be seen in each band.

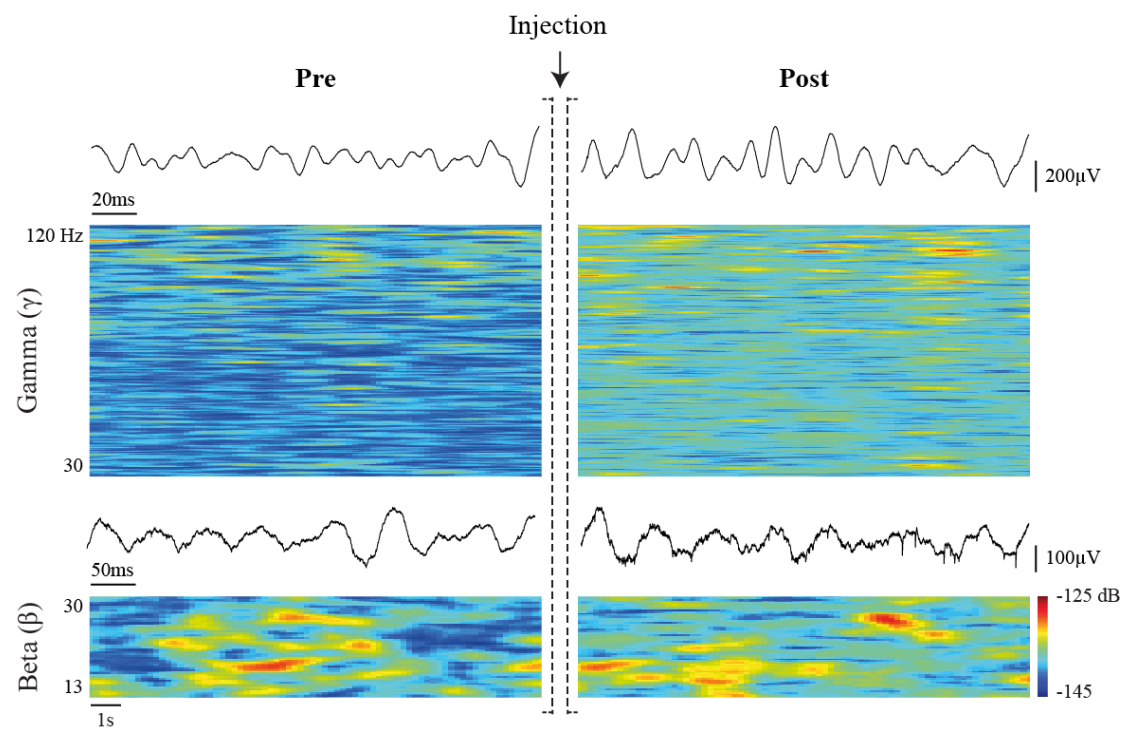


Figure 15: Example spectrograms (smoothed using a moving-average filter, span = 1.5s) of beta and gamma neural frequency bands, pre- and post- psilocybin injection. The upper panels of each spectrogram show the band-pass filtered signal of the respective frequency band. Note: Both the temporal and voltage scales differ for the sample traces shown in the upper panel so as to allow the oscillatory activity to be seen in each band.

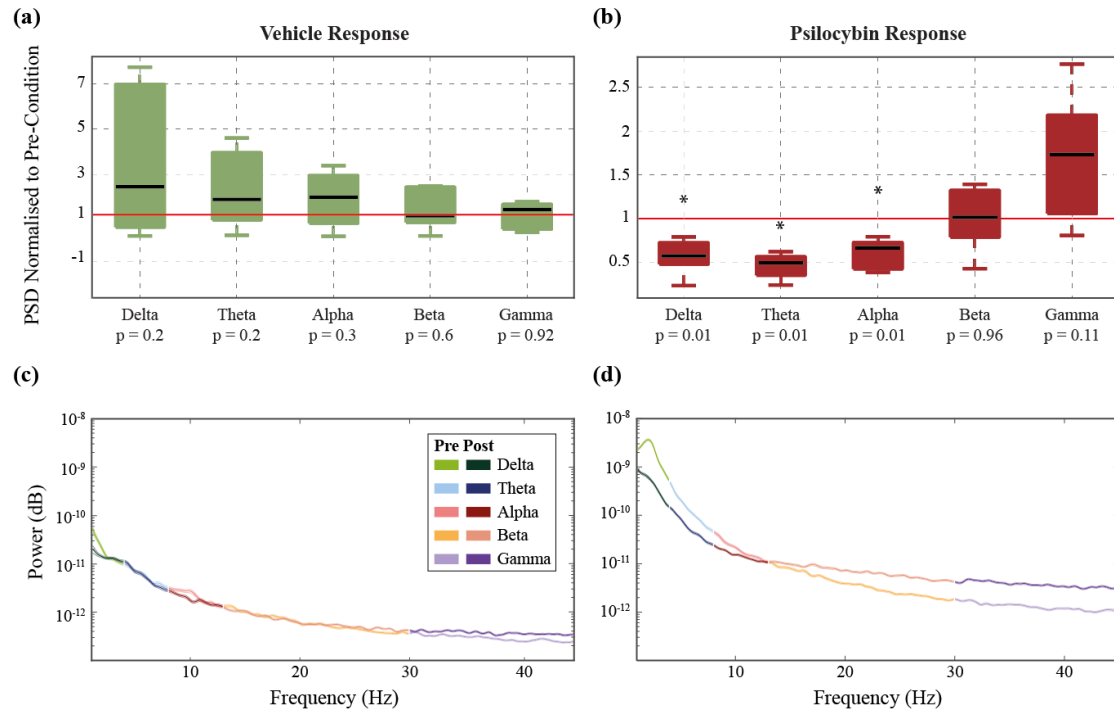


Figure 16: Psilocybin induces a reduction in power of delta, theta and alpha neuronal frequency bands. (a) The power spectral density (PSD) normalised to the pre-condition for control experiments for each neural frequency band (n = 5). (b) As in (a), for psilocybin experiments. Asterisk denotes significance in paired student t-test comparing the power spectral density in each frequency band between the pre- and post-conditions. (c) & (d) The power of the local field potential in pre- and post- conditions for a vehicle and psilocybin experiment respectively.

Effects of psilocybin on single unit activity

Activation of the serotonergic system is known to introduce inhibition in the PFC network (although it is also known to induce some activation (22,23)). On the contrary, when the population firing rate of ACC networks was compared in the pre- and post-psilocybin conditions, with the exception of one experiment, psilocybin introduced more excitation in the network (Figure 18 (c)), with the average population firing rate increasing from 28.5Hz $\pm$ 6.5Hz in the pre-condition to 51.9Hz $\pm$ 15.4Hz in the post-psilocybin condition (n = 5) (Figure 17). The outlier experiment shown in blue in Figure 18 (c) may be due to layer dependent changes in firing rate. The experiments were performed using a four shank probe that crossed layers of the PFC. In this experiment alone, cells were only detected on two shanks, hence the probe may have been more medial or more lateral than the other recordings. This may account for the altered behaviour in the single unit response. Psilocybin has the strongest affinity

for 5-HT_{2A} receptors, which have a depolarising effect on the host neuron. It is possible that the excitation may be a combination of a direct depolarisation of pyramidal cells, with a release from inhibition through the activation of 5-HT_{1A} receptors on interneurons that would induce hyperpolarisation. In both serotonergic activation (22,23), and DOI administration (83,84), a medley of activation, inhibition and null effects are seen on the ACC population. Upon closer examination of single units in the post-psilocybin condition, a similar assortment of responses was found. Approximately 1/3 of cells in each psilocybin experiment were activated, whilst the remainder showed no significant change in firing rate (Figure 18 (a,b)). The level of activation was sustained for over 20 minutes in many cases (Figure 18 (a)). The range of responses is expected given the heterogeneous expression of 5-HT_{2A} and 5-HT_{1A} receptors in neurons of the PFC (7).

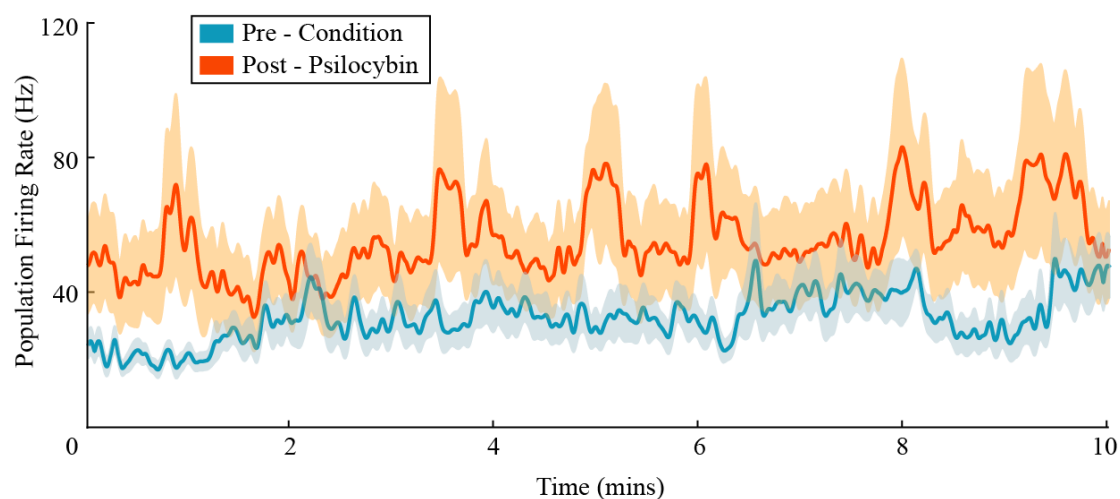


Figure 17: Psilocybin induces an increase in the mean firing rate of the ACC sample population (bin size = 200ms, moving average Gaussian window, span = 8s). Note: The population firing rate was calculated as the sum of all spikes during each time bin.

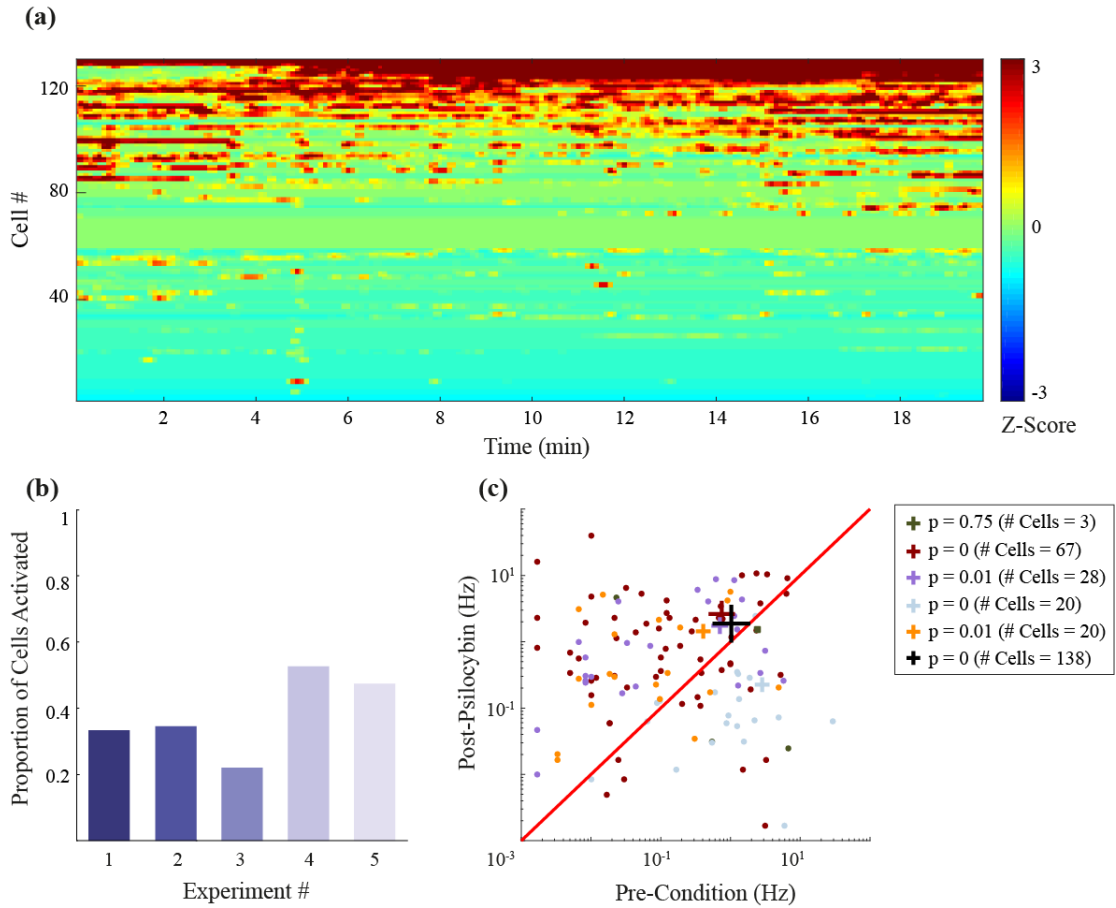


Figure 18: Single cell activation. (a) The z-score of the firing rate of each cell in the post-psilocybin condition was computed by computing the baseline firing rate during the pre-condition (nCells = 138). The cells are sorted according to peak z-score. (b) The proportion of cells activated (3+ consecutive bins with z-score $\geq [3, -3]$) for each experiment (nExp = 5). (c) The pre-post firing rate of all cells on a log-log scale to visualise neurons with low firing rates. Overall neurons showed significant activation (Wilcoxon sign rank, $p = 0.0003$). The data is colour-coded to show individual experiments. The cross for each experiment denotes the centroid of the data points for the cells of that experiment. The centroid for all cells is denoted by the black cross. Note: The size of the cross is correlated with the number of cells from which it was calculated.

The influence of psilocybin on phase modulation between single unit activity and neural frequency bands

The coherence of local field potential oscillations between brain areas is thought to facilitate a functional interaction between remote areas of cortex (186,187). When neurons of a particular region of cortex phase lock to a particular frequency band, it is postulated that this may harness their ability to

communicate with neural populations in other cortical areas (18). There is evidence for a role in cognition, as it has been shown that phase locking in the PFC to theta oscillations is enhanced during spatial working memory tasks (18). Furthermore, it has been postulated that this phase locking represents information transfer to the hippocampus, facilitating long term memory in the process (188). In the context of the findings presented here, phase locking within the ACC provides an interesting bridge between the results found on an LFP and single unit population level. The current findings are intriguing; psilocybin causes an increase in excitation in the network, whilst simultaneously decreasing the power of low frequency oscillations. In an effort to find a link between the single unit activity and the local field potential, the effect of psilocybin on the level of synchrony in the network was examined. Note, that synchrony is investigated here in terms of the level of phase modulation of cells, and the network overall, with neural frequency bands. The degree of phase modulation was calculated for each cell (quantified by the circular correlation coefficient, κ), and the median value for the network was computed to estimate the network synchrony with each LFP band. This was performed for the pre- and post-conditions. The histograms in the left panels of Figure 21 (a-e) show the distribution of κ values for the cell population to each frequency band (pooled experiments). A spectrum of phase modulation levels are seen in both the pre- and post- conditions for the population, ranging from highly phase modulated cells ($\kappa \geq 1$) to highly un-modulated cells ($\kappa = 0$). One can already see the shift towards a greater number of cells showing un-modulated spiking patterns for each band (Figure 21 (a-d)), except gamma (Figure 21 (e)). This shift between pre- and post-psilocybin conditions can be seen for all neurons in each band in Figure 19, and was quantified on a network level by measuring the change in the median population κ value for each neural frequency band (Figure 20). The network overall showed a large decrease in synchrony with LFP. In particular, significant decreases were found in the delta ($p = 0.03$), theta ($p = 0.03$), alpha ($p = 0.05$) and beta ($p = 0.03$) frequency bands, whilst the gamma band was not significantly affected ($p = 0.2$, student t-test). This bears resemblance to a transition to a desynchronised state alluded to previously with the decrease in power of low frequency bands. This desynchronised state is characteristic of awake attentiveness and REM sleep (189).

Taking a closer look at how the phase modulation level of individual cells transitioned from the pre- to post- psilocybin condition, the cell population was divided into phase un-modulated ($\kappa < 0.2$), and phase modulated ($\kappa > 0.2$) cells. Note, this threshold is heuristic based on what has been classed in

previous studies as phase-modulation (18,174). However, it is notably more conservative than the threshold of ~ 0.08 used in Lagler et al., (190). The proportion of each sub-population in the pre- and post- psilocybin conditions can be seen in the pie-charts of Figure 21 (a-e) for each neural frequency band. Of note, each neural frequency band showed a transition to a greater proportion of un-modulated cells for each LFP band. The bar chart represents the median κ values for the modulated and un-modulated sub-populations in the pre-condition (blue outline), and the median κ values for the same cells in the post-psilocybin condition (orange outline). The phase un-modulated cells in the pre-condition, largely remained un-modulated, as shown by the moderate increase in the value of κ in all cases. However, phase modulated cells in the pre-condition showed a large decrease in their mean κ value in the post-psilocybin condition. Taken together, this would lead to the postulation that the transition to a less synchronised state in the post-psilocybin condition is triggered by phase-modulated cells becoming less modulated, whilst phase un-modulated cells remaining largely unchanged. Of note, a larger proportion of cells were un-modulated to the gamma band in the pre-condition, which may contribute to the more moderate (non-significant) change in phase modulation of the population in the post-psilocybin condition. An example of the stability of the phase modulation level of both phase modulated and phase un-modulated cells in the gamma band can be seen in Figure 22 (b) and (c) respectively. This contrasts with the transition of phase modulated cells to a phase un-modulated state in the theta band, as shown in Figure 23 (b,c).

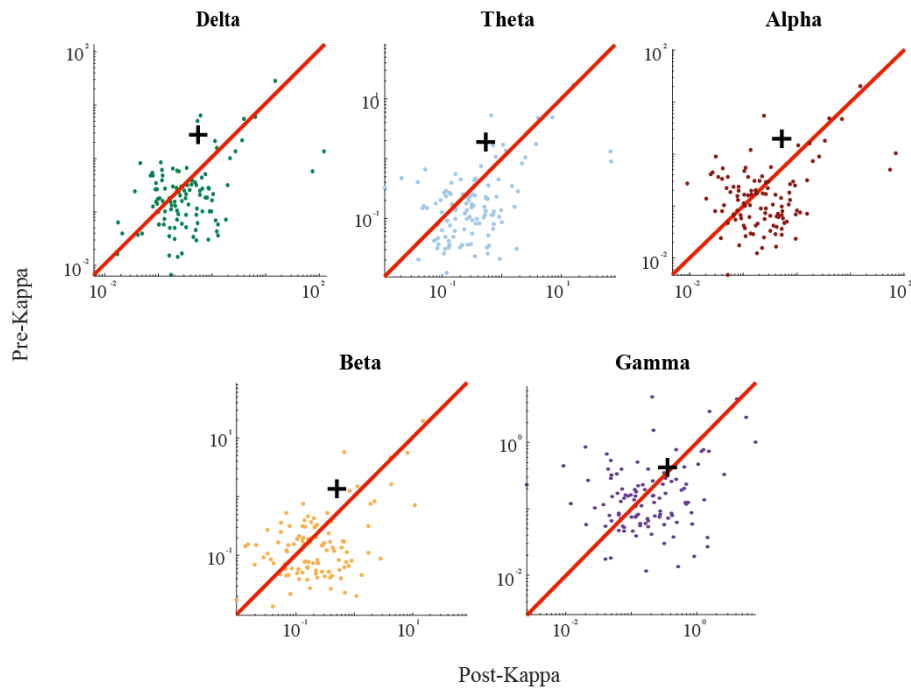


Figure 19: A scatterplot of the value of kappa for each cell pre-post psilocybin. The cross in each plot indicates the centre of mass for all data-points.

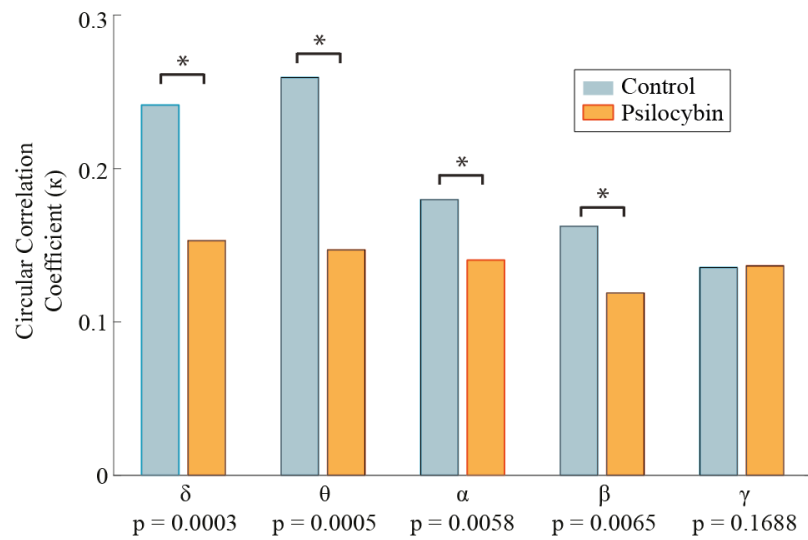


Figure 20: Psilocybin desynchronises the cell population with delta, theta, alpha and beta frequency bands. No significant change was found for the median circular correlation coefficient of the cell population and the phase of the gamma band (student t-test).

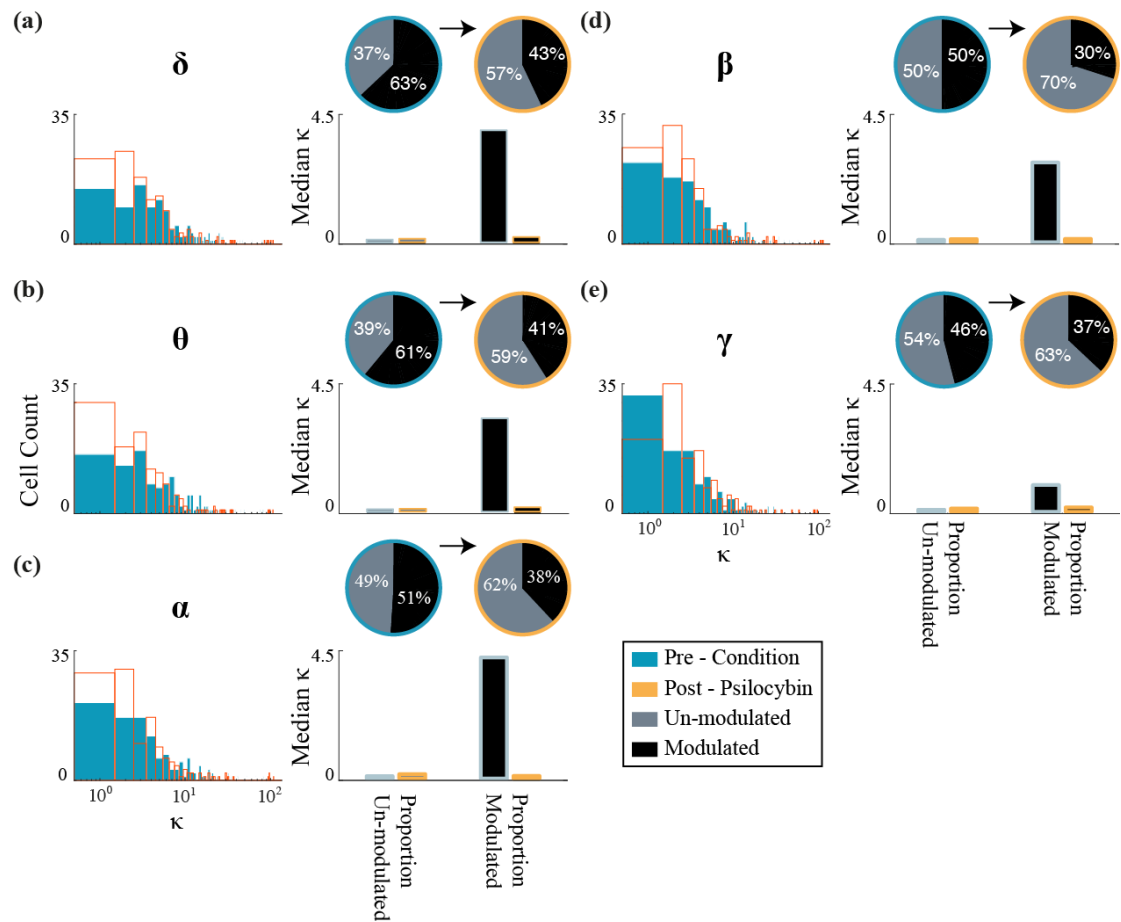


Figure 21: Modulation level of cell population to each neural frequency band. (a) Delta band; a frequency histogram of the number of cells for each κ value in the pre-condition (blue) and the post condition (orange). For all neural bands, most cells are either strongly modulated or un-modulated to the LFP. The bar plot in the adjacent panel shows the median κ value for cells classed as modulated ($\kappa > 0.2$) and un-modulated. The post condition, indicated by the bar with the orange outline, shows the median κ value for the same cells that were classed as modulated or un-modulated in the pre-condition. The pie charts show the proportion of modulated versus un-modulated cells in the pre- and post- conditions. (b) as in (a) for the theta, (c) alpha, (d) beta, and (e) gamma bands.

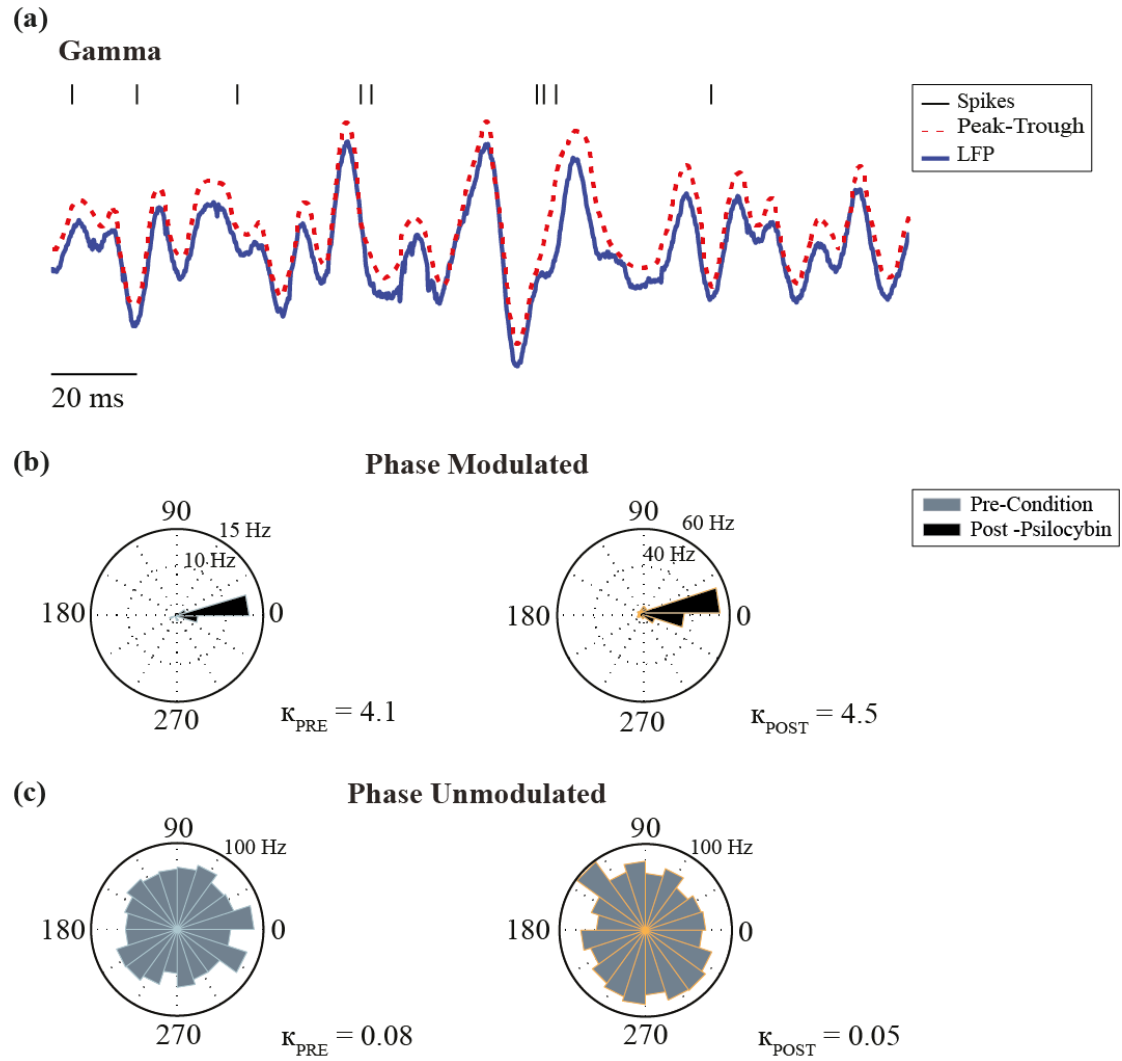


Figure 22: Phase modulation with the gamma band remains unchanged with psilocybin administration. (a) A sample trace of band-pass filtered gamma band activity with the phase envelope computed from linear interpolation of local peaks and troughs, and the timing of spikes from a single cell. **(b)** A phase-modulated cell remains modulated with a moderate increase in the circular correlation coefficient. **(c)** A phase unmodulated cell remains un-modulated.

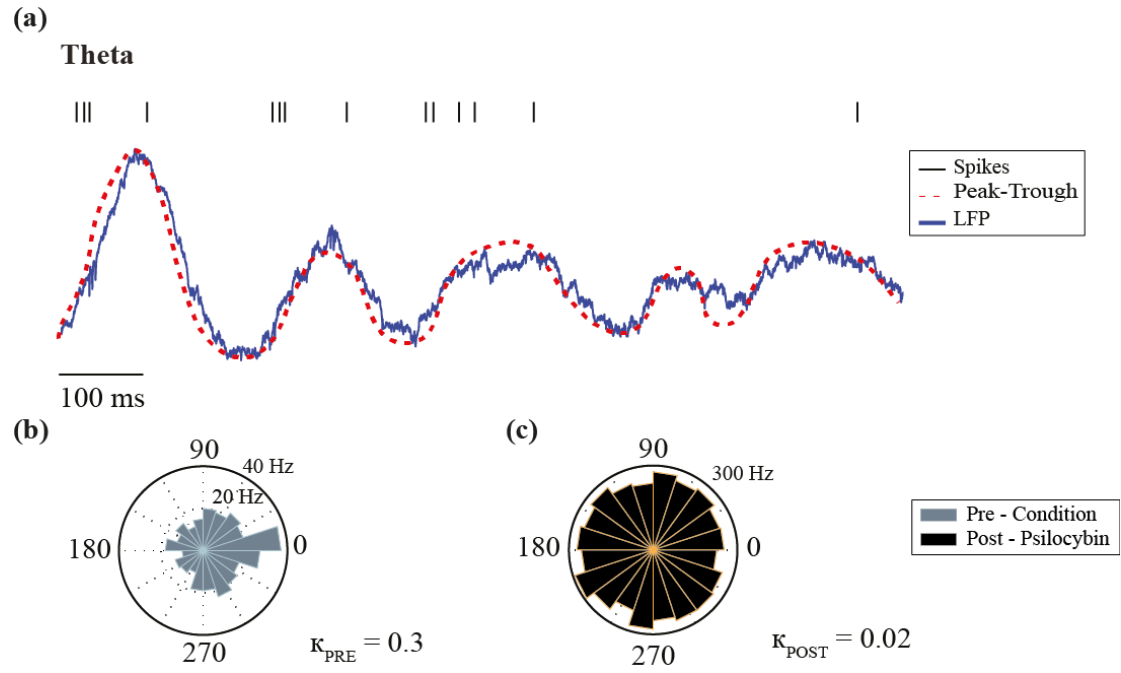


Figure 23: Phase modulation with the theta band decreases with psilocybin administration. (a) A sample trace of band-pass filtered theta band activity, with the phase envelope computed from linear interpolation of local peaks and troughs, and the timing of spikes from a single cell. (b,c) A phase modulated cell transitions to an un-modulated state following psilocybin.

Discussion

The examination of the effects of psilocybin on neural networks of the ACC provides a gateway into further understanding how the brain transitions through different states of consciousness. The primary facilitator in the case of hallucinogenic compounds is the serotonergic system, as it functions as the neurological substrate of the psychological disturbances produced by such substances. Furthermore, by studying psilocybin, a compound which has a characteristic set of affinities for a subset of serotonergic receptors, one can determine with more accuracy the effects of activation of specific serotonergic receptor subtypes. The work presented here investigates the impact of the systemic administration of psilocybin on the ACC of awake head-fixed mice, using multi-unit extracellular recordings. Psilocybin appears to induce a transition of the ACC neural network to a desynchronised state, characterised by a decrease in low frequency oscillation power, with a simultaneous increase in population firing rate, and a reduction in the level of phase-modulation with delta, theta, alpha and beta frequency bands in the network.

Psilocybin reduces power of low frequency oscillations in the anterior cingulate cortex

The local field potential has a strong role in providing functional connectivity between cortical areas (186,187). The PFC often integrates information of many different modalities to perform complex cognitive tasks such as working memory. Specifically, it has been shown that the PFC receives spatial information from the hippocampus via coherence in theta oscillations during a spatial working memory task (18). The activation of serotonergic receptors in the PFC has been shown to alter oscillatory activity. However, current knowledge relies upon data in anaesthetised states, which alters the LFP significantly from that seen in wakefulness. That said, it has been shown that blocking 5-HT_{2A} receptors decreases power in gamma oscillations, and desynchronises fast-spiking interneurons expressing 5-HT_{2A} receptors with gamma oscillations. Moreover, by blocking 5-HT_{1A} receptors, gamma oscillatory power increases, concomitantly with an increased firing rate of fast-spiking interneurons that express 5-HT_{1A} receptors. The increased activity of the interneurons increases the level of synchronisation with gamma oscillations (48). Taken together, activation of serotonergic receptors in the PFC appears to alter the excitation/inhibition balance in the circuit, and the increase or decrease in oscillatory power is dependent on the degree to which 5-HT_{1A} and 5-HT_{2A} receptors are activated. Our knowledge of the effect of psilocybin on LFP is reliant on inference from fMRI studies, and from intracerebral studies of LFP in rats in response to a similar hallucinogenic compound, DOI. However, the fMRI results are conflicting at present. Studies in humans and rats have shown a decrease in blood flow to the ACC following psilocybin administration (68,69). However, earlier PET studies found an increase in cortical metabolism in the ACC (31). Although, it has been hypothesised that the conflicting results may be due to differing time-courses, with fMRI readings operating on a significantly shorter timescale than PET recordings (32). However, even the fMRI findings in rats somewhat diverge from that found in humans, with human data showing a uniform decrease in BOLD activity following psilocybin administration (32), whereas BOLD signals in rats increased and decreased depending on cortical area. The authors postulated that this divergence may be due to interspecies differences and/or the larger effective dose used in rats (2mg/kg) compared to humans (2mg/subject) (68). It must also be noted that the relationship between BOLD signals and LFP is not linear, and indeed has not been definitively established. Whilst, a BOLD signal has been shown to correlate with gamma oscillations (191-193), it can also correlate positively with low frequency

oscillations (193), which are typically anti-correlated with gamma oscillations (189). The findings presented here show a significant decrease in low frequency LFP power (including beta band activity) in response to psilocybin (Figure 16 (b)). This is in agreement with the fMRI findings stated above, and the results of DOI investigations in the ACC (83). There was a moderate, but non-significant increase in power in the gamma oscillatory band. Following from the aforementioned results in blocking 5-HT_{2A} receptors (48), combined with the knowledge that psilocybin has the strongest affinity for 5-HT_{2A} receptors above all serotonergic receptors, it would appear that psilocybin preferentially activated neurons with 5-HT_{2A} receptors. However, examination of the effects of psilocybin on single unit activity and phase modulation with neural frequency bands revealed a more elaborate potential mechanism.

Control experiments

Before moving on to the results of the single unit activity, it is worth noting the conditions of the control experiments to ascertain the validity of the effects of psilocybin presented here. The spontaneous cortical state of the ACC in an awake animal, as with all areas of the cortex, fluctuates frequently (189). Hence in order to determine a reliable baseline from which the psilocybin effects can be compared to, (i) the parameter (e.g. power in theta band oscillations), must be averaged over a sufficiently long period to capture a reasonable sample of spontaneous fluctuations, (ii) the activity must be compared across animals for repeatability estimates, and (iii) control experiments must be performed that mimic the administration of the compound of interest (psilocybin), but be inert in the effect that it has on cortical activity. Criterion (i) was accounted for by taking a 10 minute window of analysis to compute the pre-, post- condition findings. Criterion (ii) was fulfilled by computing the average results across mice ($n = 5$, for both psilocybin and vehicle experiments). Finally, criterion (iii) was validated by conducting control experiments whereby everything mimicked the psilocybin experiments described in section Psilocybin intra-peritoneal injection, except the 200 μ L of psilocybin was replaced with 200 μ L of sterile PBS. Confirmation that the psilocybin alone was causing the changes reported in the ACC was seen in the results for the vehicle experiments shown in Figure 16 (a), where no significant change in power of any neuronal frequency band was found. This provides evidence that the results reported here are due to the effects of psilocybin, and are not simply a consequence of rudimentary fluctuations.

Psilocybin increases the activation level of cell populations in the anterior cingulate cortex

The effect of endogenous serotonin on the PFC can be seen in experiments examining the effects of activating the raphe nuclei (22,23). As per the aforementioned effects resulting from the blockage of 5-HT_{1A} and 5-HT_{2A} receptors, stimulation of the raphe nuclei caused a medley of effects on the ACC, with 66% of pyramidal cells being inhibited, 13% being excited, and 20% showing a bi-phasic response. This may be dependent on the relative activation of 5-HT_{2A} and 5-HT_{1A} receptors, as it has been shown that these receptors induce depolarisation and hyperpolarisation respectively (41-43), and that a portion of PFC neurons show co-expression of the two receptors (45), which may account for the bi-phasic response. The results presented here echo this disruption in the balance of excitation and inhibition in the network. Approximately 1/3 of cells in each experiment were significantly activated in response to psilocybin administration (Figure 18 (b)). Psilocybin has the strongest affinity for 5-HT_{2A} receptors, which are known to have an excitatory effect, whilst also activating 5-HT_{1A} receptors, which have an inhibitory effect on host neurons. Taken together, it may be possible that inhibition in the network caused by the activation of 5-HT_{1A} receptors may have induced a release from inhibition of the pyramidal cells, which were then further boosted by the depolarising effects of the activation of 5-HT_{2A} receptors.

The locomotion of the mice was examined to ascertain whether the increased in population activity shown here was correlated with a change in speed.

Figure 24 shows the average speed of the mice in the pre- and post-psilocybin period. The mice displayed little movement in either epoch, hence showing no change between the pre- and post-conditions (Wilcoxon signed-rank pre/post psilocybin, $p = 0.44$). The lack of motion was likely attributed to the lack of training on the treadmill prior to the experiment. Whilst the mice were habituated to the environment to alleviate any stress response, they had not undergone training to become accustomed to running on the head-fixed setup. A comparison on the mean speed profile of mice from the working memory task (pre-tone presentation), is given as a reference in Figure 24 to show the speed relative to a trained mouse.

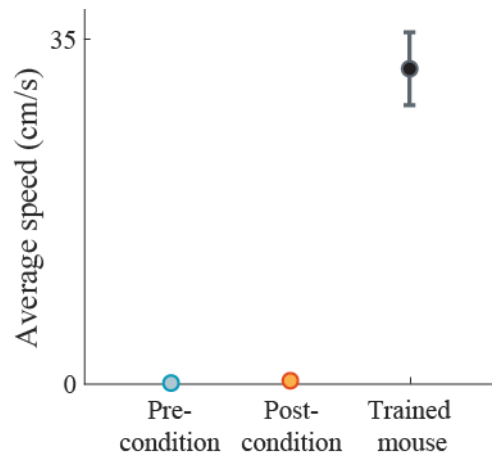


Figure 24: No change in locomotion following psilocybin administration. The average speed of mice in the pre-condition was compared to the post-psilocybin condition (Wilcoxon signed rank, $p = 0.44$). The average speed of mice trained on the treadmills is shown for reference. The error bars denote standard deviation.

Decrease in network synchrony in response to psilocybin administration

An increase in cell population firing rates with a simultaneous decrease in power of low frequency oscillations may appear somewhat counter-intuitive. However, closer inspection of the synchrony of the network via phase modulation with neural frequency bands yields some interesting results. Whilst the activity of the network increases, the degree of phase modulation in the population with each neural frequency band decreases significantly, with the exception of gamma oscillations (Figure 20). Whilst previously un-modulated cells remain unchanged in their phase modulation strength, the average phase modulation strength of modulated cells decreases strongly, often to a phase un-modulated state (as per the example Figure 23 (b-c)). This reduction in synchrony may contribute to the reduced power seen in low frequency bands following psilocybin administration.

Furthermore, the lack of increased synchrony in gamma oscillations may provide more evidence for the argument of the release of inhibition of pyramidal cells via suppressed interneuron activity. As previously discussed, serotonergic receptor activation that causes an increase in firing rate of fast-spiking interneurons, induces a concurrent increase in the power of gamma oscillations, and a sharper synchrony with the phase of gamma oscillations (48). However, interneurons also express 5-HT_{1A} receptors, which have a hyperpolarising effect. Given that gamma oscillation power did not increase significantly, nor did the level of phase modulation of the cell population with gamma oscillations

increase, it would appear that interneurons within the ACC were in fact being inhibited as a result of psilocybin administration. This would lead to the release from inhibition shown in the increased overall activation level of the population (Figure 17 and Figure 18).

Intra-cortical injection of psilocybin experiments

The neurological changes induced by psilocybin presented here may be due to local effects within the ACC, or upstream effects on other cortical and/or subcortical areas. In an effort to disentangle these two mechanisms, the effect of psilocybin on network activity of the ACC was investigated on two levels: (i) systemic administration, (ii) local administration. The systemic administration via i. p. injection has a number of advantages:

1. It closely mimics the entry mechanism of psilocybin when ingested by humans.
2. It provides a more realistic effect of the compound in the ACC as it will spread in an unbiased manner to other neural tissue in addition to the ACC. Given the interconnected nature of the cortex, the effects of psilocybin on other brain structures is highly likely to effect network activity in the ACC.

However, the disadvantage lies in determining specifically how the ACC is influenced by psilocybin, as multiple influencing factors are occurring simultaneously (see advantage (2) above). Hence, to more finely examine the effects of this compound, localised i. c. injections of psilocybin were performed. However, it must be acknowledged that psilocybin would rarely encounter a cortical region in isolation. The experiments were performed as described in section Intra-cortical injection of a serotonergic agonist, however an important caveat was discovered. The margin for error in this experiment is extremely small, as it is very difficult to locate the probe in such close proximity to the cannula opening, blindly, *in vivo*. The proximity of the probe to the cannula was verified by injecting CNQX (AMPA/kainate receptor antagonist) at the end of the experiment. An illustration of this can be seen in the population activity plots shown in Figure 25 (a). The effect of CNQX can be seen clearly at the end of the experiment, verifying that the probe was recording from neurons that were being affected by the compound that was injected through the cannula. However, the margin for error was even smaller than originally anticipated due to the caveat that occurred when the probe was too close to the cannula opening. When this occurred, the volume of liquid entering the cortex (1 μ L), caused a brief

inhibition in the circuit, which was independent of what compound was injected, as it occurred for both psilocybin and vehicle experiments. This effect can be seen in Figure 25 (b), with the subsequent CNQX showing that the probe was indeed next to the cannula. Hence, whilst sufficient vehicle ($n = 5$), and psilocybin ($n = 5$) experiments were collected with successful CNQX verification, numerous experiments were discarded for the aforementioned volume artifact. Hence, the dataset only contained $n = 2$ vehicle experiments, and $n = 2$ psilocybin experiments where this caveat did not occur. Thus, analysis could not be performed until further data is collected.

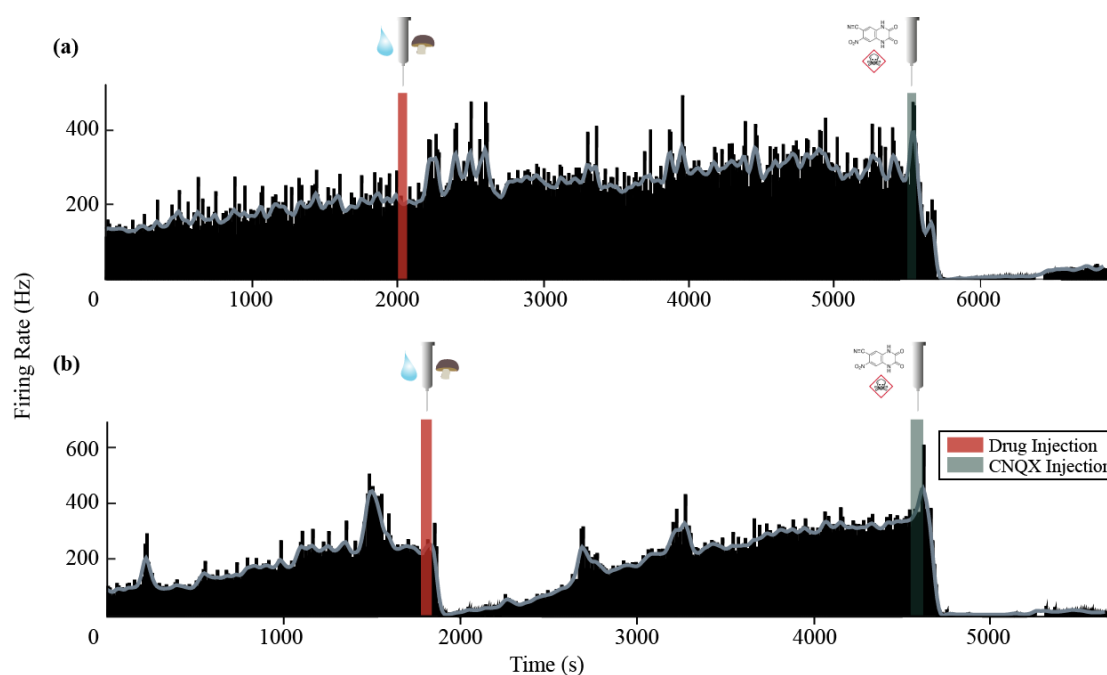


Figure 25: Verification of location of IC psilocybin recording using CNQX. (a) The successful recording of neuronal data can be seen by the sharp reduction in neuronal activity of the population post CNQX injection. (b) The method however, has the confound that if the injection site is too close to the recorded neuronal population, the volume effects of the injection can disrupt the network, independent of what compound is injected.

Conclusion

The effects of psilocybin on a single neuron level would suggest that the administration of psilocybin disrupted the excitation/inhibition balance in the ACC via a release from inhibition of pyramidal neurons. This increased the overall activity level of the cell population and de-synchronised single unit

activity with LFP oscillations. This may reflect the decrease in functional connectivity between brain areas observed in fMRI studies of psilocybin administration in humans (32). It is worth noting that these results are in agreement with that of DOI studies that found that DOI decreased phase modulation of neurons with gamma oscillations (83,84). However, one must be cognisant to the fact that whilst DOI is a structural analog of psilocybin, it is a different compound, and in the same manner in which other structural analogs that do not produce hallucinogenic effects, activate a different biochemical pathway via serotonergic receptors, it is likely that DOI and psilocybin differ in the mechanism in which they act on the cortex. Furthermore, the incorporation of the effects on the LFP would suggest that psilocybin induces a transition to a desynchronised cortical state in the ACC. A desynchronised state is characterised by a decrease in low frequency power and an increase in gamma oscillatory power (189). The systemic administration of psilocybin caused a similar decrease in power of low frequency oscillations with a moderate increase in gamma oscillatory power. It is worth noting that stimulation of the serotonergic system via the raphe nuclei induces a simultaneous decrease in low frequency and gamma oscillations (48), which differs from that which is seen in psilocybin. These findings, in combination with the behavioural effects of psilocybin (flat body posture), would indicate that of the two states associated with desynchronised cortical activity; attentiveness and REM sleep (194), it would appear that psilocybin is inducing a state similar to that of REM sleep (Figure 26).

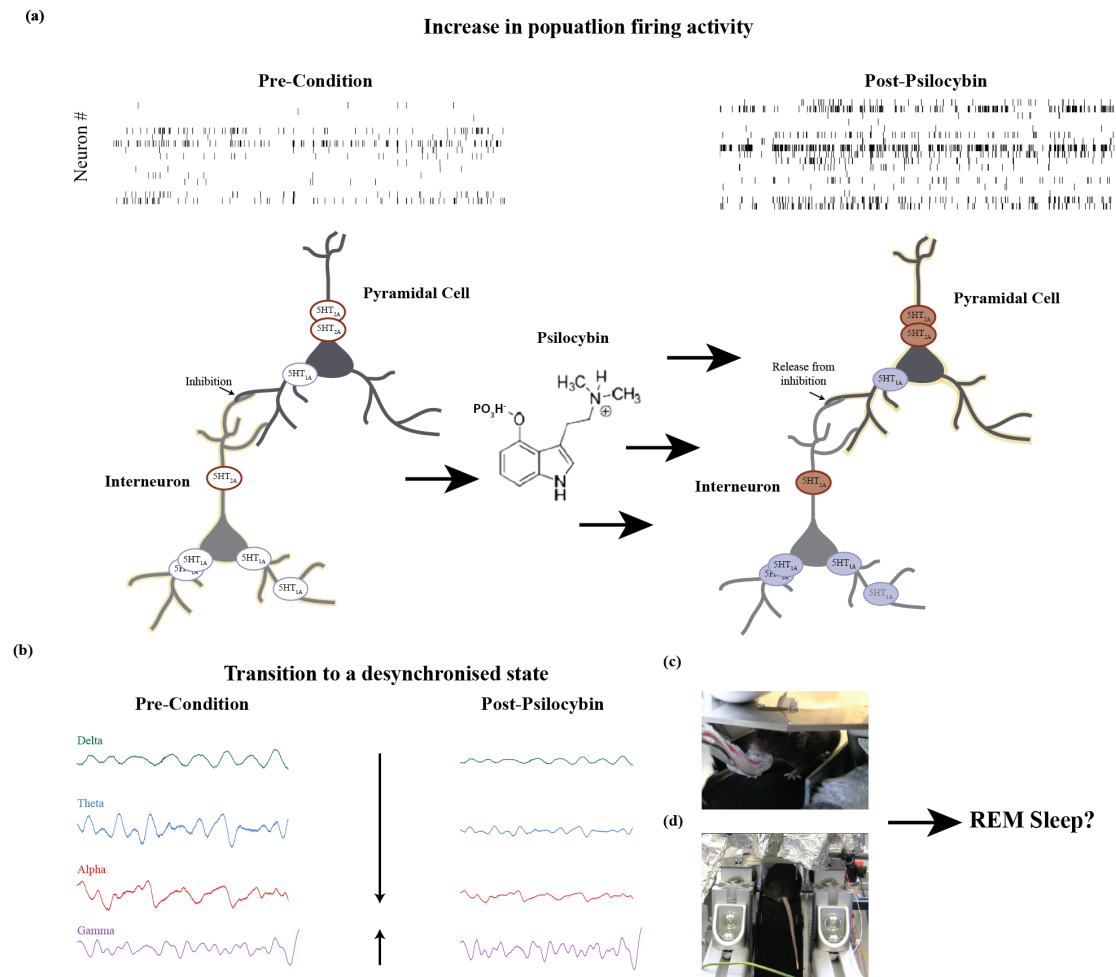


Figure 26: A schematic of the potential mechanism by which psilocybin effects the neural circuit of the ACC. (a) Neurons of the ACC show a heterogeneous distribution of 5-HT_{2A} and 5-HT_{1A} receptors on both pyramidal cells and interneurons. Following the administration of psilocybin, a strong excitatory effect may be produced in the network as 5-HT_{2A} receptors of pyramidal cells are activated causing increased depolarisation, concomitantly with the activation of 5-HT_{1A} receptors of interneurons, causing increased hyperpolarisation. This hyperpolarisation of interneurons provides a release from inhibition for pyramidal cells, and so further contributes to increased excitation in the network. (b) The activation of the serotonergic system induced by psilocybin decreases the power of low frequency oscillations, and produces a moderate increase in gamma oscillations. In combination this leads to a transition of the network to a desynchronised state that may be representative of REM sleep or awake attentiveness. (c) Furthermore, the behavioural response of the mouse from alert locomotion during the pre-injection epoch to a (d) flat posture, low locomotive state following psilocybin injection may indicate that the cortical network has transitioned into a state that is similar to REM sleep.

Chapter 4: Working memory within the mouse anterior cingulate cortex

Introduction

Working memory has been the target of scientific research for over four decades. These investigations have unveiled many clues into the mechanism behind this complex phenomenon, however many nuances continue to be unclear. Some of the most interesting challenges highlighted here are determining:

- The hierarchical role of the PFC in working memory
- Single unit versus population encoding mechanisms
- Presence and ratio of encoding of high and low level information
- The mechanism of mnemonic encoding in the delay period; persistent activity or transient activation of a population.

The overarching conundrum lies in the role of the PFC in working memory. Contrary to primary sensory areas of cortex, the PFC appears to contain a sparse representation of diverse information that can rapidly switch encoding patterns when required. Indeed, there has been much evidence for stimulus representation, decision information, expectation information, and rule information (18,21,94,101). However the question remains; is the PFC the first point of integration of these disparate information representations, or is it a downstream puppet of another part of the cortex? Is it orchestrating the decision making or simply representing a result that is computed elsewhere? The answer to this is unknown, and will not be addressed in work presented here, as it would rely on an exhaustive knowledge of neural syntax. However, this is an important point to be cognisant of whilst observing the information encoded in the PFC, to avoid the assumption of confounding an integrative role with a representative role.

Single neurons of the PFC can exhibit strong response profiles to discrete elements of working memory tasks, such as cue responsive cells and delay period responsive cells (94). Upon closer inspection of these responses, many appear to be fluid in their representations, with neurons shifting the information they represent depending on high-level changes, such as the relevant information to the task, as

opposed to the shape or colour of the stimulus itself (197). Interestingly, a study has shown evidence that parsing information encoded in a working memory task, such as distinguishing the upcoming choice from the stimulus inputs, is significantly clearer on a population level than when examined on a single unit level. Decoding the upcoming choice produced an unclear result when determined from the response profiles of single units, however, when the response of the population was considered, the information became clearer (103). Of note, the distinction that is made in the work presented here is as follows: The information from a collection of cells is a summary of their response profiles, whereas examining the population as a whole, contains this information in addition to the interactions between cells within the population. Together, this leads to the postulation that the fidelity of PFC encoding of WM information is much stronger when examined on a population perspective. However, this may be dependent on the type/function of the information to be presented. Perhaps low level information e.g. the frequency of an auditory cue, may have a strong representation on a single neuron level, but high-level information such as upcoming choice, may have stronger fidelity in the population. This is discussed further in the discussion regarding the mnemonic mechanism behind working memory. Furthermore, the mechanism by which the memory of the stimulus is encoded in the delay period remains ambiguous, as there is much evidence to show memory retention during the delay period via persistent activity (94), and other evidence to show the transient activation of a population of cells to encode the memory information (123).

The aim of the work presented here is to examine the response properties of cell populations of the ACC, using extracellular multi-unit recordings in awake, head-fixed mice, performing a WM task. This work provides an opportunity to detect both types of encoding mechanisms (persistent activity and transient activation) during the delay period of the working memory task, and furthermore, determine if there is a temporal structure to such encoding. This will be facilitated by the variable delay period inherent in the task. Moreover, the extracellular recordings will facilitate investigation on both a single neuron and population level. The delayed response task on the treadmill rig will also allow examination of the ability of PFC neurons to change the information they encode on a second timescale. The optogenetic perturbation experiments have dual functionality: (i) they provide a proof of principle of how this technique may be used to determine mechanisms underlying neural circuit phenomena, and (ii) they provide neuron identification confirmation via opto-tagging of pv-interneurons.

The experimental results presented here are from a series of extracellular electrophysiology datasets collected in mice performing a delayed-response working memory task. The data can be split into two groups:

- i. Extracellular multi-unit recordings, using a 4x2 tetrode probe, in the ACC, of performing C57BL6 and Pvalb-Cre mice performing a DR task (Figure 27 (a)).
- ii. In an experiment similar to (i), optogenetic perturbation of parvalbumin interneurons was introduced in the ACC using ChR2. The perturbation was administered by means of a 100ms light pulse via an optrode, 400ms after tone onset in the DR task. Mice were given an initial 60 trial window with no perturbation, followed by stimulation trials interspersed with catch trials for the remainder of the session.

Delayed-response working memory task

The working memory paradigm employed in this study was a delayed response task. The mice received one of two pure tones (cue), followed by a delay period during which they had to remember which tone was presented. Upon reaching the reward zone, they responded according to which tone was presented. Of the many working memory tasks employed in scientific studies (see section Working memory behavioural paradigms), all exhibit confounds in their ability to engage working memory capacity. With this caveat in mind, the DR task performed here was specifically chosen to maximise the following criteria:

- 1) Induce sufficient cognitive load to reduce the propensity for automation of sub-elements of the task.
- 2) The task must not be able to be performed using a learned motor action, which can be the case in alternation tasks (see Delayed alternation task).
- 3) The outcome and/or cues of the previous trials should have no correlation with the current trial, to prevent the use of reference memory instead of WM (195).
- 4) Mice should be resistant to over-training – error trials are vital to discern WM activity from noise.
- 5) Mice should perform a sufficient number of trials for each trial type (T1/T2 trials) for statistical analysis.

- 6) The task should facilitate a high trial count before animal satiation.

It should be noted from Figure 27 (b) that mice performing this task showed a stereotyped speed profile depending on trial type. T1 and T2 trials can be differentiated based on locomotion data from early in the delay period, namely the increase in speed that occurs in T2 trials. This is interesting on two accounts; firstly, T2 trials, in which the correct response of the mouse requires an absence of action i.e. the mouse has to not stop in the reward zone, elicits a change in locomotion compared to that of the pre-tone period. Hence, the mice are performing a deliberate action during T2 trials. Furthermore, this phenotype of speed highlights the necessity to delineate locomotion influences on neuronal response profiles recorded in the ACC. Looking further into the speed profiles of sub-trial types [Hit, Miss, FA, CR], it is interesting to note that the speed profile for Hit and FA trials display a strong similarity (middle panel of Figure 27 (b)), as do CR and Miss trials (right-hand panel of Figure 27 (b)). This is an early indication that incorrect trials i.e. FA and Miss trials, may be due to the mouse mistaking the type of tone that was presented. For example, on a FA trial where tone T2 was presented, the mouse displays the same speed profile as when T1 was presented. This will be investigated further to see if there is evidence for this in the neural data.

The advantages of the task/setup presented here are:

- 1) The pseudorandom presentation of the two tones highly reduces the possibility of the task being completed by motor action alone; criteria (1), (2) & (3).
- 2) The spatial, auditory and visual cues following tone offset to the reward zone are identical in all trial types, hence, there are no indications of trial type in the external environment after the tone has been presented; criteria (1).
- 3) The delay period duration can be tailored on a mouse specific basis, thus the working memory load can be increased until the mouse performance reaches a desired level of correct-error trials; criteria (1) & (4).
- 4) The hardware of the setup facilitates head-fixed electrophysiology recordings during behaviour. The fluid nature of the locomotion encourages a high trial count, and the water delivery system in combination with an appropriate training protocol prevents animal satiation pre session cessation; criteria (5) & (6).

Mouse training & performance

The DR response task described in section Delayed response task, is the culmination of numerous iterations of the strategy taken to implement the task. The treadmill setup is a novel experimental rig with numerous benefits for implementing WM paradigms, for example, it provides a stable platform for maintaining neuronal signals in a head-fixed setup whilst the mice are performing a very active task, and versatility in controlling task parameters such as delay period length. However, with novelty comes the unknown. There were a number of uncertain factors that were refined through iterations of the task (Note: this is not an exhaustive list):

1. Would the complexity level provide too much of a cognitive load for the mice to perform the final expression of the task?
2. What would the duration of training be?
3. Would the mice perform a sufficient number of trials to facilitate viable analysis?
4. What are the factors that perturb mice during training?
5. How can the yield, i.e. the number of mice per batch that would successfully complete the full expression of the task, be maximised?
6. Given the highly volatile nature of mouse behaviour in complex tasks, how can the recording session be sufficiently incognito such that the mouse does not perceive anything different to a regular training session?

The protocol outlined in section Training Protocol builds on the knowledge obtained for all of the above factors to produce a smooth behavioural training protocol with an average yield of 70% of mice per batch trained. The training protocol was continuously adapted until the learning curve of mice followed a moderately linear trajectory in the penultimate stage of training. Note: The penultimate stage refers to when the mice are being presented with tone T1 or T2 at random but with a short delay period (no longer than 10cm). The mean learning curve for each batch is shown in Figure 28 (a). The cumulative learning curves for all mice ($n = 12$) is shown in Figure 28 (b), with the individual d' trace for each mouse shown in grey. The learning curve profile became more linear with less variability with each batch of mice that were trained Figure 28 (a). Note: For the purposes of mouse averaging the number of sessions was normalised to the start and end of the learning curve. However, the occluded

information is revealed in the boxplot in Figure 28 (c), which shows the number of sessions taken for each mouse to reach the end of the learning curve. Note: The learning curve is defined as the point from which the penultimate stage of training began, to when the d' of the mouse surpassed threshold. The dispersion of learning curve duration is shown cumulatively for all mice and for individual batches to further elucidate the progression in mouse training with each batch. The mean learning curve duration drops significantly from Batch 1 (mean duration = 14 sessions), to Batch 2 (mean duration = 10 sessions), from which it plateaus (Figure 28 (c)).

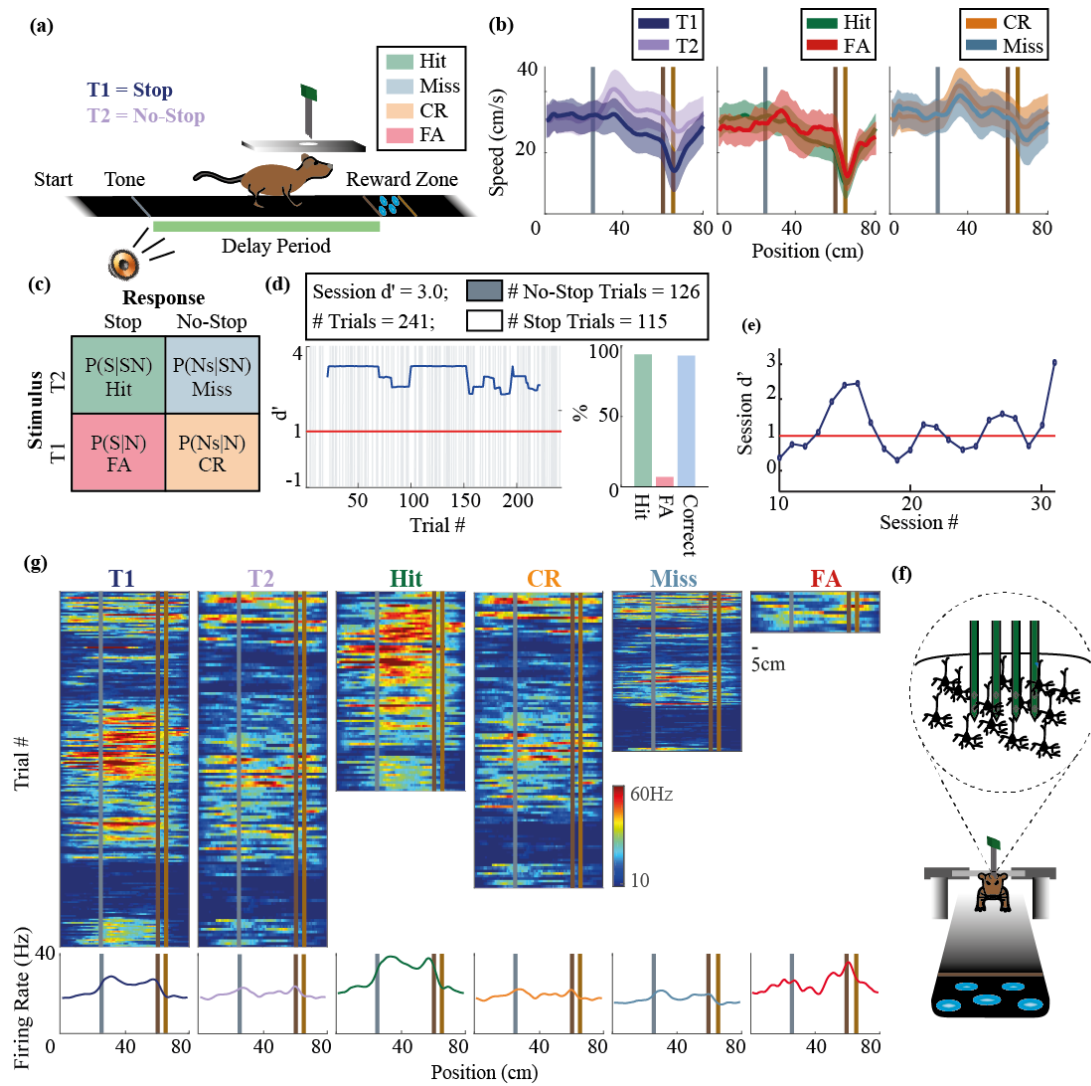


Figure 27: Recordings of neuronal activity in the ACC during a delayed-response task. (a) Mice were head-fixed to a treadmill during recording. Upon reaching the start position, a tone [T1, T2] was presented, following which the mouse had to run to the reward zone and respond to the tone (T1 – Stop, T2 – No-Stop). (b) Mice showed strong differences in speed profiles between T1 and T2 trials. However, the speed profiles of Hit and FA trials showed strong similarities, as was the case in CR and Miss trials. (c) The four potential trial outcomes [Hit, Miss, CR, FA] for the two stimuli [T1, T2], with the corresponding Hit, Miss, FA and

CR rates as defined in signal detection theory, by taking T1 trials as the signal (S), and T2 trials as the noise (N). (d) An example session showing rolling d' (computed over a 40 trial window) over the behavioural session. The performance threshold of $d' = 1$ is shown in red. The average d' across the session is given in the legend, along with the total number of trials, and the number of T1 and T2 trials presented. The bar chart shows the corresponding Hit and FA rates, and the % Correct for this session. (e) The average d' across sessions for an example mouse. Note: The fluctuations in performance level are explained in the increased working memory load explained in Figure 8. (f) Extracellular multi-unit activity was recorded using a 4x2 tetrode probe. (g) An example response of a single neuron to T1 and T2 trials, and subdivided into correct trials (Hit and CR), during the behavioural session shown in (d). The mean firing rate trace for each trial type is shown in the lower panels. Whilst the mean firing rate can differentiate T1 and T2 trials, a more pronounced difference can be seen when comparing Hit and CR trials. Note: For all diagrams, the grey vertical line indicates tone presentation, the dark brown line indicates reward zone entry, and the gold line indicates reward zone exit.

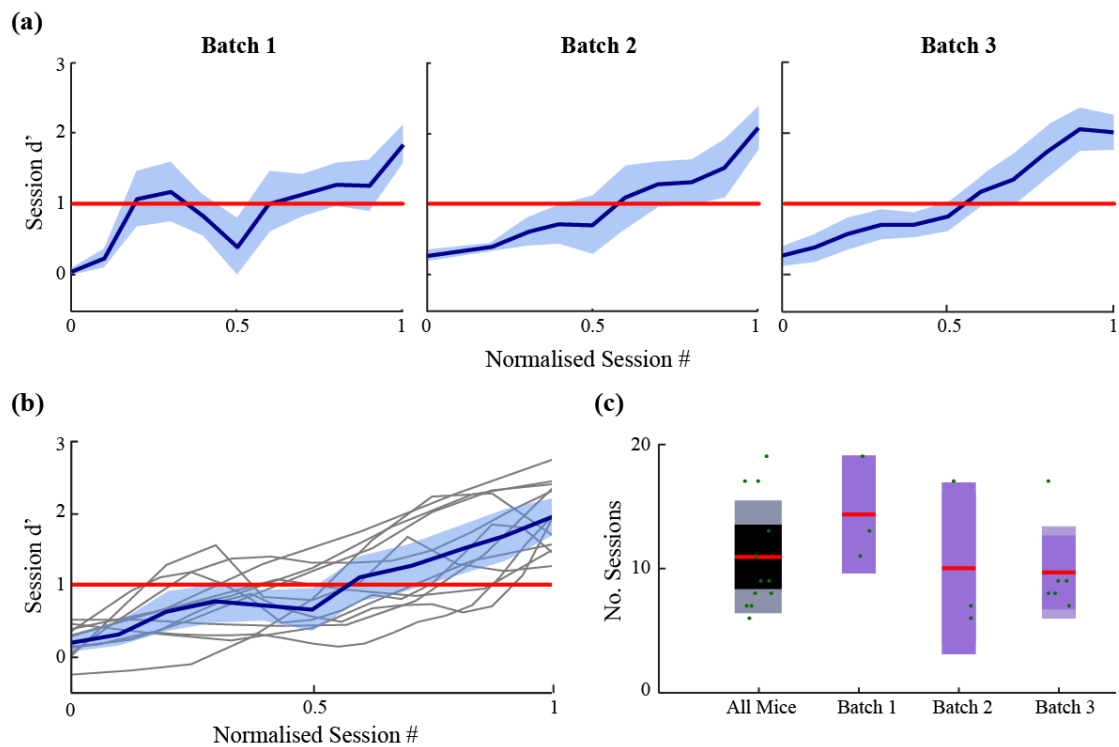


Figure 28: Learning curve profile and duration. (a) Mean learning curve and standard deviation for all mice in each batch represented by the average d' value for each session (normalised session number). The red line represents the d' threshold of 1. (b) Mean learning curve and standard deviation for all mice, individual learning curves shown in grey. (c) Distribution of learning curve duration for all mice (# Mice = 12), and for each batch (Batch 1; # Mice = 3, Batch 2; # Mice = 3, Batch 3; # Mice = 6). The red line shows the median, and the standard deviation is shown in grey/pale purple. The raw data is superimposed as green points on each boxplot.

Neural correlates of working memory in the anterior cingulate cortex

Single unit activity

Whilst a large volume of literature exists to predicate the involvement of PFC neurons in WM, it was necessary to check that the specific neuronal population that was recorded was key to the task at hand. For this, there are two fundamental requirements:

1. Neurons are responsive to elements of the task, e.g. cue, and delay period.
2. Neurons show a discernible response profile between T1 and T2 trials.

Statement (1) was verified via Z-score analysis of each recorded population. Figure 29 (a) shows the pooled data from all experiments (no. cells = 177). As reported previously, a sample population from the PFC will have a proportion of cells that are task-modulated, and a portion that are deemed task irrelevant (see section Neuronal responses in the prefrontal cortex during working memory tasks). Of the recorded populations in the ACC, $82.8 \pm 7.3\%$ ($n = 7$) were task modulated, similar to Lagler et al., (190). The conditions upon which neurons are classed as task-modulated are highly specific to individual laboratories, hence the distinction expressed here must be interpreted with the knowledge of how the segregation criteria were set. For the purposes of this analysis, a task modulated cell exhibited a significant change in firing rate, determined by a z-score, for at least one position bin (1cm), post tone onset. Note, firing rate was computed in spatial bins (bin size = 1cm), as trials were consistent across the spatial domain and inconsistent in the temporal domain due to the speed of the mouse dictating the length of the delay period. If no significant change was found, or the most significant change was found between the trial start position and cue presentation, i.e. the period in which everything is identical between T1 and T2 trials, then the neuron was classed as task-irrelevant. Though, as per the argument above, this was task-irrelevant as per this analysis, I would not seek to say that this is definitively the case. The z-score peak locations for each experiment can be seen in Figure 29 (c). This illustrates the broad range of responses found in each recorded population, in agreement with the wide range of responses found in the PFC to such tasks (190). The first hint of trial-specific modulation can be seen in Figure 29 (b), which shows how the distribution of peak responses changes depending on trial type [T1, T2]. An example of a cell which shows a change in the location of the peak response between T1 and T2 trials is shown in Figure 30 (a).

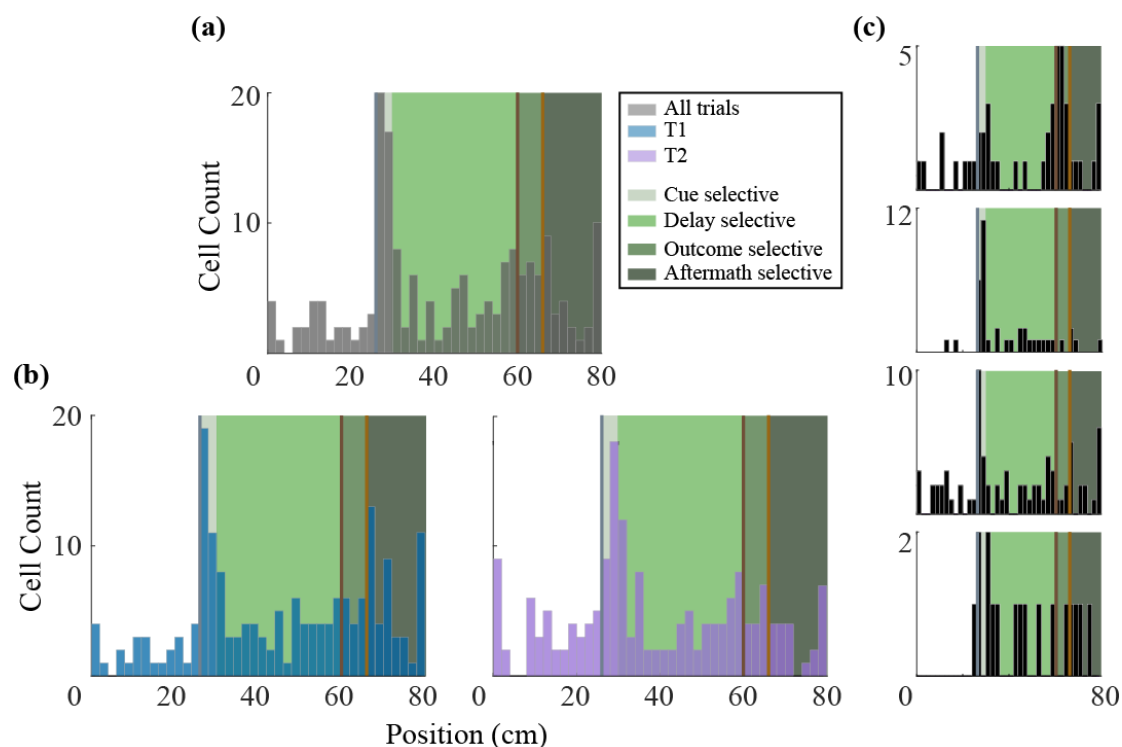


Figure 29: Segregation of task-responsive and task-unresponsive neurons. (a) Z-score peak location for all cells, analysed across all trials. The task epochs (cue, delay, outcome, aftermath) were defined approximately as shown in the diagram. (b) As in (a), split into T1 and T2 trials. (c) As in (a), for individual experiments. For all plots, the grey line indicates tone onset, the brown line indicates reward zone entry, and the gold line indicates reward zone exit. This nomenclature will persist for the remainder of the figures.

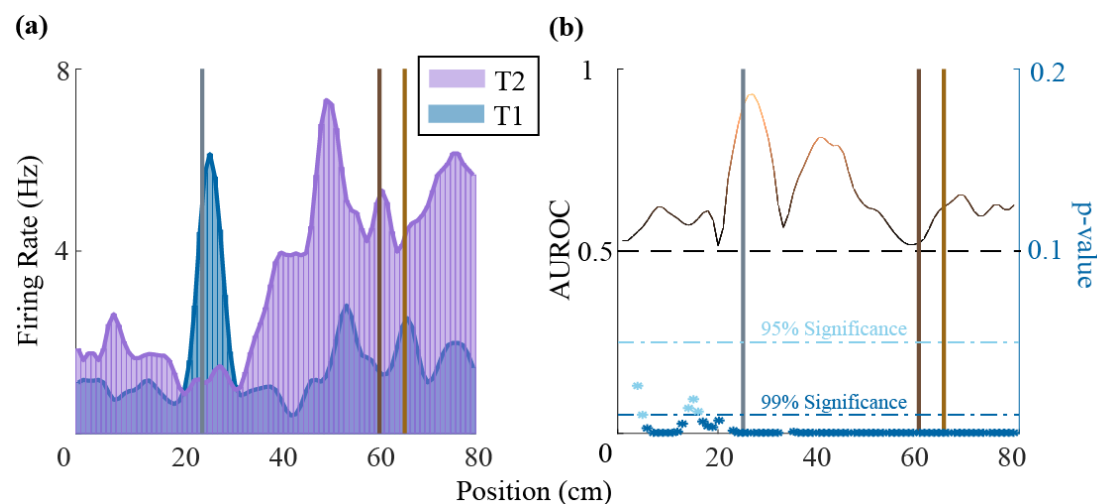


Figure 30: Shift in peak response location between T1 and T2 trials. (a) An example neuron shows a shift in location of peak response between T1 and T2 trials. (b) This difference is emphasised by the strong AUROC values in two locations in the delay period.

Statement (2) was further examined via receiver operating characteristic (ROC) analysis (98,180). As previously discussed, PFC neurons exhibit diverse, often multi-modal and frequently transient, response profiles during WM tasks (95,146). As such, whilst the location of the peak z-score reveals useful information in initially differentiating task-responsive neurons from task-unresponsive neurons, the nuances of this activity can be more closely examined via ROC analysis. The AUROC value determined the firing rate discriminability between two trial types. The p-value corresponds to the logistic regression model computed to determine the prediction power of the firing rate to the trial outcome. The probabilities from this model were used to compute the ROC curve.

Neuron response epochs

Studies of working memory paradigms such as that employed here, often segregate single neurons into different response periods e.g. cue, delay, response activity (146,196). In the context of this analysis, the response period of each neuron was determined by examining three characteristics; (i)-(ii) peak z-score location in T1 and T2 trials (iii) the location of peak discriminability between T1 and T2 trials via the AUROC value. As in previous studies, neurons were divided into the following response epochs:

- Cue selective
- Delay period selective
- Outcome selective
- Aftermath selective

An example of a cue selective cell is seen in Figure 31 (a). The cue was administered as a discrete pure tone (duration = 200ms) of T1 = 1kHz, T2 = 9kHz. A cue-responsive neuron was differentiated from a delay period neuron by showing a unimodal, brief response (<500ms, otherwise it is interpreted as delay period activity), during, or immediately after tone presentation. Figure 32 (a, c) show examples of tone echo in T1 and T2 trials respectively. Tone echo is characterised by a tone response, as described above, accompanied by a repetition of this response close to, or within the reward zone. The diversity of delay period neurons can be seen by the sustained and transient response profiles shown in Figure 33 (a) and (b-c) respectively. Early DP neurons are those that respond in the first half of the delay period, and late DP neurons respond in the subsequent half. Reward-zone responsive and aftermath responsive cells show the greatest degree of overlap. The former was defined as a neuron

with a peak response (by AUROC or z-score) that lay primarily in the reward zone, potentially including the surrounding area. Whilst the latter was defined as a response whereby the peak AUROC discrimination value lay between the end of the reward zone and the termination of the trial.

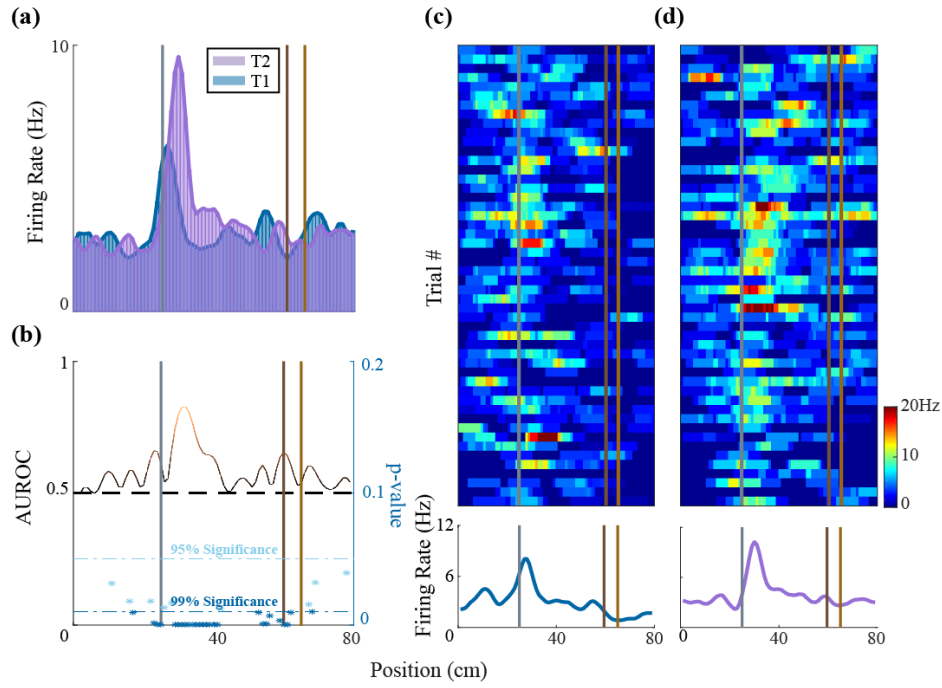


Figure 31: Tone-responsive neuron. (a) PSPH, and (b) corresponding AUROC profile. For all AUROC plots, the pale blue line indicates 95% significance for the logistic regression model used to compute the ROC curve, the dark blue line indicates 99% significance, and the black dashed line indicates the AUROC value for random discrimination. Note: The significance value represent how well the logistic regression of the predictor (the firing rate), predicts the response function (trial type). Hence, the p-value refers to how well the firing rate predicts the trial type. (c) The upper panel shows the firing rate over 50 T1 trials for this cell, with the mean trace shown in the lower panel. Note: The mean trace corresponds to the PSPH of T1 trials in (a), except in (a) the mean is taken over all T1 trials. (d) As in (c) for T2 trials.

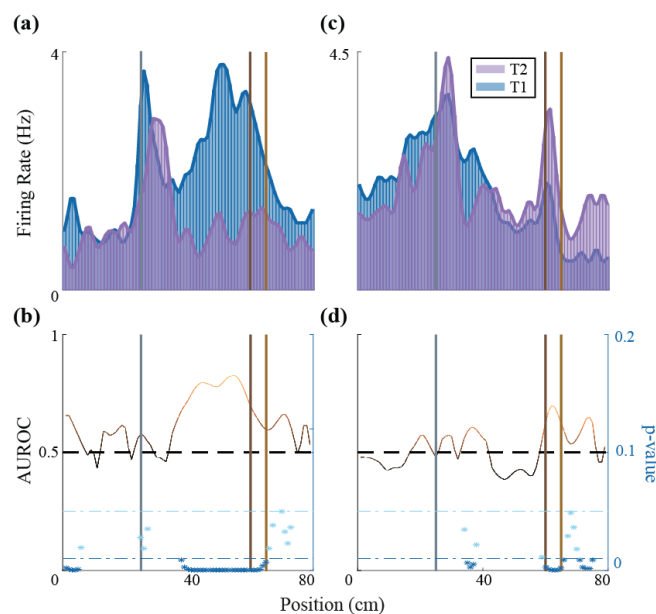


Figure 32: Tone-echo neurons. (a) PSPH, and (b) corresponding AUROC profile for T1 trial tone echo cell. (c) As in (a) and (d) as in (b) for a T2 trial tone echo cell. For all AUROC plots, the pale blue line indicates 95% significance for the logistic regression model used to compute the ROC curve, the dark blue line indicates 99% significance, and the black dashed line indicates the AUROC value for random discrimination.

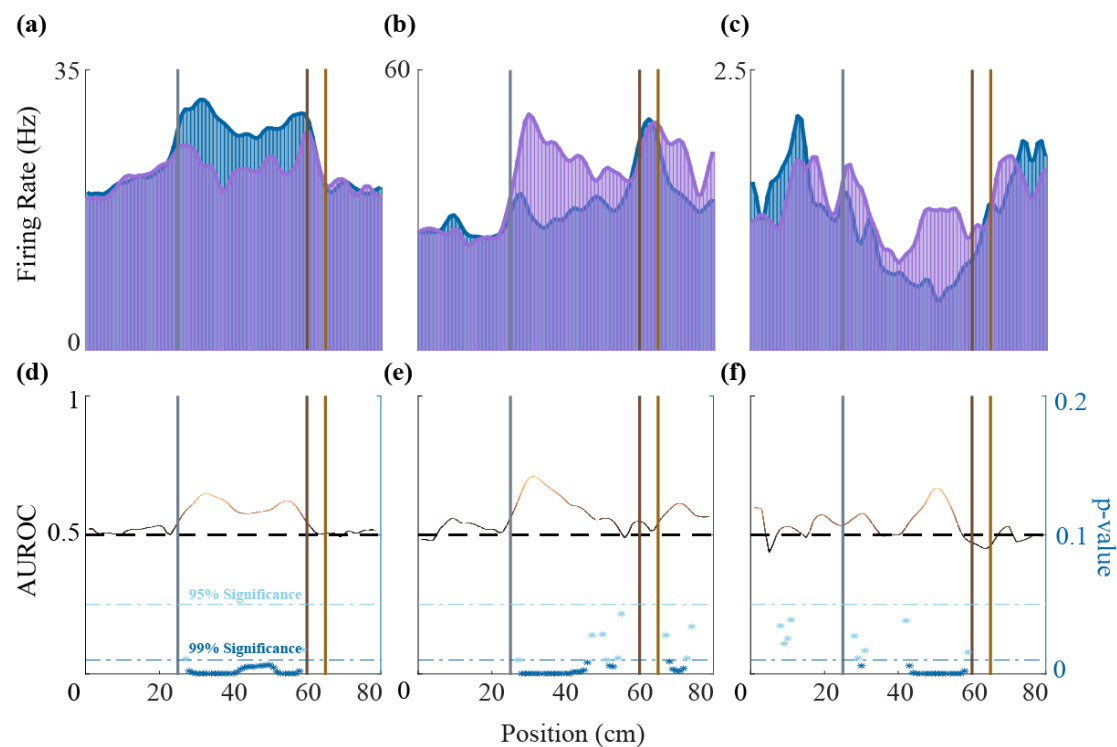


Figure 33: Delay period-selective neurons. (a) PSPH of sustained activity with (d) corresponding AUROC curve. (b) As in (a), with the corresponding AUROC trace (e) for an early DP neuron. (c) As in (a), with the corresponding AUROC trace (f) for a late DP neuron.

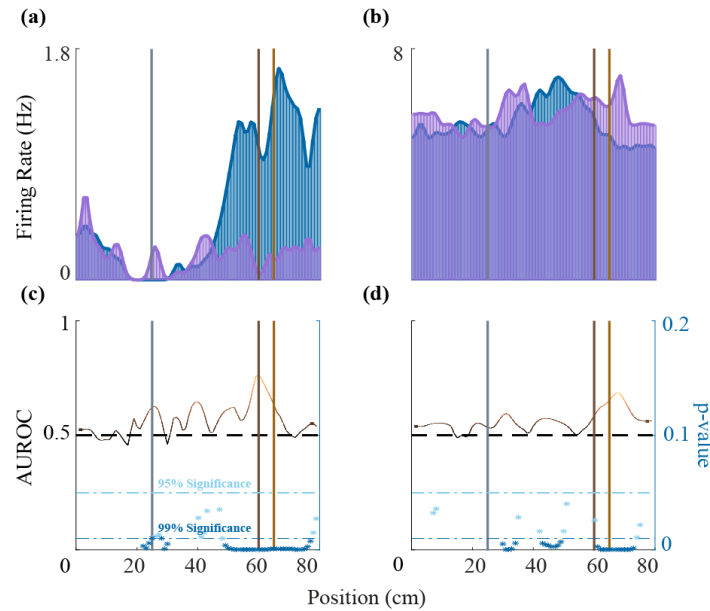


Figure 34: Reward zone-modulated and aftermath-modulated neurons. (a) PSPH of reward zone-responsive cell with (c) corresponding AUROC curve. (b), (d) As before for an aftermath-responsive neuron.

The AUROC values for discriminability between T1 and T2 trials for all delay period cells of a neural population is shown in the upper panel of Figure 35. The cells were ordered based on peak AUROC values. This figure illustrates the large heterogeneity that is present in the response profiles of each population. For comparison, the AUROC values for shuffled firing rates are shown in the lower panel of Figure 35.

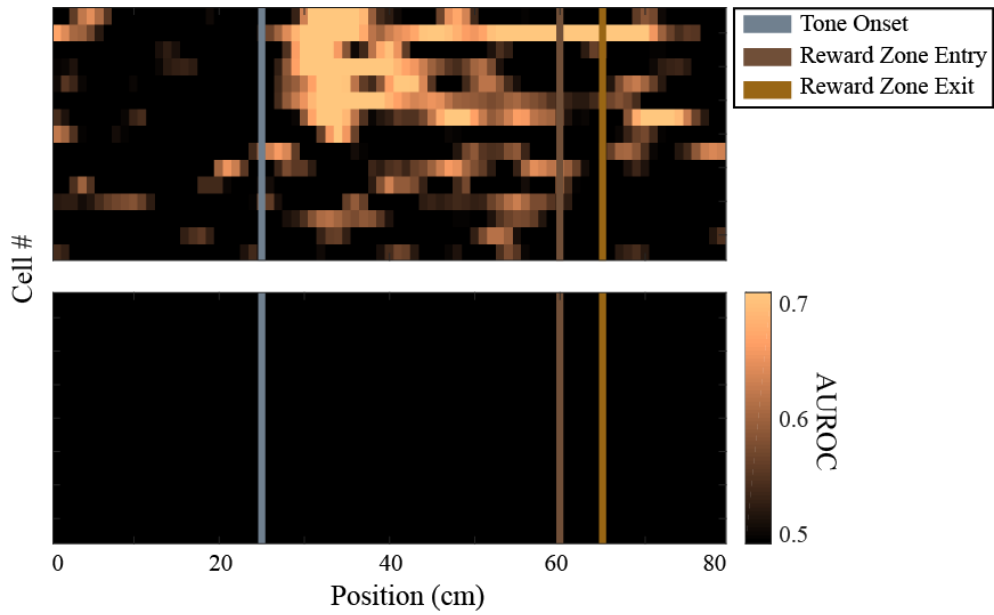


Figure 35: Population discriminability of delay period cells between T1 and T2 trials. Upper panel: The firing rate for each cell over all trials was used to determine the AUROC value for each position (for each cell). An AUROC value of 0.7 shows strong discriminability between trial types. Lower panel: The firing rate for each position was shuffled across trials ($n_{\text{Shuffle}} = 500$), for comparison. As expected an AUROC value of 0.5 was found for all cells, for all positions, corresponding to a chance level discrimination.

Delay period encoding; low level information or high-level information?

As previously described, there is an increasing amount of evidence to show that the delay period activity in the PFC is encoding high level information such as choice, in addition to mnemonic stimulus information. In the data presented here, there is potential evidence for both stimulus encoding (Figure 36), and high-level cognition encoding in the form of response prediction (Figure 37), and reward anticipation (Figure 38). However, it would be necessary to delineate the neural response from locomotion to ensure that the response profiles are not a product of motor activity. Reward anticipating neurons were classed as those displaying ramping behaviour (197). Notice the two distinct peaks that are only present in the Hit and FA response profiles of Figure 38 (b). This may be indicative of a delayed reward anticipation signal in FA trials. Together, this indicates that a combination of low level (stimulus), and high level (cognition) information is encoded by ACC neurons during the delay period of a WM task.

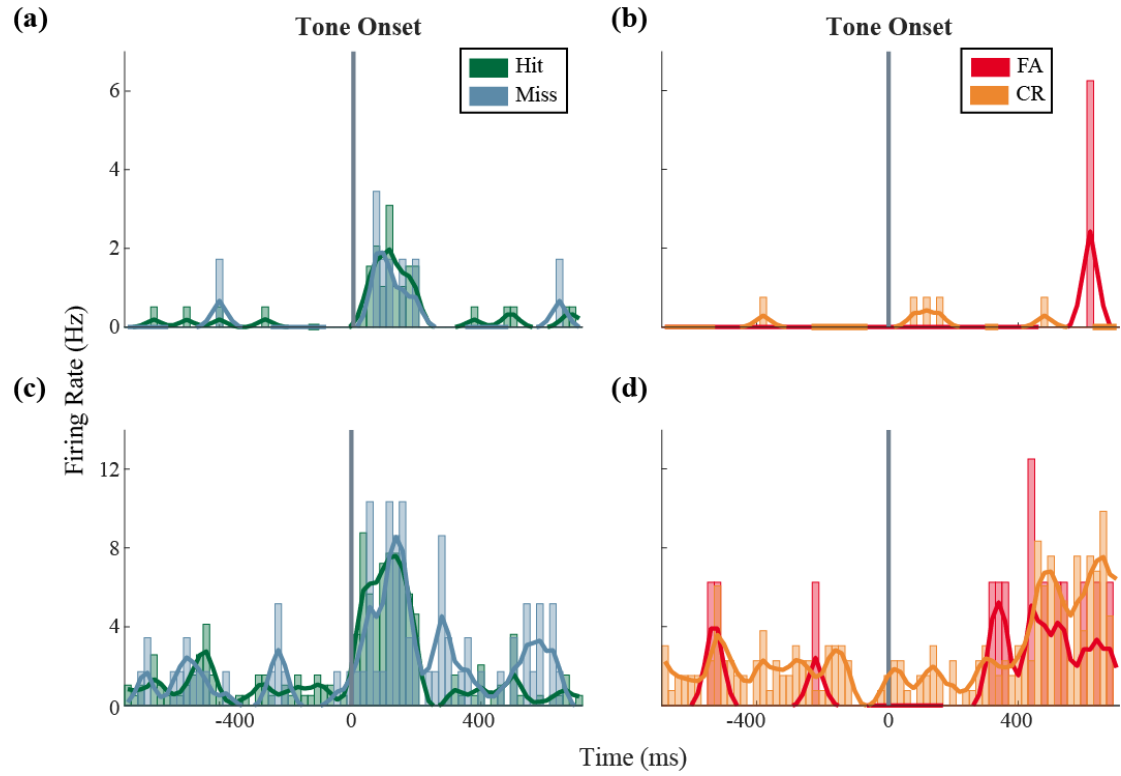


Figure 36: Neuronal response profiles of two stimulus-specific neurons representing low-level information encoding. (a) The response profile (bin size = 20ms) between Hit and Miss trials is indiscernible following tone presentation. Tone T1 was presented in both instances. (b) Similar responses were also found for FA and CR trials in which tone T2 was presented in both instances. (c) and (d) As in (a) and (b) for another stimulus-specific cell.

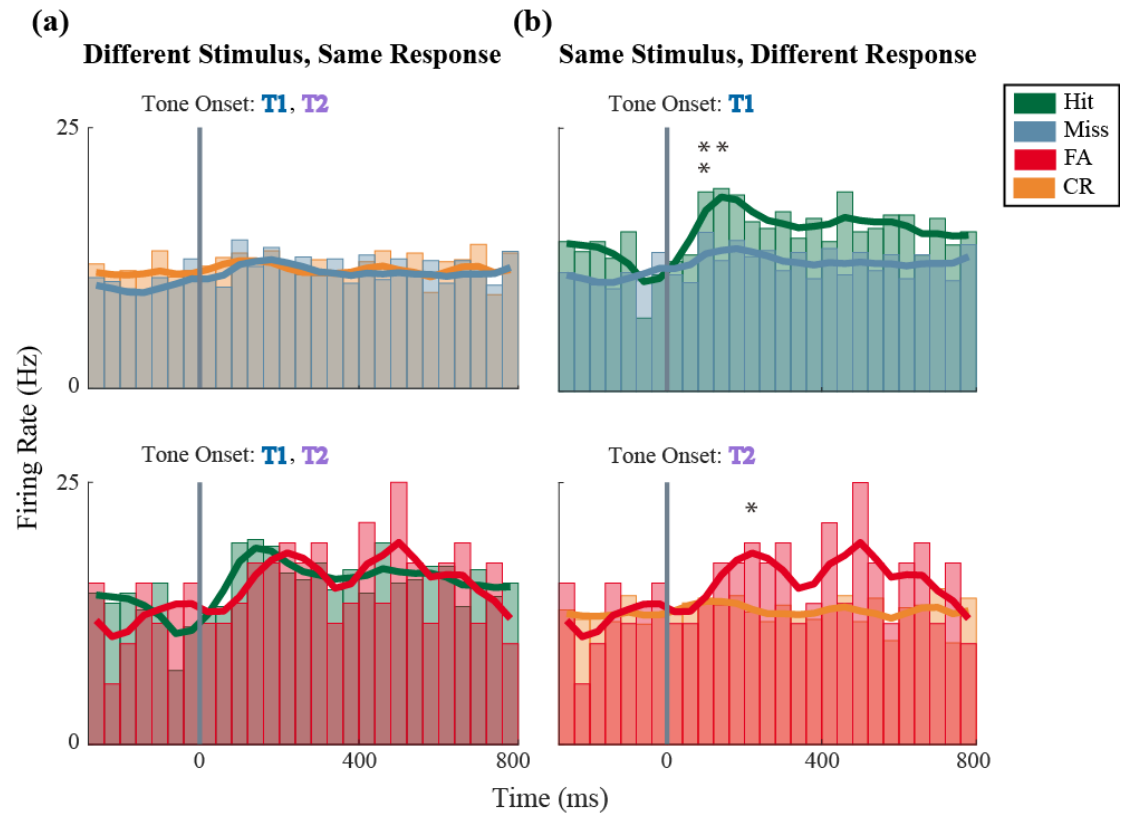


Figure 37: Neuronal response for a prediction neuron. (a) The upper and lower panels show a PSTH for sub-trial types whereby a different tone was presented in each case. The upper panel shows the PSTH for CR (T2) and Miss (T1) trials, and the lower panel shows the psth for Hit (T1), and FA (T2) trials. There was no significant difference in firing rate in either case (p-value determined from ROC analysis – see Methods). (b) As in (a) except the tone presented is the same. In the upper panel, despite tone T1 being presented in both the Hit and Miss trials, the firing rate showed a significant difference after 120ms. In the lower panel, despite T2 being presented in both cases, the firing rate showed a significant different between FA and CR trials after 240ms. One asterisk indicate 95% significance, two asterisks indicate 99% significance. All data is from the same neuron.

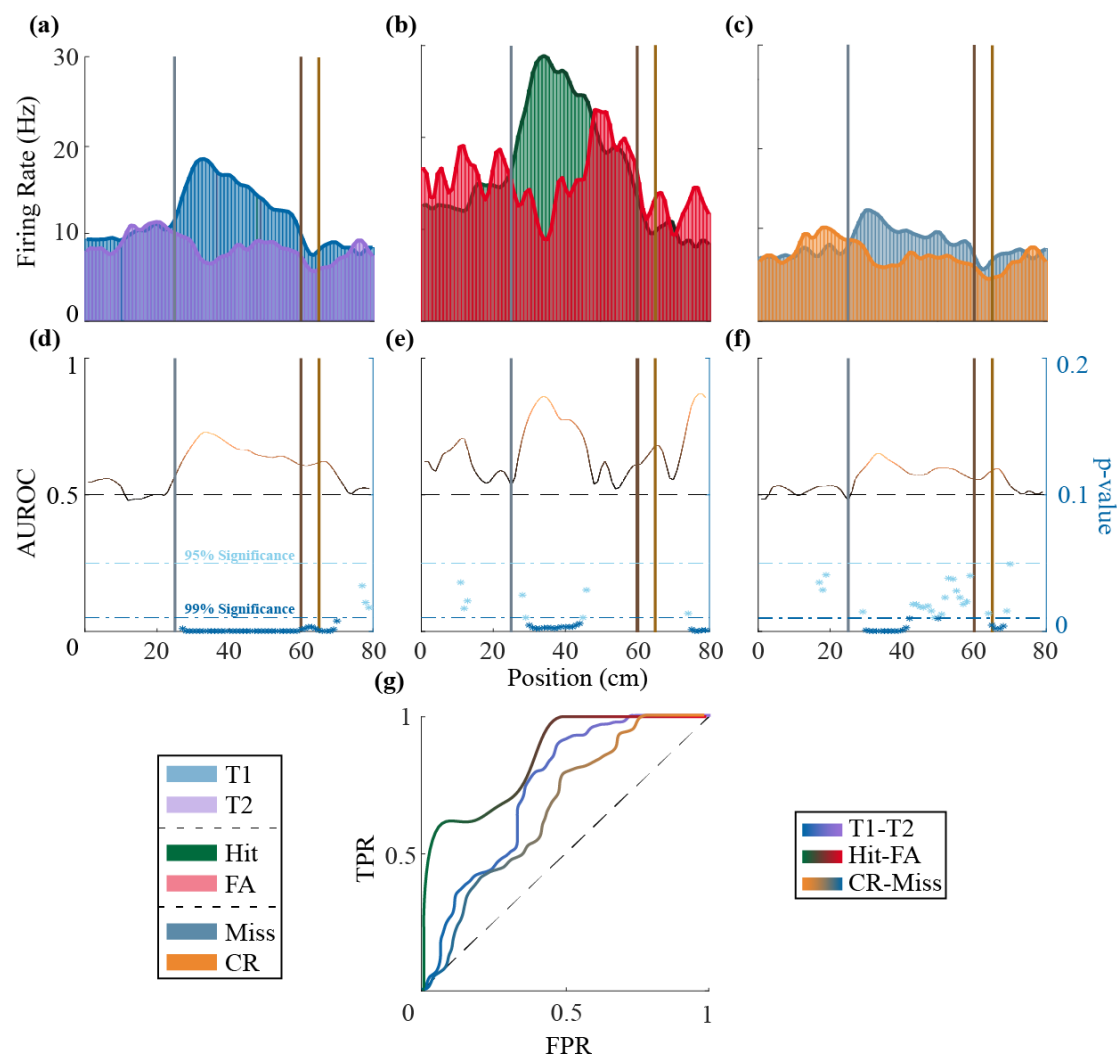


Figure 38: Reward-anticipation neuron. (a) The response profile between T1 and T2 trials shows a strong decreasing ramp response to the T1 (potentially rewarded) trials. (b) Upon closer inspection this response is most strongly present in Hit trials, with FA trials showing a similar response with a longer latency. (c) The Miss and CR trials are absent of this response. (d-f) The corresponding AUROC values for each trial type in the upper panel. Of note, despite the strongest response within Hit trials, the Miss and CR trials are also distinguishable with this neuron as shown by the significance values in (f). (g) A comparison between the peak ROC curves (i.e. chosen at the location of the peak AUROC value) shows the strongest discriminability between Hit and FA trials. (T1-T2, peak AUROC value = 0.73, position = 33cm, p-value = 0.00, Hit-FA; peak AUROC value = 0.87, position = 77cm, p-value = 0.00, Miss-CR; peak AUROC value: 0.65, position = 33cm, p-value = 0.00).

It is important to delineate the aforementioned neuronal responses with a simple correlation with running speed. As shown in Figure 27 (b), there is a characteristic speed profile between T1 and T2 trials. A linear regression between the speed profile in T1 and T2 trials and the response profile of each neuron was performed to determine if the response profile could be explained by the speed of the mouse. The results for other experiments can be found Figure 75 -76 of the Appendix. An example

population can be seen in Figure 39 for T1 trials, and Figure 40 for T2 trials. The coefficient of determination (R^2) is quoted for each cell. Whilst some cells show a moderate R^2 , the highest value is shown for cell ID: 8027 in T1 trials (Figure 39). This correlation is not repeated in T2 trials where the R^2 for this neuron is 0.04. Thus, whilst the speed can be predictive of a portion of the response profile a small number of neurons, the predictive power does not translate across T1 and T2 trials, i.e. and it can only predict a portion of the response profile in each case. Hence, when considering a linear relationship, the neuronal response profile cannot be explained by locomotion alone. However, one must consider that there may be other non-linear relationships present.

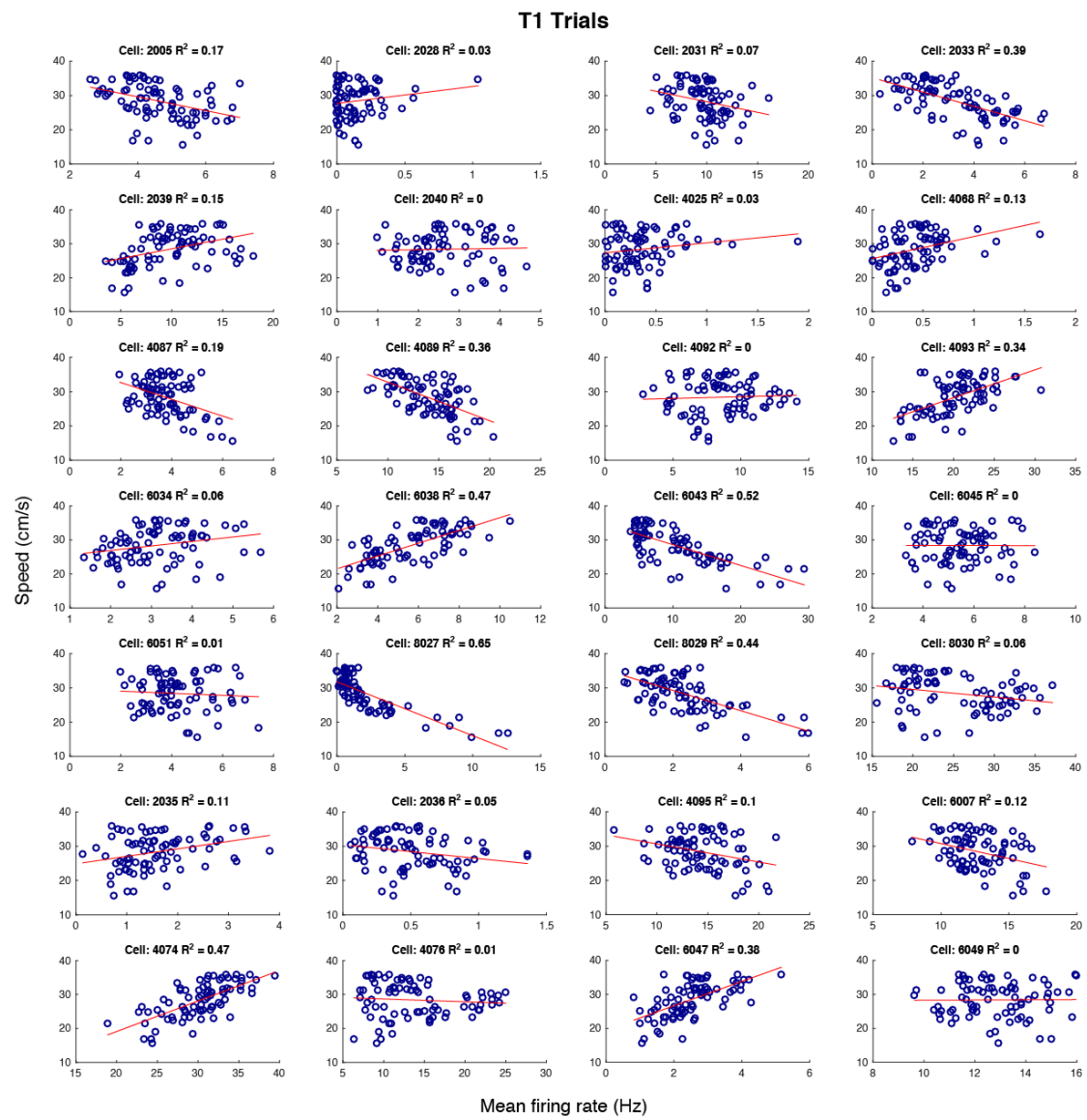


Figure 39: Linear regression between the mean speed profile in T1 trials and the mean firing rate of each neuron in T1 trials. The raw data is shown in blue, and the linear regression is shown in by the red line. The coefficient of determination (R^2) is quoted in the title of each plot.

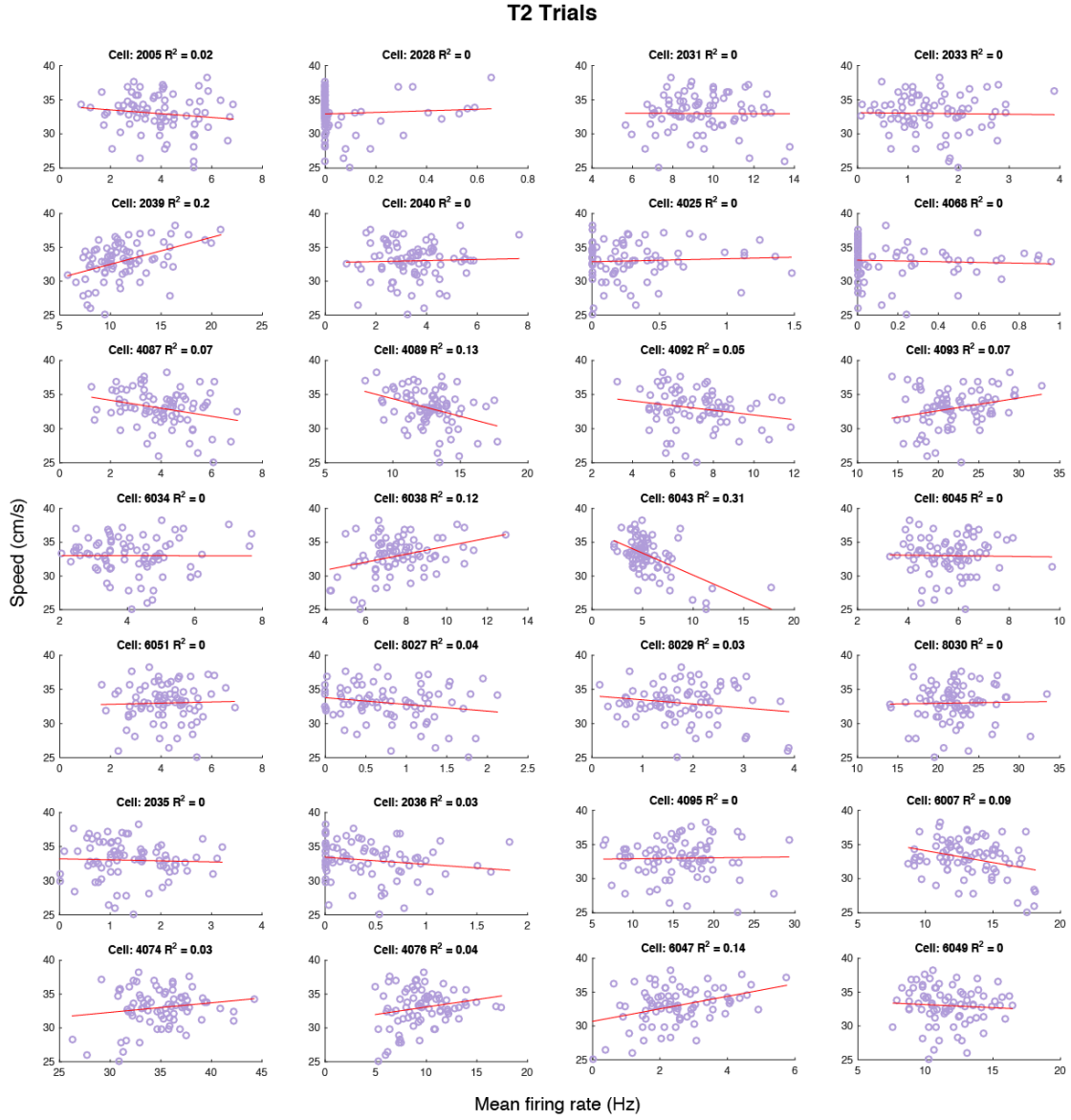


Figure 40: Linear regression between the mean speed profile in T2 trials and the mean firing rate of each neuron in T2 trials. The raw data is shown in blue, and the linear regression is shown in by the red line. The coefficient of determination (R^2) is quoted in the title of each plot.

Population activity during a working memory task

As per previous reports, the dataset presented here shows similar levels of diversity on a single neuron level. This prompted investigations into population level dynamics that may lead to further information regarding WM dynamics.

Given the evidence for multiple transient peaks in delay period cells, some of which differ in their peak location depending on trial type (Figure 30 (a)), combined with evidence in the literature of relay-race

sequential activation during the delay period (123), each population was examined for a trial-specific pattern of activation during the delay period.

The analysis was based on the method employed by Harvey et al., (146). A matrix of spatially-binned, normalised, mean neural firing rates for the task-modulated cells of each population were ordered according to the location of their peak firing rate in a particular trial type, e.g. T1 trials. Leave q-out cross validation ($n_{\text{CrossVal}} = 1000$, $q = 0.5$, where q is the proportion of trials assigned to the training set) was performed to determine the robustness of the repeatability of the trial-specific order, and to generate the most likely sequence for each trial type. For each iteration, the trial-specific dataset e.g. T1 trials, was randomly divided into training and test datasets. The sequence was generated on the training dataset (Set 1) and examined on the test dataset (Set 2). The sequence was then tested on the opposing trial type, e.g. T2 trials. Figure 41 (a-c) shows the T1 sequence tested on the T1 test data set and T2 trials. Figure 42 (a) shows a strong maintenance of the location of the peak FR for each cell throughout the trial when comparing T1 training and test dataset. Whereas, when the comparison is made with T2 trials, the early delay period and the post reward zone period show strong inconsistencies Figure 42 (b). This analysis was performed for the T2 sequence in Figure 41 (d-f). The T2 is noticeably less robust than the T1 sequence (Figure 42(c)).

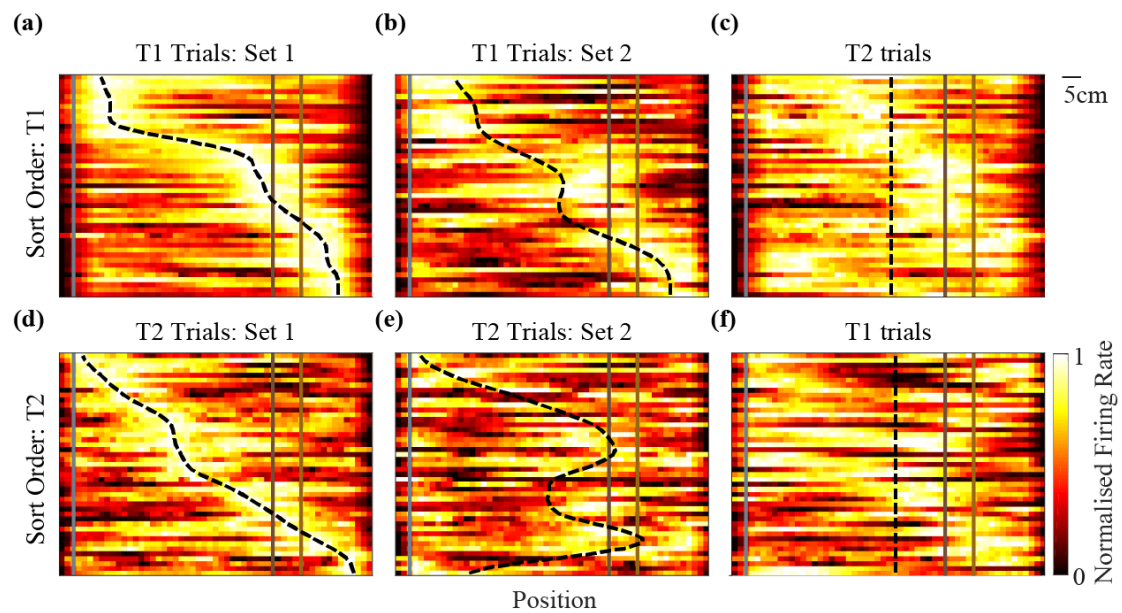


Figure 41: Context-dependent sequence of activation in T1 and T2 trial in neuronal population. (a) Neurons were sorted according to location of peak firing rate in T1 trials (Set 1 containing 50% of trials – training set). The black dashed line shows the line of best fit for the peak response locations. **(b)** The cell order was

maintained when plotting the firing rate of cells in the Set 2 (test set) of T1 trials (the remaining 50% of trials). (c) The cell order was maintained when plotting the firing rate of cells in T2 trials. (d) As in (a) for T2 trials sorted according to the location of the peak firing rate in T2 trials (Set 1 containing 50% of trials – training set). (e) The cell order was maintained when plotting the firing rate of cells in the Set 2 (test set) of T2 trials (the remaining 50% of trials). (f) The cell order was maintained when plotting the firing rate of cells in T1 trials.

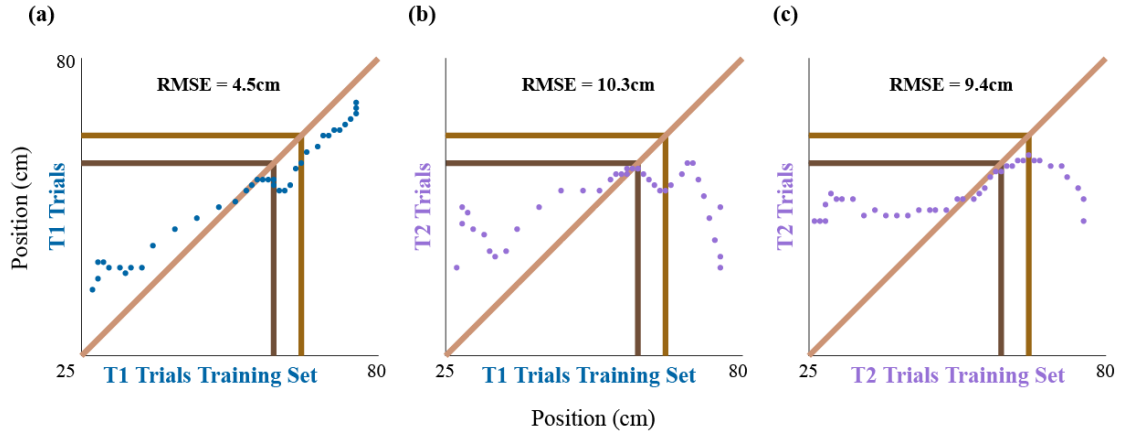


Figure 42: Context-dependent sequence of activation is robust in T1 trials but not T2 trials. (a) The mean position of the peak firing rate in the T1 trial test set (over cross validated dataset $n_{CrossVal} = 1000$), relative to the T1 trial training set. The root mean squared error is 4.5cm. **(b)** The mean position of the peak firing rate in the T2 trial test set (over cross validated dataset $n_{CrossVal} = 1000$), relative to the T1 trial training set. The root mean squared error is 10.3cm. **(c)** The mean position of the peak firing rate in the T2 trial test set (over cross validated dataset $n_{CrossVal} = 1000$), relative to the T2 trial training set. The root mean squared error is 9.4cm. Note: The scatter plot of T1 trial test set versus the T2 trial train set was excluded as the T2 trial sequence was not robust against the T2 trial test set. The locations are shown from the delay period (position 25) to the end of the trial, with the dark brown lines indicated reward zone entry and the gold lines indicating reward zone exit.

The validity of each sequence was examined by computing the mean and standard deviation of the spearman correlation coefficient of the cell sequences generated by cross validation. The significantly high value of ρ in Figure 43 (d) for T1 trials, and low value for T2 trials, indicates the robustness of the sequence generated in the T1 dataset when compared with itself, and the non-repeatability of the sequence in T2 trials. In contrast, the T2 sequence shows only a moderate correlation with itself and T1 trials, both at significance level. Thus, the T2 sequence appears to be only moderately robust, and can bear resemblance to a T1 sequence. Note, the large standard deviation in this metric (Figure 44 (d)). To assess how deterministic the sequence of activation was, the “best” sequence for T1 and T2 trials was estimated by computing the probability of a cell being located at a particular index in the sequence,

$p(\text{cell}|\text{Index})$, from the dataset generated during cross-validation. The cells were then ordered according to largest probability and then allocated a sequence index based on the index that had the highest probability for that cell. The probability of each cell at each index for this “best” sequence is shown in panel (e) of Figure 43 and Figure 44. The low probability values for all cells in both cases would indicate that the optimal sequence was not found for this method. However, this may not mean that the optimal sequence is not deterministic, but rather that a more adept approximation of this sequence would be required to estimate it more accurately.

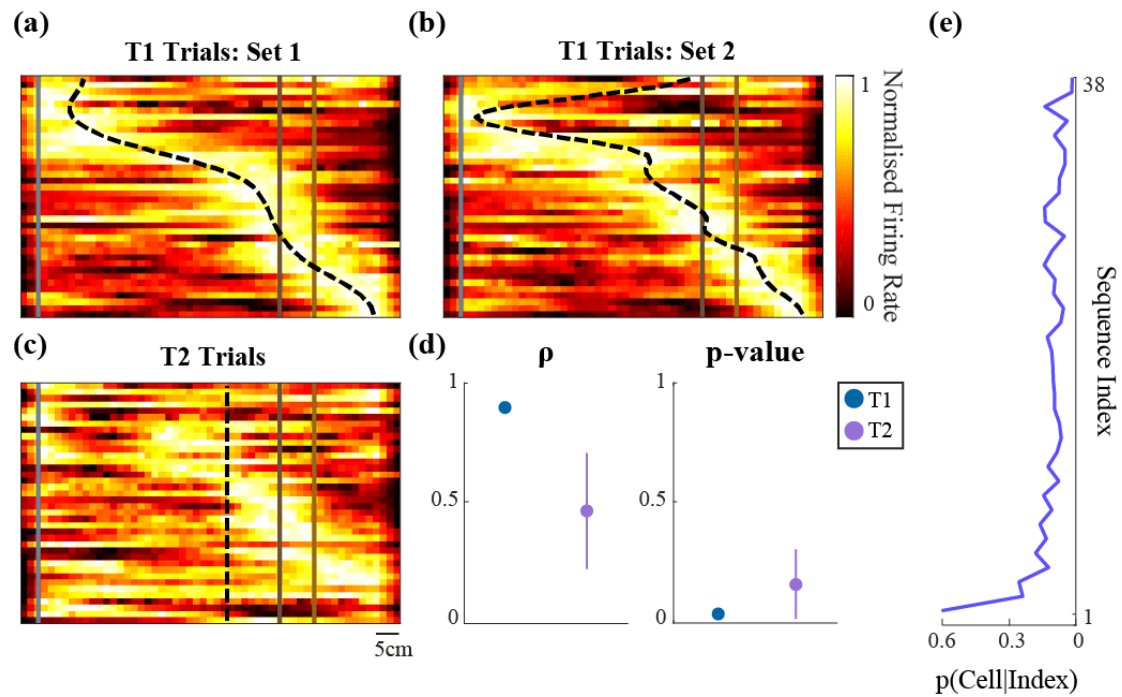


Figure 43: The normalised mean firing rate of neurons was ordered according to the T1 “best” sequence. T1 trials were divided into two equal sets. (a) The normalised firing rate of neurons on set 1 of T1 trials ordered according to the T1 “best” sequence. (b) As in (a) for set 2 of T1 trials. (c) The T1 “best” sequence was tested on T2 trials. (d) The T1 sequence was generated via cross validation ($n\text{Iterations} = 1000$). T1 trials were randomly split in half to generate a training dataset and a test dataset. A sequence was computed from the training dataset and tested on the test dataset, and the T2 trials. The similarity between the line of best fit in the training dataset and test datasets, and T2 trials was compared using Spearman’s correlation coefficient, ρ (ρ). The blue data point quantifies the similarity between the line of best fit of peak responses in training and test datasets of T1 trials when ordered according to the T1 sequence generated in the training dataset ($\rho = 0.96$, $p = 0.00$). The purple data point quantifies the similarity between the line of best fit in the T1 training dataset and the T2 dataset when ordered according to the T1 sequence ($\rho = 0.48$, $p = 0.14$). The corresponding p-values for each measurement are shown in the right-hand panel. (e) The probability of each cell being located at the allocated index for the T1 “best” sequence.

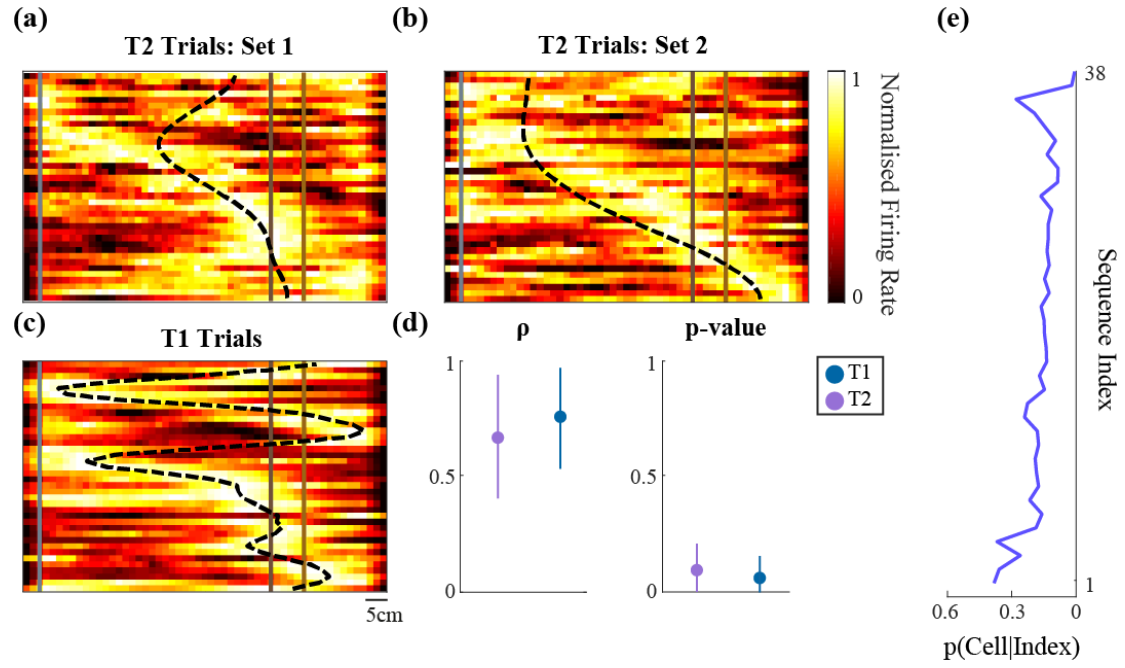


Figure 44: The normalised mean firing rate of neurons was ordered according to the T2 “best” sequence. T2 trials were divided into two equal sets. (a) The normalised firing rate of neurons on set 1 of T2 trials ordered according to the T2 “best” sequence. (b) As in (a) for set 2 of T2 trials. (c) The T2 “best” sequence was tested on T1 trials. (d) The T2 sequence was generated via cross validation (nIterations = 1000). T2 trials were randomly split in half to generate a training dataset and a test dataset. A sequence was computed from the training dataset and tested on the test dataset, and the T1 trials. The similarity between the line of best fit in the training dataset and test datasets, and T1 trials was compared using Spearman’s correlation coefficient, rho (ρ). The blue data point quantifies the similarity between the line of best fit of peak responses in training dataset of T2 trials and the T1 dataset when ordered according to the T2 sequence generated in the training dataset ($\rho = 0.77$, $p = 0.08$). The purple data point quantifies the similarity between the line of best fit in the T2 training and test datasets when ordered according to the T2 sequence ($\rho = 0.69$, $p = 0.04$). The corresponding p-values for each measurement is show in the right-hand panel. (e) The probability of each cell being located at the allocated index for the T2 “best” sequence.

Outcome prediction

Based on the preceding evidence for population encoding in the WM task, the ability of population dynamics to predict task outcome was investigated. The method employed here was based on the principal component analysis (PCA) classifier used in Harvey et al., (146).

PCA classifier

A binary classifier was constructed by performing principal component analysis (PCA) on the mean firing rate per belt position, of each neuron in a given population, and examining the trajectories of the first 3 principal components in state space. The aim was to ascertain whether the dominant characteristics of the firing rate patterns in the population were indicative of the upcoming decision of the mouse. To construct the classifier, the data (all trials) was divided in half to produce training and test datasets. The mean firing rate for each task-responsive cell was computed, for each position on the belt, for each trial type e.g. [T1, T2]. Dimensionality reduction was performed using PCA. The viability of this method was assessed via two measures; (i) The amount of variance of the population that was explained with the selected PCs, (ii) the distance between the two trajectories in state space to facilitate discrimination. For the classifier illustrated here, the percentage of variance explained by the first three PCs in T1 trials was $> 75\%$. Figure 45 shows that 76% and 46% of the variance was accounted for in T1 and T2 trials respectively. The evolution of each principal component across belt position for T1 and T2 trials is given in Figure 47. The ability to discriminate the two trajectories in state space was measured via the Euclidean distance (Figure 46 (b)). An illustration of the neural trajectories in state space for T1 and T2 trials is shown in (Figure 46 (a)). Note the proximity of the early portion of the trial trajectory until tone presentation, from which point the two trajectories bifurcate strongly. To understand the degree to which the neural trajectories in state space could be influenced by statistical fluctuations, a bootstrapped estimate ($n_{\text{Boot}} = 800$) was computed for each trial type. The robustness of a trajectory was quantified by measuring the normalised root mean square error (NMRSE) between the observed trajectory and the bootstrapped estimate. Interestingly, T1 trials provided a much more robust estimate in state space than the T2 counterpart for all experiments. This is in accordance with the poor repeatability of the sequence of activation seen in Figure 43 (d) in T2 trials. It is essential that the classifier is robust to “noise” in the circuit, i.e. the underlying variability in the neural circuit trajectories in state space. Hence, there must be a greater distance between the T1 and T2 trajectories, than with each trial type and the bootstrapped estimate. Any distance greater than that of the bootstrapped estimates would increase the discrimination ability at that position.

The neural trajectory of the first three principal components was explored in state space (downsampled to the n th sample, $n = 15$ or 20 depending on the experiment, smoothed using cubic spline

interpolation). The decision criterion was constructed by assigning the central plane of the two trajectories as the decision line, with a decision boundary in the form a volume surrounding the decision plane. The decision boundary was assigned by computing the selectivity index for each position for shuffled trials ($n_{\text{Shuffle}} = 800$). The absolute value of the mean shuffled selectivity index was used as a symmetric decision boundary around the decision line. An alternative approach of an asymmetric decision boundary (whereby the decision line itself would form part of the boundary as the volume of the decision boundary would be determined by the sign of the average selectivity index of the shuffled results) was avoided, as it would be a more conservative decision boundary. Given the inherently high degree of variability within PFC networks, a more conservative decision boundary would increase the robustness of the classifier to noise within the system. A collapsed view of the decision criteria can be seen in the 2D examples of Figure 55. Classification of a test trial was determined by the selectivity index (Equation 3), which was defined as the distance from the test trial data-point to each of the trial-type state-space trajectories, normalised to the distance between the state-space trajectories for that position. Hence, by this definition, an example classification would be T1 trial = 1, T2 trials = -1. An inconclusive trial was one in which the selectivity index lay within the decision boundary.

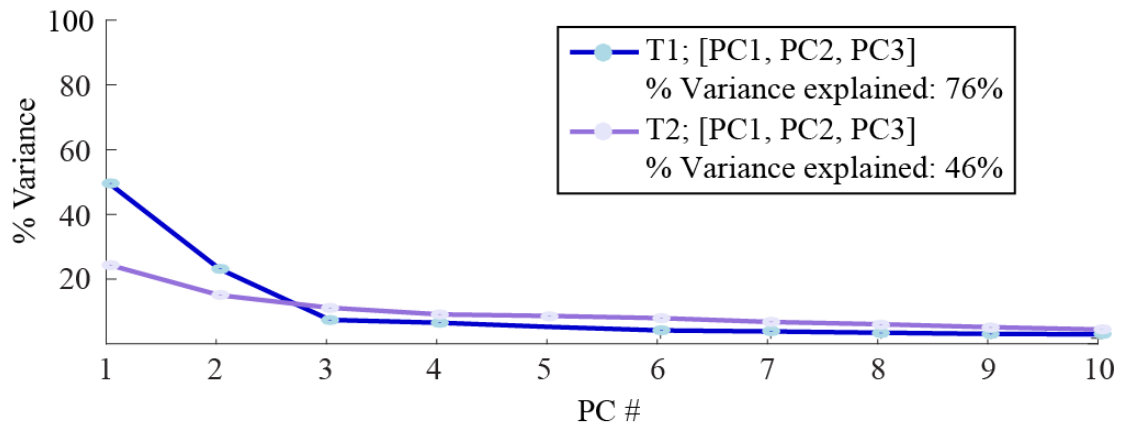


Figure 45: Proportion of neural population variance explained by [PC1, PC2, PC3].

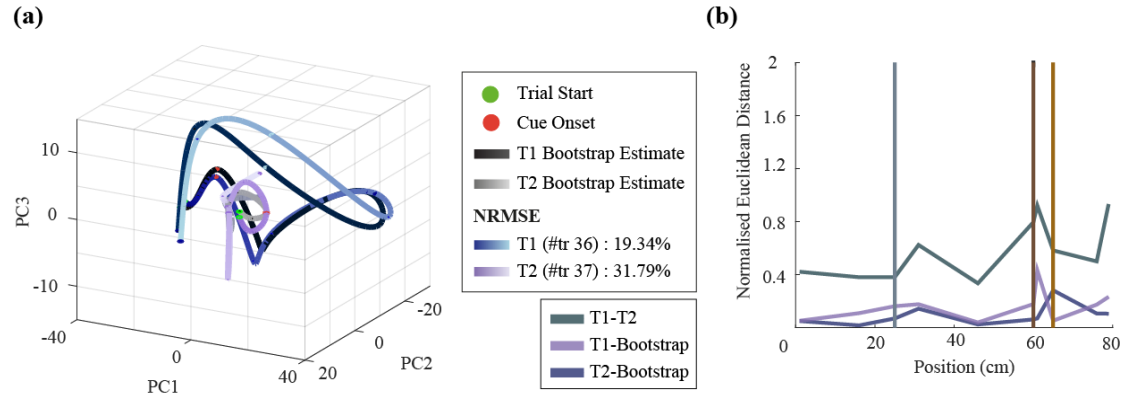


Figure 46: PCA classifier. (a) Neural trajectories in state space for T1 and T2 trials, and their bootstrapped estimates. The locations of the trial start and cue onset are highlighted on all four trajectories. (b) The Euclidean distance between T1 and T2 trials for each belt position. The Euclidean distance between the T1 neural trajectory and the T1 bootstrapped estimate is also shown, in addition to the Euclidean distance between the T2 neural trajectory and the T2 bootstrapped estimate.

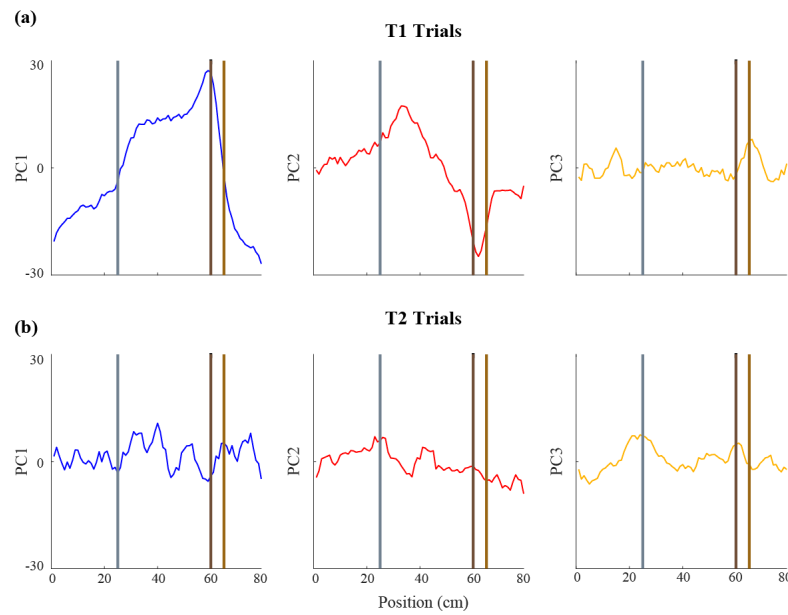


Figure 47: The profile of principal components 1-3 across trial position, for (a) T1 trials, and (b) T2 trials.

The score for each cell, i.e. the weighting of each PC for each cell used in the classifier was computed to determine whether the classifier was being directed by a population of cells or only a small subset of neurons. Figure 48 shows that for both T1 and T2 trial state space trajectories, whilst there are certainly dominant neurons, the trajectory is influenced by a subpopulation of quite a large number of cells in the population.

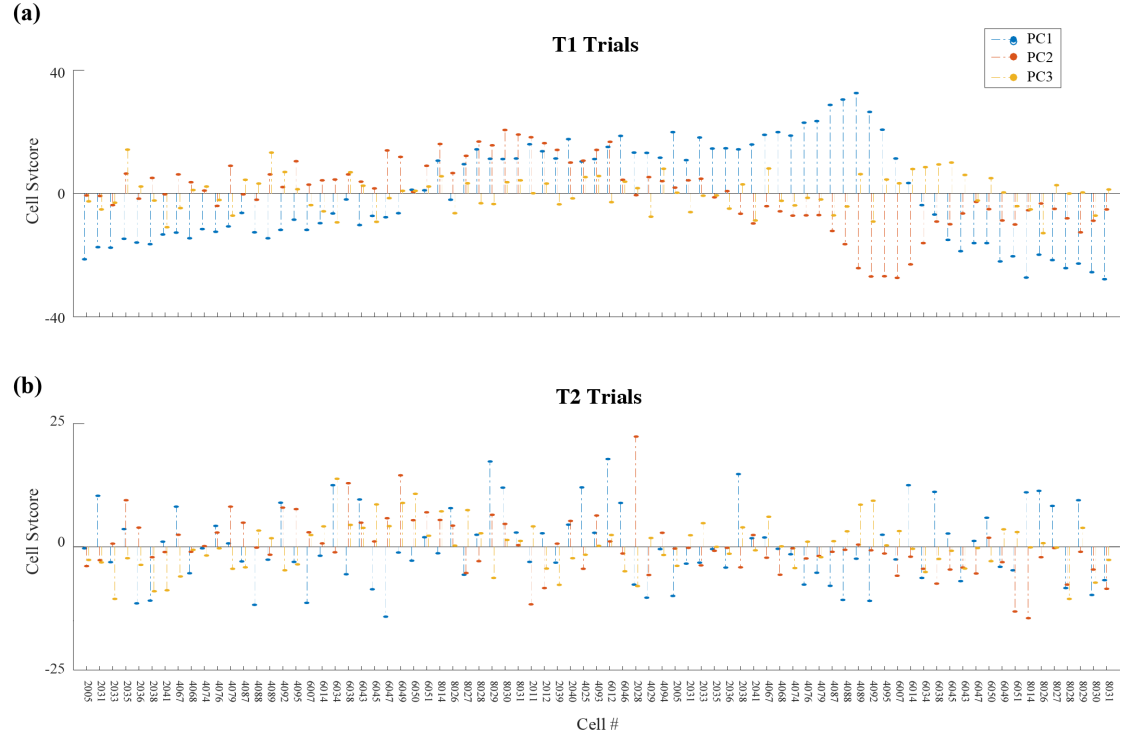


Figure 48: (a) The scores of each neuron for the first 3 PCs are shown for the T1 neural trajectory in state space. The scores represent the contribution of each neuron to a given PC. (b) As in (a) for the T2 neural trajectory in state space.

$$SI = \frac{(d_{mean\ traj, traj2} - d_{mean\ traj, traj1})}{d_{mean\ traj, traj1} + d_{mean\ traj, traj2}}$$

Equation 3: Selectivity index.

PCA classifier performance

The outcome of the classifier was evaluated by two metrics:

- The overall classification based on the average outcome predicted in the delay period.
- The spatial profile of classifier outcome i.e. the speed and accuracy with which it can correctly predict the trial outcome.

The performance of each metric was assessed overall for all trial types (Figure 49), and subdivided into specific trial types (Figure 50). The classifier was trained on correct trials only; Hit (T1) and CR (T2)

trials. Note the strong discrimination power between Hit and CR trials (Figure 51 (b)), and Hit and Miss trials (Figure 52 (b)). However, when evaluating classifier performance, the classifier was tested on the ability to predict all 4 outcomes; Hit, Miss (T1), CR and FA (T2). The peak performance for the classifier was can be seen in Figure 49 shortly after the tone was presented. This may be due to the presence of predictive cells such as that shown in Figure 37. Despite only being trained on correct trials [Hit, CR], the classifier was able to correctly predict the behavioural outcome of the mice in Miss trials from early in the delay period (Figure 50 (a)). It is interesting to note in Figure 50 (c) that the classification of Hit trials is poor despite the classifier being trained on these trial types (see Miss trial classification for comparison (Figure 50 (b)). The ability of the classifier to determine four outcomes [Hit, Miss, FA, CR] whilst being trained only on two [Hit, CR], is likely to be due to a similarity between Hit and FA trials, and CR and Miss trials. If this is the case, the poor performance of the classifier for Hit trials in comparison with Miss trials, may be due to a greater similarity between CR and Miss trials, than Hit and FA trials, which does indeed appear to be the case in Figure 53 (b) and Figure 54 (b). Indeed, this is highly likely given the low trial count of FA trials and the high variability that occurs, as seen in Figure 54 (a). An example of how test trials were classed (collapsed into 2-dimensional space) for each of the aforementioned outcomes is shown in Figure 55. As found in Harvey et al. (146), the selectivity index of a given trial often fluctuates across the decision boundary. This appears to occur most often for FA trials (Figure 55 (d)).

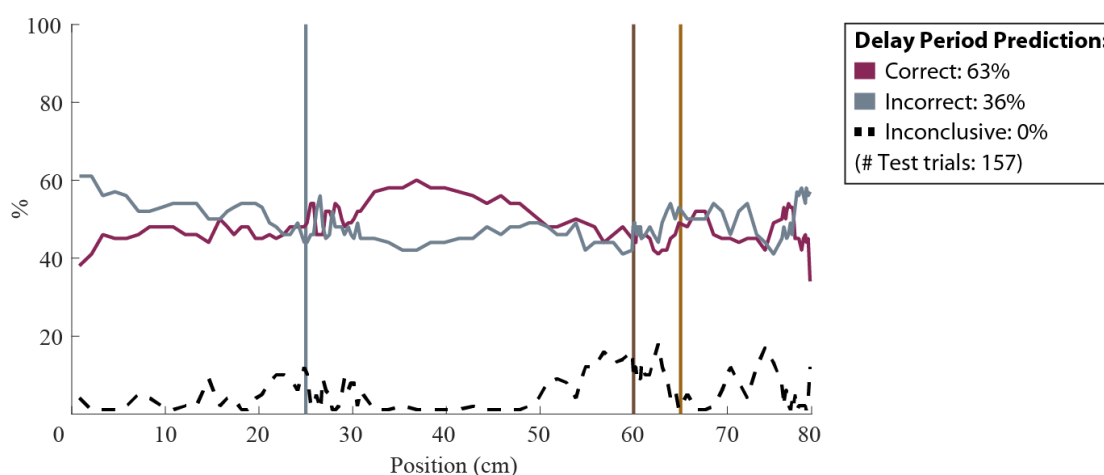


Figure 49: The performance of the PCA classifier in predicting all four outcomes [Hit, Miss, FA, CR] was computed on a per position basis (plot traces) and an overall classification based on the average trial outcome predicted during the delay period (shown in figure legend). Note the peak in classifier performance soon after tone presentation.

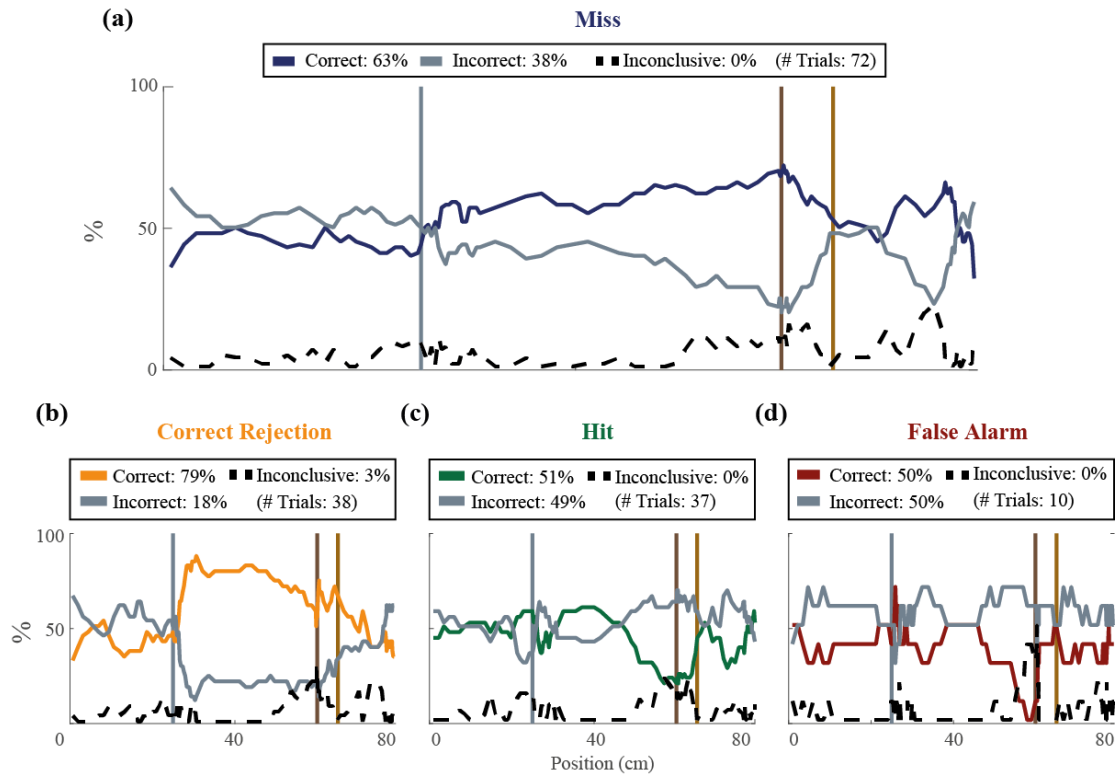


Figure 50: (a) The performance of the PCA classifier in Miss trials was quantified on a per position basis (plot traces) and an overall classification based on the average trial outcome predicted during the delay period (shown in figure legend). (b) As in (a) for CR trials. (c) As in (a) for Hit trials. (d) As in (a) for FA trials.

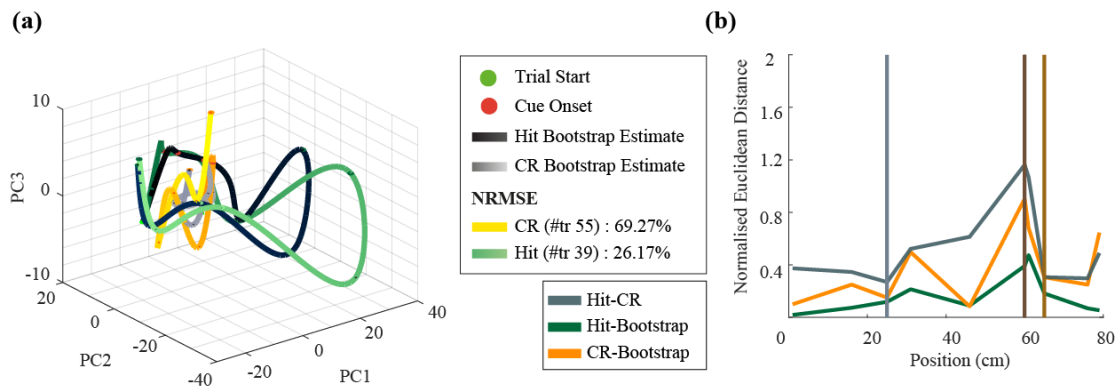


Figure 51: (a) Neural trajectories in state space for Hit and CR trials, and their bootstrapped estimates. The locations of the trial start and cue onset are highlighted on all four trajectories. (b) The Euclidean distance between Hit and CR trials for each belt position. The Euclidean distance between the Hit neural trajectory and the Hit bootstrapped estimate is also shown, in addition to the Euclidean distance between the CR neural trajectory and the CR bootstrapped estimate.

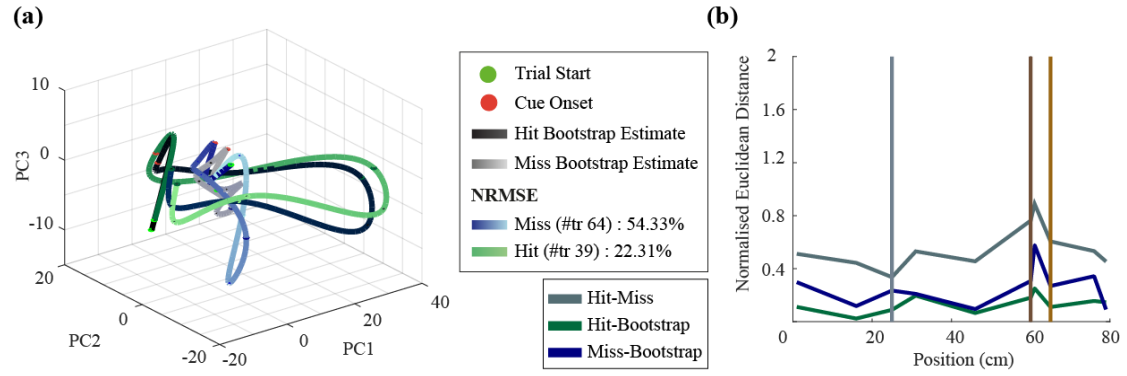


Figure 52: (a) Neural trajectories in state space for Hit and Miss trials, and their bootstrapped estimates. The locations of the trial start and cue onset are highlighted on all four trajectories. (b) The Euclidean distance between Hit and Miss trials for each belt position. The Euclidean distance between the Hit neural trajectory and the Hit bootstrapped estimate is also shown, in addition to the Euclidean distance between the Miss neural trajectory and the Miss bootstrapped estimate.

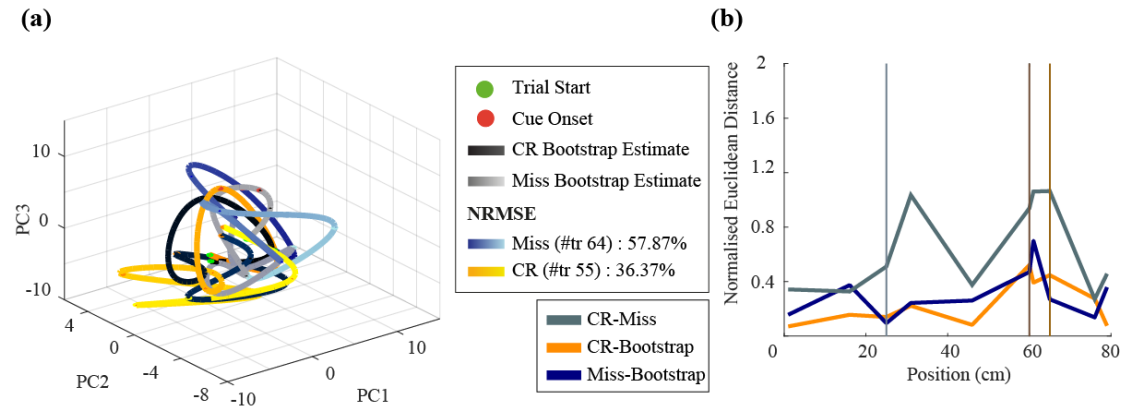


Figure 53: (a) Neural trajectories in state space for CR and Miss trials, and their bootstrapped estimates. The locations of the trial start and cue onset are highlighted on all four trajectories. (b) The Euclidean distance between CR and Miss trials for each belt position. The Euclidean distance between the CR neural trajectory and the CR bootstrapped estimate is also shown, in addition to the Euclidean distance between the Miss neural trajectory and the Miss bootstrapped estimate.

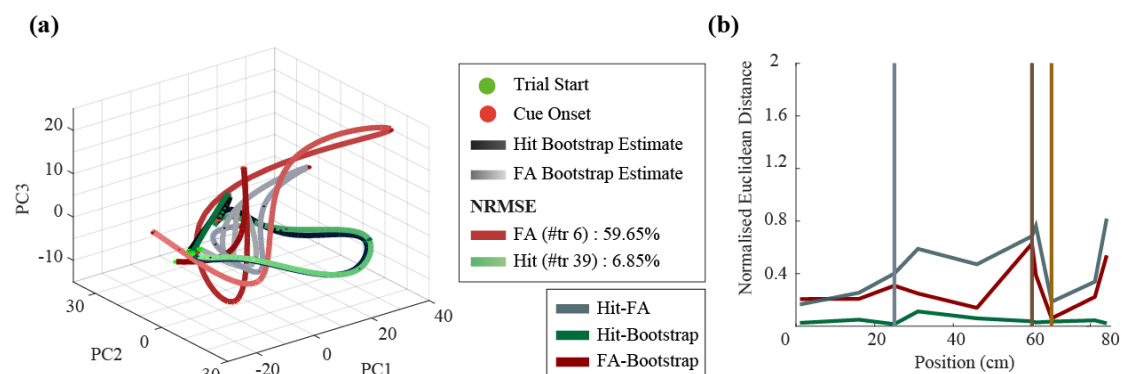


Figure 54: (a) Neural trajectories in state space for Hit and FA trials, and their bootstrapped estimates. The locations of the trial start and cue onset are highlighted on all four trajectories. **(b)** The Euclidean distance between Hit and FA trials for each belt position. The Euclidean distance between the Hit neural trajectory and the Hit bootstrapped estimate is also shown, in addition to the Euclidean distance between the FA neural trajectory and the FA bootstrapped estimate.

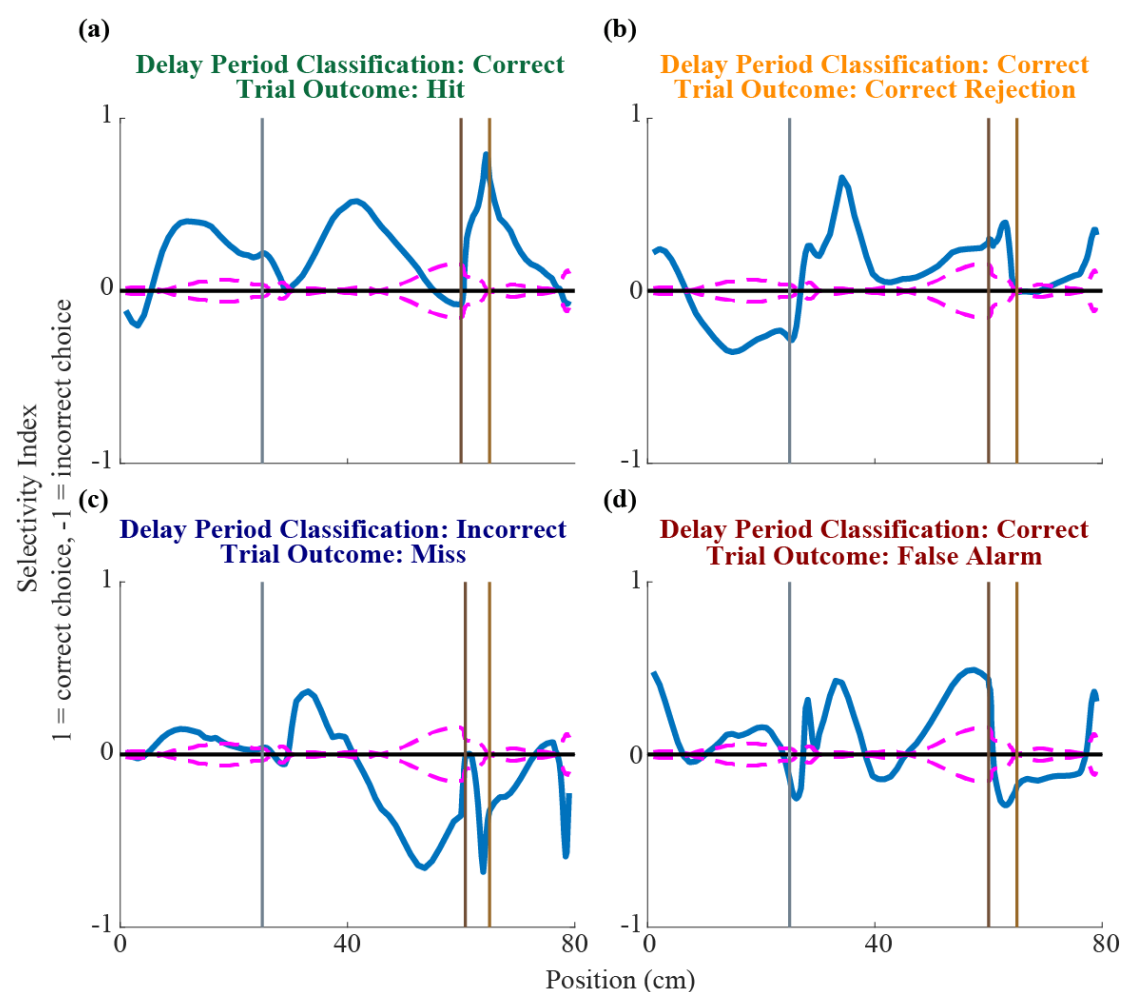


Figure 55: Classification via the selectivity index. (a) The selectivity index (blue trace) for a sample Hit trial classed correctly as a Hit trial by the PCA classifier. The black line represents the decision plane, the

magenta trace represents the decision boundary (a point within this region will be classed as inconclusive). (b) As in (a) for a correctly classed CR trial. (c) As in (a) for a correctly classed Miss trial. (d) As in (a) for an incorrectly classed FA trial. The classifier classed the FA trial as “correct” i.e. a Hit trial. This can be seen from the largely positive values for the selectivity index across belt positions for this trial. Of note, although wrongly classified, the selectivity index of the FA trial shows the most fluctuations across the decision boundary.

Locomotion classifier

A similar classifier was built on the locomotion data, namely the mean speed profiles for the two different trial types [T1, T2] (Figure 56 (a)). As before, the classifier was trained on correct trials only and a training-test ratio of 0.8:0.2 was chosen. Classification was performed via the selectivity index previously described. The locomotion classifier outperformed the PC classifier in terms of overall accuracy of classification (75% correct in comparison with 67% - Figure 57), however the PC classifier was much faster in it’s ability to discriminate trial types. Whilst the locomotion classifier performs well for all trial types (Figure 58), the neural classifier is superior in terms the speed at which it can correctly classify an outcome.

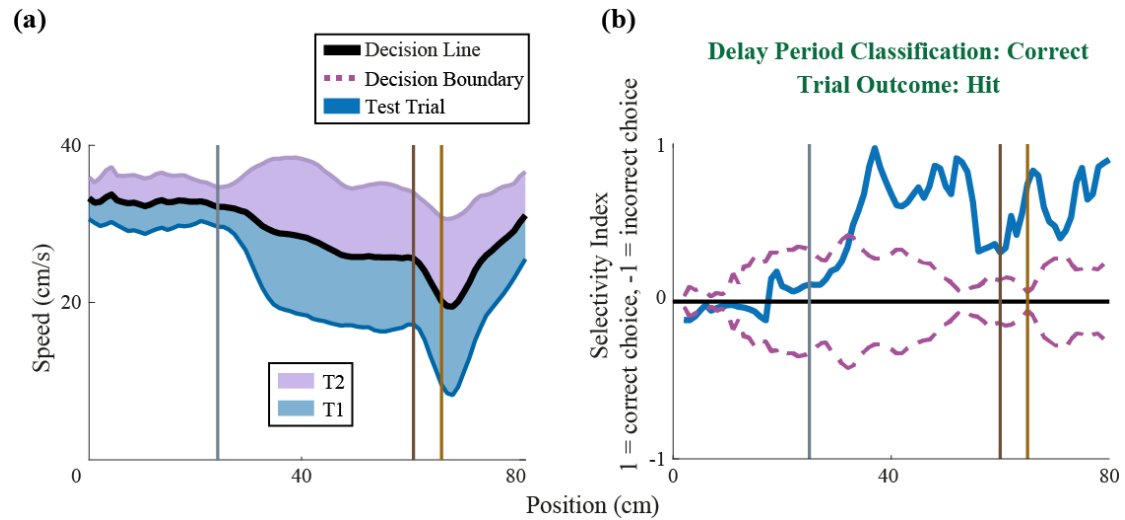


Figure 56: Locomotion classifier. (a) The behavioural classifier was built on the mean speed profiles for T1 and T2 trials, (smoothed using a moving average filter, span = 6cm). The decision boundary was defined as the midpoints between the two speed traces at each position. **(b)** The selectivity index (blue trace) for a sample Hit trial classed correctly as a Hit trial by the behavioural classifier. The black line represents the decision line, the magenta trace represents the decision boundary (a point within this region will be classed as inconclusive).

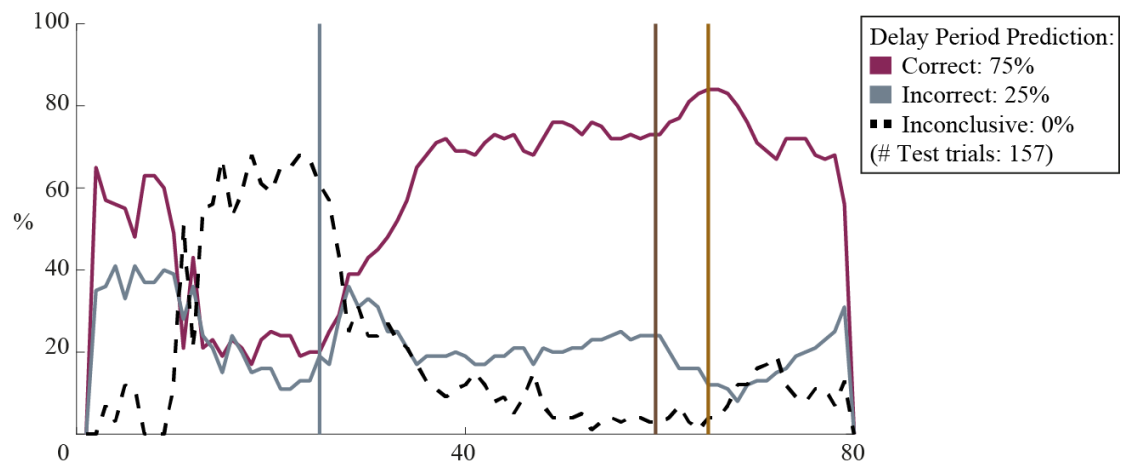


Figure 57: The performance of the locomotion classifier in predicting all four outcomes [Hit, Miss, FA, CR] was computed on a per position basis (plot traces) and an overall classification based on the average trial outcome predicted during the delay period (shown in figure legend).

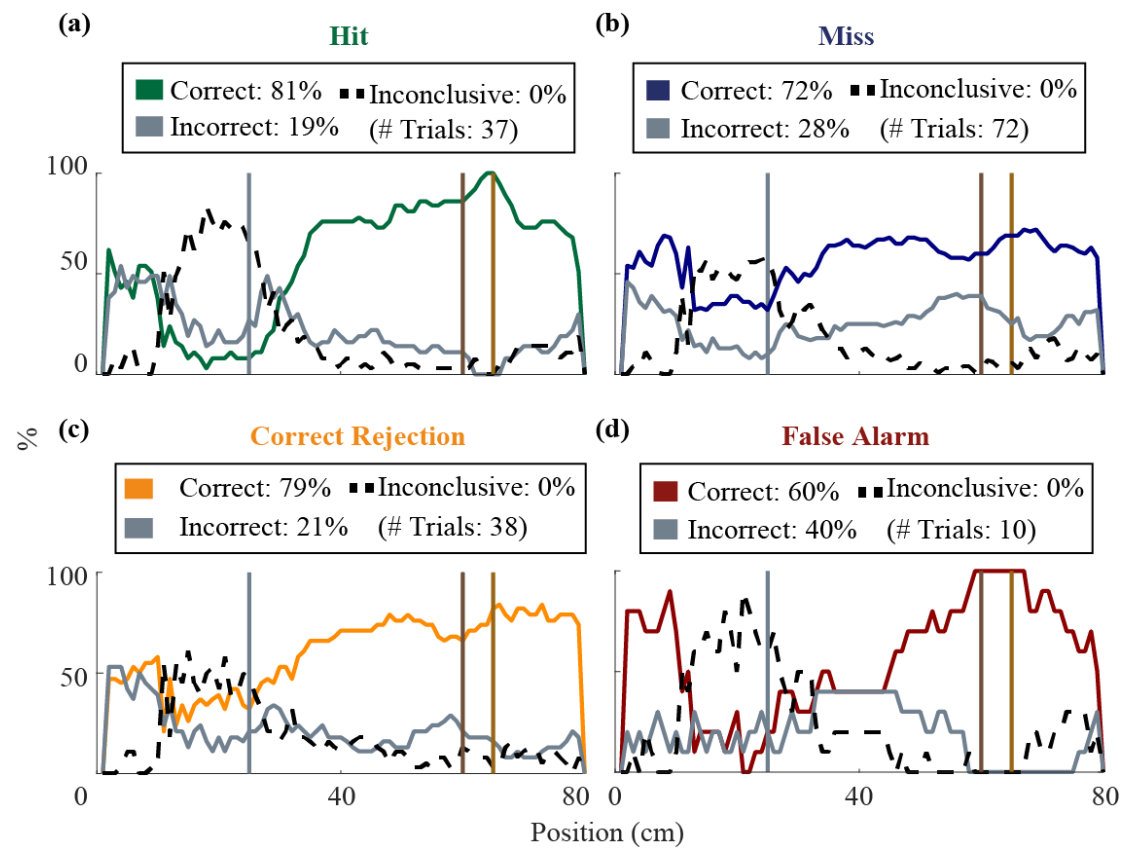


Figure 58: (a) The performance of the locomotion classifier in Hit trials was quantified on a per position basis (plot traces) and an overall classification based on the average trial outcome predicted during the delay period (shown in figure legend). (b) As in (a) for Miss trials. (c) As in (a) for CR trials. (d) As in (a) for FA trials.

Temporal influence on neural response profiles

Given the potential evidence for a sequential pattern of activation in an ACC neuronal population that differentiates T1 and T2 trials, the structure of this activation was assessed by determining the influence of time and space on the response profile of each neuron. In order to determine if the response profile of a neuron was independent of time, the response profiles within trials that have short delay periods was compared that of trials with long delay periods. As shown in the example neurons in Figure 60, there was a spread of delay period lengths across a given recording, yielding a sufficient number of long and short delay periods to quantify neuronal responses within the two. As described in the behavioural protocol, the delay period length was variable as it was dependent on the speed of the mouse in any given trial. In each experiment, there was a range of delay period lengths, an example of which is shown in Figure 59. The delay period durations were divided into intervals of 0.3s, with the first 4 intervals [0-1.2s] defining the short delay period, and the final 4 intervals [1.8-maxDelayPeriodDuration s] defining the long delay period. The response profiles were compared by examining the PSPH and the AUROC values per position as previously described. There was evidence for both temporally dependent and temporally independent coding regimes. A neuron with a temporally independent response profile in T1 and T2 trials is shown in Figure 61. Note that no significant AUROC value is found in the delay period between short and long T1 (Figure 61 (e)) and T2 trials (Figure 61 (f)). An example of the temporal dependency is shown in the T1 trials of Figure 62 (b) & (e), whereby the response profile is altered across short and long delay periods. The response profile of T2 trials however, shows a temporally independent response profile as the profile is maintained across delay period lengths. This may be an indication of a spatially encoded cell. The temporal dependency of neural response profiles within specific trial types, T1 and T2 trials, was examined by assessing the AUROC values of short and long T1 trials for all cells (Figure 63 (a)) and T2 trials (Figure 63 (b)). Of interest, the T1 trials show a mixture of temporal encoding and potential spatial encoding within the cell population, whereas T2 trials show a strong temporal encoding. This could potentially be connected to the larger variability/non-repeatability of T2 trials in the context map and the PCA classifier, as both sets of analysis were performed in the spatial domain i.e. the firing rate was binned in position bins of 1cm. Based on the findings here, some cells are stable in the spatial domain in T1 trials, but very few, if any, are stable in the spatial domain in T2 trials. Thus, the process of computing

the firing rate on a position bin basis, as was the case for the context map and PCA classifier analysis, would introduce noise into a signal that was in fact stable in the temporal domain. Thus, analysing T2 trials through this lens would make the response profile appear more variable and less robust.

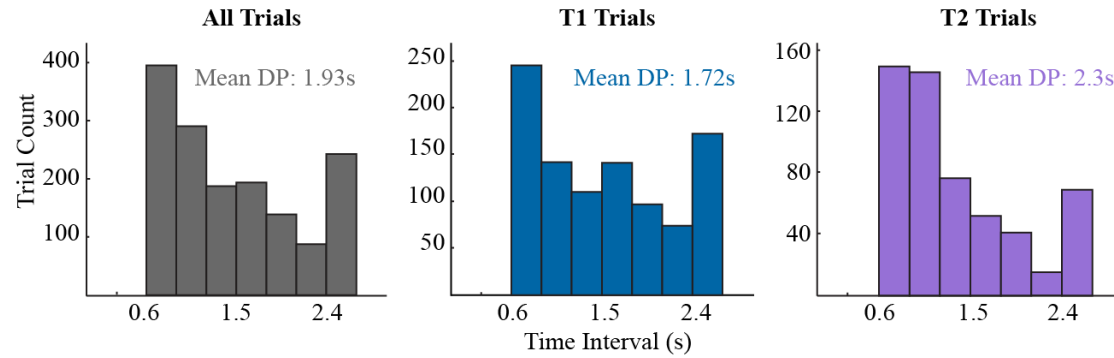


Figure 59: (a) The distribution of delay period duration for all trials (bin size = 0.3s). (b) As in (a) for T1 trials. (c) As in (a) for T2 trials. Note: For each plot the final time bin includes all trials with a delay period greater than 2.4s.

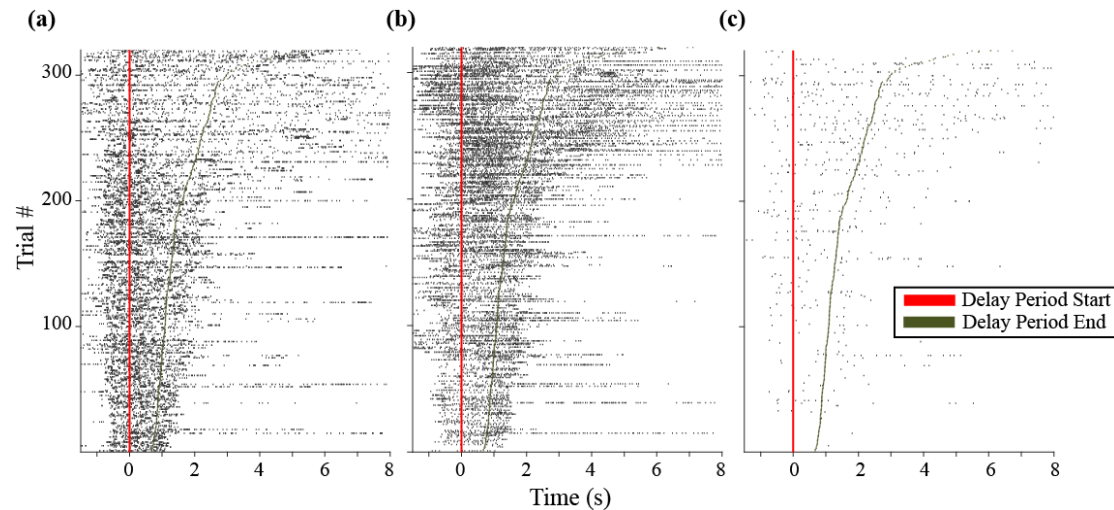


Figure 60: (a-c) Raster plots for three sample cells aligned to tone presentation (start of the delay period). Trials were ordered according to delay period length.

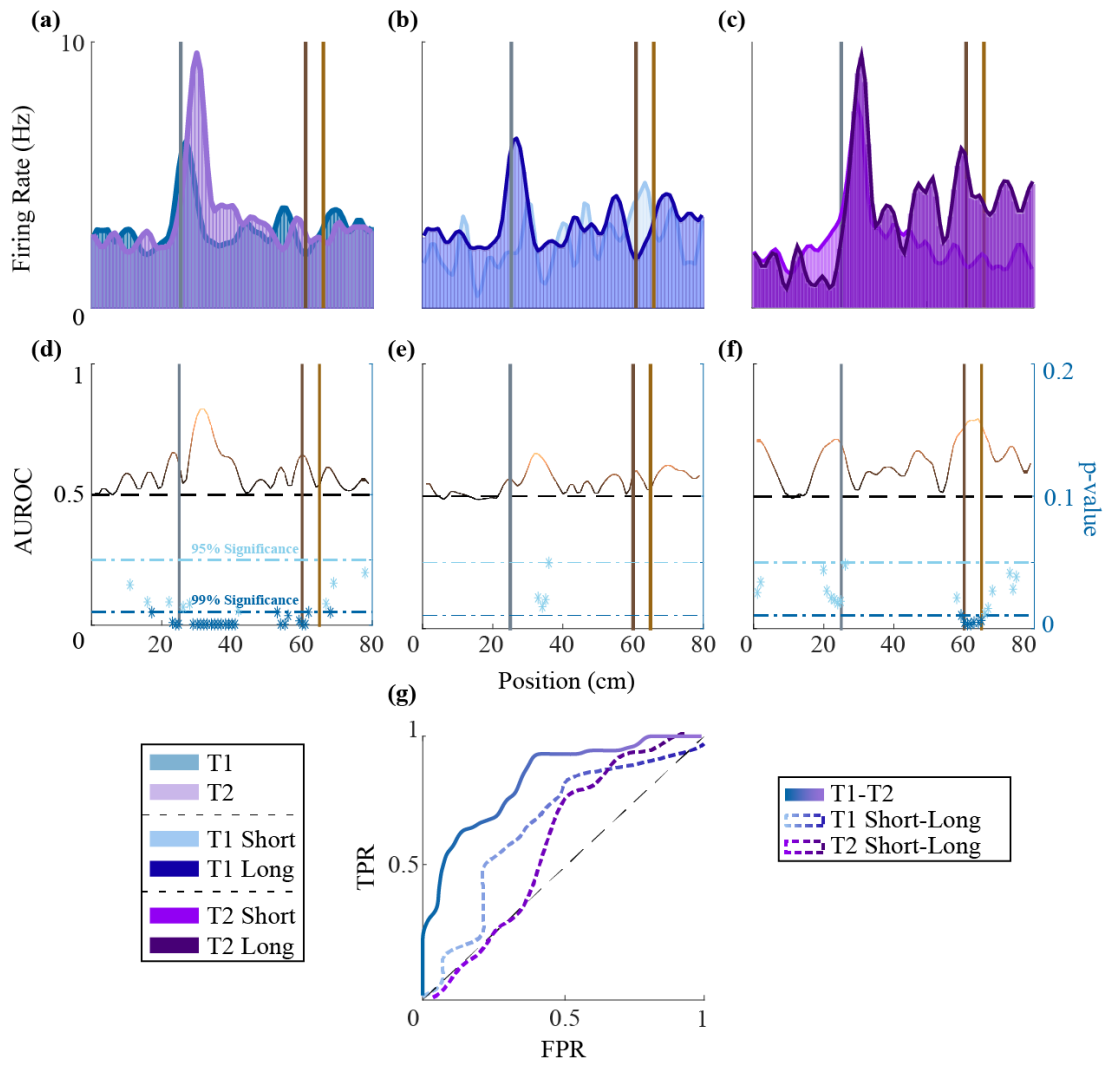


Figure 61: Temporally independent delay period response profile. (a) PSH for all T1 and T2 trials, with (d) corresponding AUROC profile. (b) PSH for T1 trials, divided into short and long delay periods, with (e) corresponding AUROC profile. Note the lack of any significant discrimination in the response profile of the neuron between T1 trials with short and long delay periods. (c) As in (b) for T2 trials, with (f) corresponding AUROC profile. Note the lack of significant differentiation in the delay period of the response profile of the neuron between T2 trials with short and long delay periods. (g) The peak AUROC value in (d) occurs at position = 32cm. The ROC curve for this position is plotted for T1-T2 trials, T1 short – T1 long trials, and T2 short – T2 long trials. Note the reduction in discrimination ability at this position in the response profiles of the neuron between short and long trials.

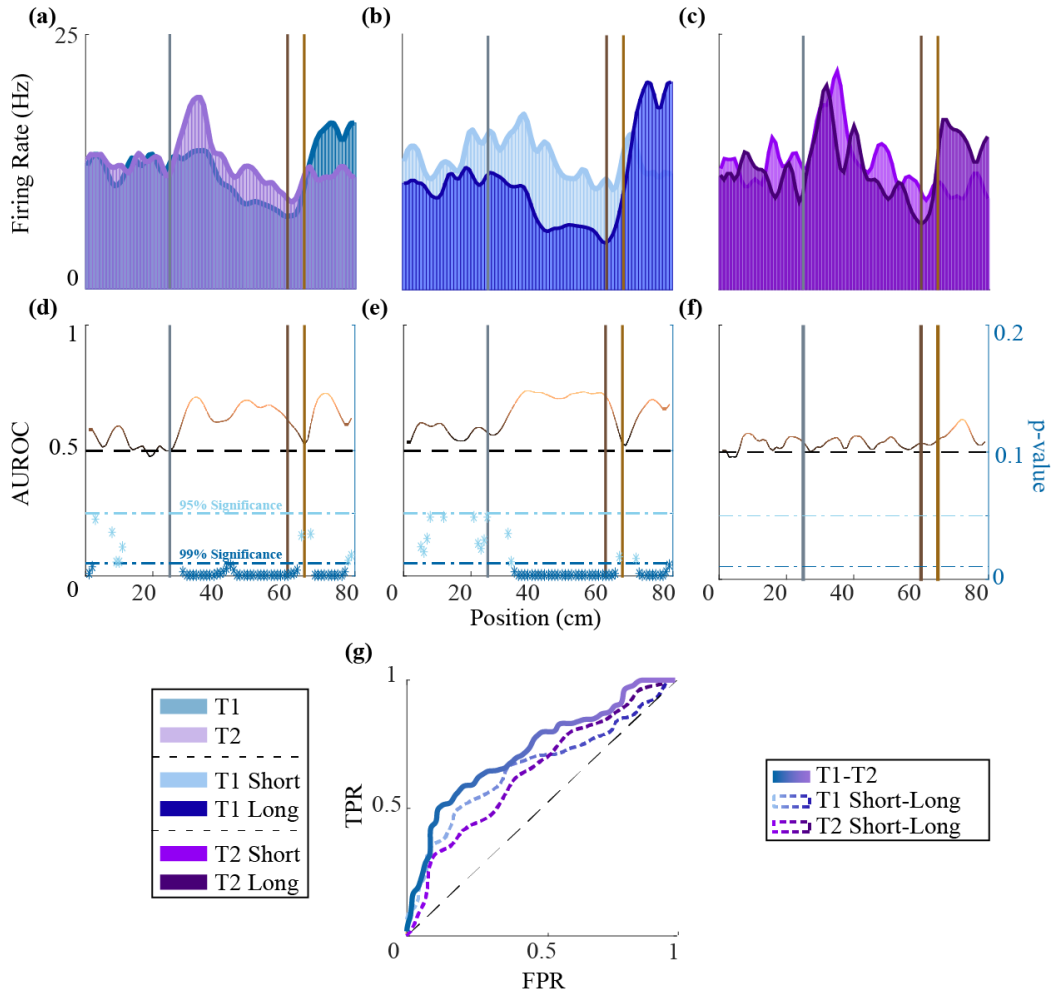


Figure 62: Temporally dependent delay period response profile. (a) PSPH for all T1 and T2 trials, with (d) corresponding AUROC profile. (b) PSPH for T1 trials, divided into short and long delay periods, with (e) corresponding AUROC profile. Note the significant discrimination in the response profile of the neuron between T1 trials with short and long delay periods. (c) As in (b) for T2 trials, with (f) corresponding AUROC profile. Note the lack of significant differentiation in the response profile of the neuron between T2 trials with short and long delay periods. (g) The ROC curves for the peak AUROC value in (d) are compared between T1-T2 trials, T1 short – T1 long trials, and T2 short – T2 long trials. Note: The temporal influence is only evident in the response profile during T1 trials. The response profile appears to be temporally independent in T2 trials.

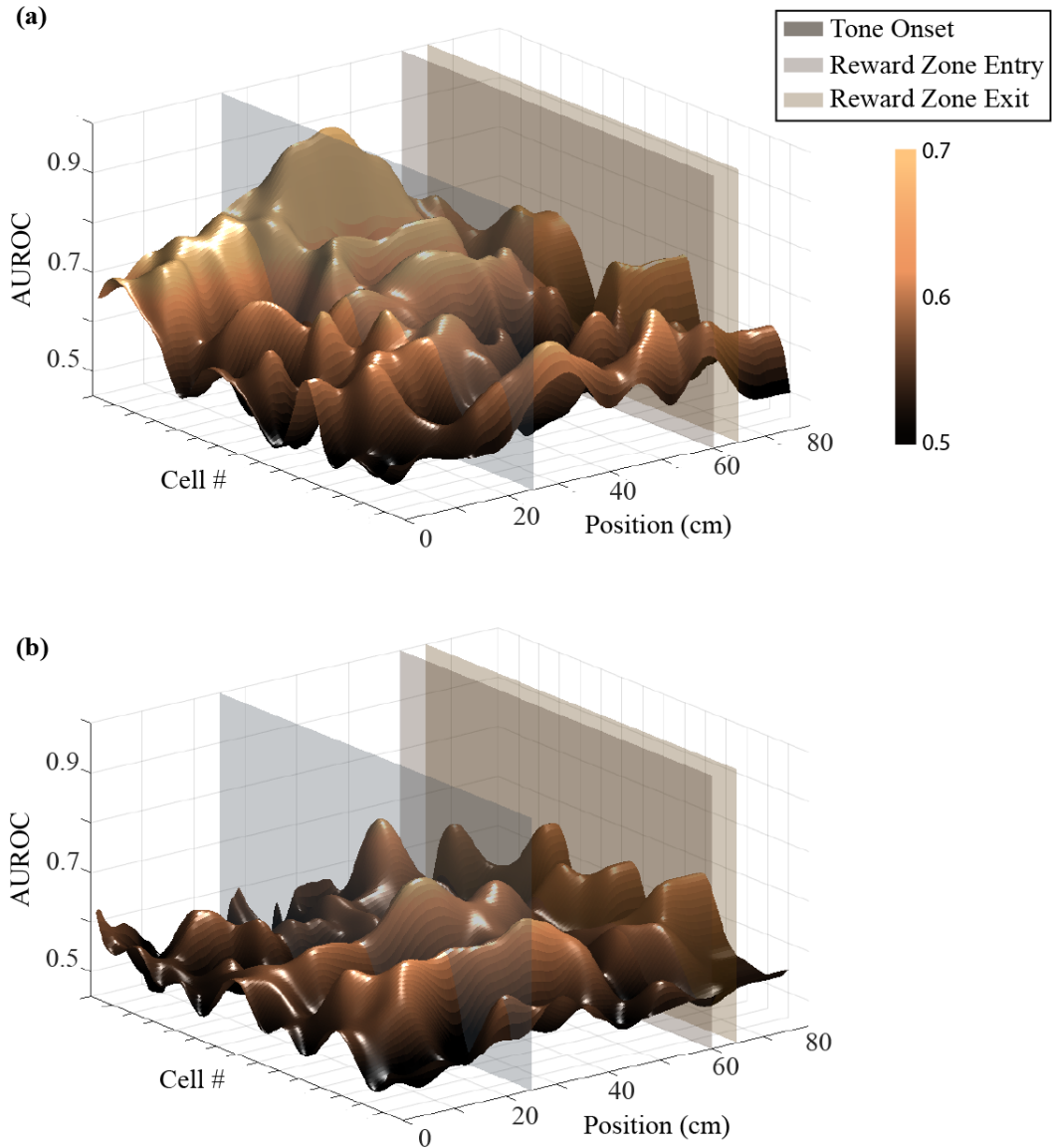


Figure 63: (a) The AUROC values for each belt position showing discriminability between T1 trials with short delay periods and long delay periods. (b) As in (a) for T2 trials.

Optogenetic perturbation of delay period activity

The results presented here provide evidence for many different working memory phenomena. The results show single unit encoding of working memory information in conjunction with population level encoding. Moreover the single unit encoding does not appear to be restricted to low-level information, with single units such as that shown in Figure 37 exhibiting predictive properties. The distribution of scores across multiple neurons in the PCA classifier between T1 and T2 cells in Figure 48 show that at least in state space, the bifurcation between T1 and T2 trials relies on more than a few predictive cells.

Furthermore, much of the results lead towards a postulation that different encoding properties are present between T1 and T2 trials. T1 trials appear to have a strong temporal influence (Figure 63), whereas the response profiles in T2 trials appear to be more temporally independent. The next step to delve further into these findings is to perturb the network whilst the mouse is performing a working memory task. This was performed using optogenetics in conjunction with a genetic mouse line (Pvalb-Cre). A subpopulation of the ACC were directly indirectly silenced during the delay period by activating the pv-interneuron population, and thereby introducing a strong inhibition in the circuit and silencing the pyramidal cells. This technique has the potential to uncover the mechanistic phenomenon behind the mnemonic, decision, and perception responses that have been highlighted thus far.

The results presented here show evidence of delay period encoding via both persistent activity (Figure 33 (a)), and transient activation (Figure 35). The perturbation technique presented here involves a single laser pulse for 100ms, 400ms after the tone is presented. This allows sufficient time for the cue information to be integrated, whilst allowing the effect of perturbation to subside before the end of the delay period. For neurons that display persistent activity, could they maintain their neural representations despite being perturbed in the middle of the delay period? Moreover, for the neurons that exhibit transient activation during the delay period, is this a sequential population code that could be disrupted by temporarily silencing the network? Will this result in the re-organisation of a sequence? Or are the neurons that are transiently activated independent of their neighbours' activity and hence will always respond at a particular position/time in the delay period despite what happened to the neurons in the previous part of the sequence. If true, this hypothesis could represent a certain redundancy in the mnemonic encoding mechanism of working memory. The ability of neurons to respond more independently of their peers may represent a mechanism by which the network can readily update to a new relevant input, and also be vulnerable to noise/perturbation if a sufficient number of these neurons are perturbed.

The presence of predictive neurons early in the delay period (e.g. within ~120ms in Figure 37), and the ability of the PCA classifier to classify the outcome of a given trial shortly after cue presentation, would lead one to question the potential redundancy of the activity during the remainder of the delay period. It may be possible that the integration of the cue information, and the upcoming decision occurs

early in the delay period and that this information is recalled just before the response is required (see the tone echo cells of Figure 32 (b-c)). If this is the case, the perturbation performed here will induce a negligible change in performance, as the information being encoded during the middle of the delay period provides a redundant function, i.e. the neural activity in the latter portion of the delay period may increase the robustness of memory encoded or the upcoming decision, but not be crucial to the correct execution of the task. Hence, whether this perturbation disrupts the behaviour, introduces more noise into the behaviour, or does not disrupt the behaviour whatsoever, will inform us in terms of the important epochs of working memory encoding, and indeed indicate whether there is redundancy present in the network. Furthermore, the potential sequence of activation shown in Figure 42 could be probed to examine whether the perturbation produces a large reorganisation of the sequence of activation, or indeed if this method of encoding is abolished entirely. The latter may point to a stronger reliance of the network on single unit encoding of mnemonic information, as it may be more robust to this type of perturbation. These are few of many different hypotheses that can be probed using this technique. For example, a multitude of interesting observations would be found by simply altering the location of the perturbation within the trial. For the results presented here, the efficacy of this technique to perturb neural activity of the ACC during the delay period of a working memory task will be evaluated. Also, some preliminary results will show the effect on the behaviour of the mouse, and how this is reflected by the response profiles of the neural population.

Optogenetics has been successfully utilised to perturb cortical network activity in numerous studies (161,173,198-200). It has the distinct advantages over techniques such as muscimol injection and cooling of neural tissue, of having high temporal precision (ms timescale), reversibility, targeted stimulation, and high reliability (172). The chosen opsin, ChR2, is a non-specific cation channel that acts to depolarise the cell when stimulated using blue light ($\lambda = 450-490\text{nm}$) (201). The opsin is injected into the target brain region of a Pvalb-Cre mouse line, via an AAV. This will ensure that the opsin will only be expressed in the pv-interneurons of the brain region in which the virus was injected. Thus, the presentation of blue light to this neural tissue will activate pv-interneurons. PV-interneurons have been shown to have a modulatory role on pyramidal cell activity in cortical circuits (8,9). Hence, activation of this sub-population is highly likely to induce network-wide disruption by introducing inhibition to the circuit. A single laser pulse (duration = 100ms, intensity = 2.25mW at optic fibre tip)

was applied to the ACC network via optrode stimulation, 400ms after cue presentation. The stimulation was administered during the delay period with a sufficient offset from the cue to avoid interfering with cortical processing of the tone, whilst also allowing sufficient time for network activity to recover before the end of the delay period. The advantage of using an optrode to stimulate the neurons lies in the highly targeted nature of the technique, as it allows the acquisition of neural signals from the population receiving light stimulation.

Another significant factor in this technique is the evidence for redundancy across hemispheres. A study by Li et al. (162), examined the perturbation potential of preparatory motor activity in the premotor cortex to unilateral and bilateral silencing. Unilateral stimulation proved insufficient in disrupting premotor neural activity. In this case, the response profiles of neurons were found to recover to a state comparable to that of unperturbed trials following laser stimulation. However, bilateral stimulation radically reduced behavioural performance and produced a strong perturbation on an electrophysiology level. Whilst neurons were found to recover their firing rate following bilateral stimulation, their selectivity for task epochs was greatly diminished, contrary to unilateral stimulation. The findings of this study render it unlikely that the stimulation of a small sub-population that an optrode would target in a single hemisphere, would be sufficient to cause a behavioural shift in performance. Nonetheless, it still has merit as a technique for assessing the degree of perturbation required to suppress neuronal responses to tone presentations and upcoming choice predictions.

The chief advantage of this technique is the versatility. Stimulation via an optrode can be replaced with bilateral optic fibres to compensate for redundancy that may be present across hemispheres. Furthermore, with either technique, the timing of the perturbation during the delay period, and the duration of the perturbation can be used to determine the robustness of the network to perturbations, and of single neuron response profiles. Thus, this technique has the potential to unveil the mechanistic function behind working memory.

Experiment outline

The experiments were performed as per the working memory experiments, with the addition of optogenetic stimulation during specific trials. The laser stimulation protocol was as follows:

- **Pre-Laser Epoch:** Trials 1:60 – DR Task as normal, no laser stimulation.
- **Post-Laser Epoch:** Trials 61:session end – Laser trials interleaved with non-laser trials with a probability of $p(\text{tr}_{\text{laser}}) = 0.7$, $p(\text{tr}_{\text{non-laser}}) = 0.3$ respectively.

A laser trial consisted of a laser pulse (duration = 100ms, intensity = 2.25mW at optic fibre tip), 400ms following tone onset.

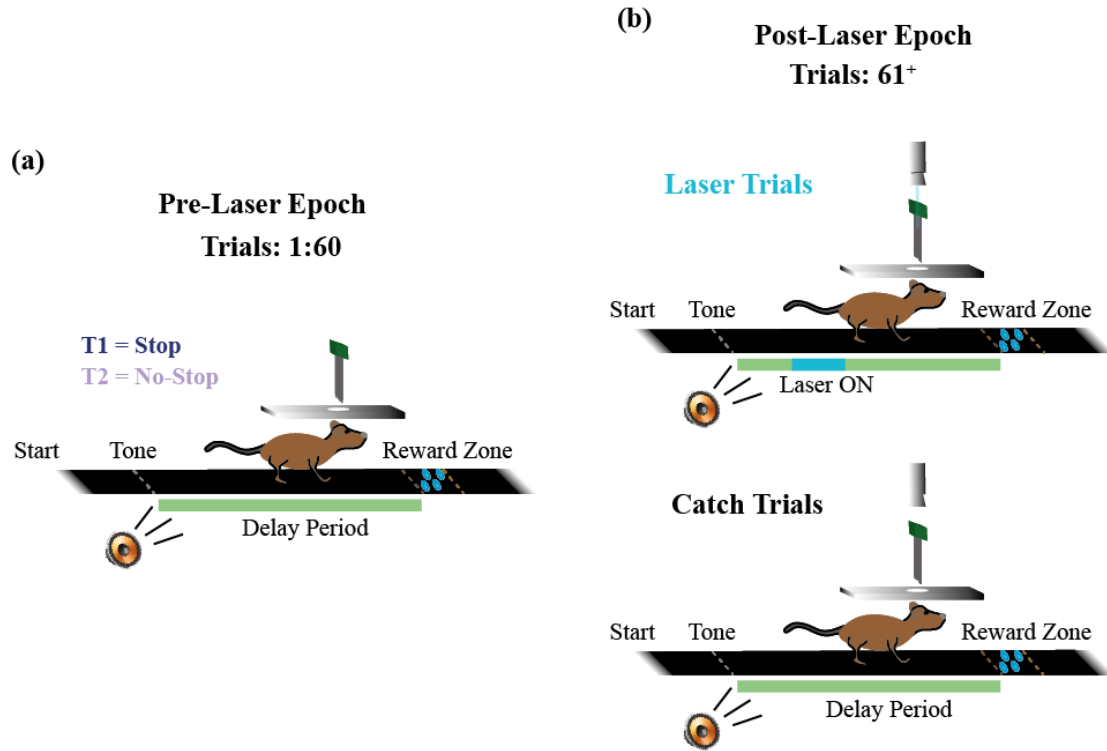


Figure 64: Recordings of neuronal activity in the ACC during a delayed-response task using optogenetic perturbation. (a) Pre-laser epoch: Trials 1-60 of the experiment were performed as in Figure 27. (b) Post-laser epoch: Trials 61⁺ were composed of interleaved laser trials and catch trials. The probability of the presentation of a laser trial was ~ 0.72 , and the probability of a non-laser trial was ~ 0.28 . The probabilities are approximate due to the fact that T1 and T2 trials are presented with a probability of 0.5 each. A laser trial was presented with the aforementioned probability with the inclusion that if the previous laser trial was a T1 trial, the subsequent laser trial would be a T2 trial. Hence, the probability of a laser trial fluctuated slightly between experiments. During a laser trial, a 100ms pulse of blue light was presented 400ms after the tone.

Verification of neural stimulation

A test experiment was performed to verify sufficient opsin transfection to induce neural depolarisation upon activation, and to calibrate the intensity of the laser required to perturb the network. By

manufacturer specifications (NeuroNexus), 75% of the light intensity at the tip of the optic fibre is transmitted to the optic fibre terminal on the optrode ($\varnothing = 50\mu\text{m}$), equating to 2.25mW. This light intensity is large enough to activate ChR2 (172), without damaging neural tissue (173), whilst being comparable with other experimental protocols (162). Although, it is worth noting that the light intensity will not be uniform across all targeted neurons as it will be dependent on the volume of neural tissue it penetrates before reaching a particular cell.

The direct effect of light stimulation on optogenetically-tagged pv-interneurons is shown in Figure 65 (a). The onset time for stimulation is within 20ms, with a rebound effect lasting $\sim 100\text{ms}$ following stimulation offset. The indirect inhibition of putative pyramidal cells is shown in Figure 65 (c). The latency of this onset response is $>20\text{ms}$, which is expected given that it is a minimum of a 2nd degree effect of the stimulation i.e. the light activated an interneuron(s) that inhibited the pyramidal cells. As previously mentioned this time period was crucial as it was within the duration of the delay period. Hence in theory, the neuronal activity would have time to recover before the end of the delay period.

A demonstration of the opto-tagging function of this technique is shown in the pv-interneurons and putative pyramidal cells identified in Figure 65 (b) & (d). This technique can be used to identify these two sub-populations via their responses to stimulation, and determine if WM response profiles are specific to certain populations (see Appendix Figure 74).

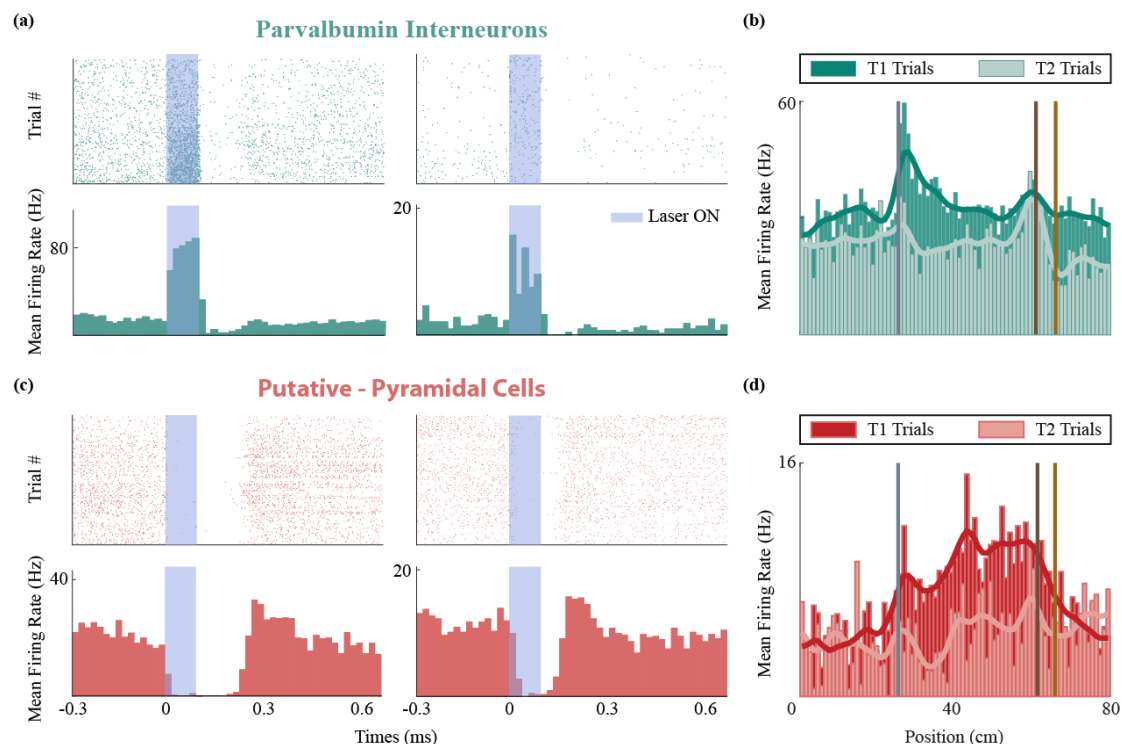


Figure 65: Verification of perturbation. (a) Upper panel: A raster plot of an opto-tagged pv-interneurons showing the response for a laser pulse of duration = 100ms, $\lambda = 430-490\text{nm}$, intensity = 2.25mW. Lower panel: A psth of the same activity (binsize = 20ms). (b) The response of an opto-tagged pv-interneuron during the DR Task. (c) As in (a) for putative pyramidal cells. (d) As in (b) for a putative pyramidal cell.

Perturbation effect on working memory performance

The effect of perturbation on the behavioural performance was evaluated on two levels:

1. The effect of perturbation on laser trials.
2. The effect of perturbation on the confidence level of the mouse.

In summary, the perturbation of the neural circuit induced a moderate (non-significant) decrease in task performance when comparing the performance in the pre-laser epoch and the laser trials of the post-laser epoch. The speed profile of T1 and T2 trials typically showed a drop in speed following tone presentation. Interestingly, the speed profiles were similar in both T1 and T2 laser trials, where this speed drop was absent (Figure 66 (c)). Furthermore, a higher frequency of mistakes in the laser trials would be likely to impact the confidence of the mouse in catch trials (i.e. non-laser trials in the post-laser epoch). When comparing the pre-laser epoch to the non-laser trials of the post-laser epoch, a

decrease (non-significant) in performance was found, accompanied by a significant decrease in stopping frequency (Figure 67(a)).

The neuronal population that is stimulated by an optrode is small when compared to that of an optic fibre. This local perturbation has the advantage of targeting the neurons that are being recorded, however, from the perspective of disrupting behavioural performance, it is unlikely to be strong enough to illicit a significant change. Moreover, as previously discussed, the redundancy in the cortex across hemispheres would necessitate a bi-hemisphere perturbation to cause a significant drop in performance (162). The perturbation performed here induced a downward trend in working memory performance between the pre-laser epoch and the laser trials (not significant) (Figure 66 (a)). To examine the effect of laser stimulation, the hit, false alarm, and stopping rate were examined (Figure 66 (b)). Of note the Hit rate decreases but the FA rate remains constant. Further examination of the speed profiles show that the mice omit the typical decrease in speed following tone presentation in laser trials (Figure 66 (c)). This may be an indication of a disruption to the decision portion of the delay period activity.

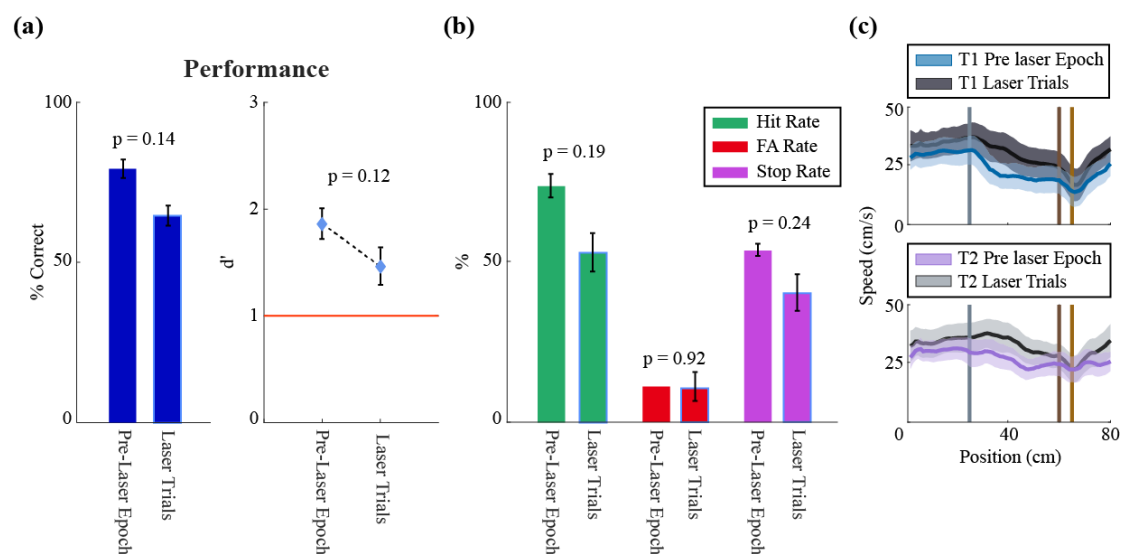


Figure 66: Minor decrease in behavioural performance in response to laser stimulation (n = 3). (a) Left panel: the percent correct for pre-laser epoch versus laser trials. Right panel: d' between pre-laser epoch and laser trials. The red line shows the d' threshold for performance. (b) The Hit, FA and Stopping rate between the pre-laser epoch and laser trials. (c) The speed profiles for T1 and T2 trials comparing the pre-laser epoch and laser trials.

Whilst the neurological effects of the perturbation are highly reversible (the rebound effect lasting for ~100ms), the non-laser trials in the post-laser epoch were examined to determine if the decreased performance in the laser trials influenced the confidence level of the mouse and thus caused a similar decrease in performance as seen in the laser trials. When comparing the non-laser trials (in the post laser epoch) with the pre-laser epoch, the stopping frequency showed a significant decrease ($p = 0.08$). This was accounted for by a drop in both the Hit and FA rates Figure 67 (a). The performance also showed a notable, but non-significant drop from the pre-laser epoch Figure 67 (c).

Overall, the perturbation did not produce a significant change in mouse behaviour despite causing neurological disruption. However, whilst it did not cause a significant decrease in mouse performance in laser trials, the perturbation may have been sufficient to affect the confidence level of the mouse in executing a decision. This may be reflected in the absence of the drop in speed after tone presentation also present in non-laser trials in the post-laser epoch. The minor drop in performance is similar to that which is shown in Li et al., (162) for unilateral perturbation. It is possible that in order to produce a strong behavioural effect, the perturbation would need to be performed in bilaterally, to compensate for the potential redundancy across hemispheres in the ACC.

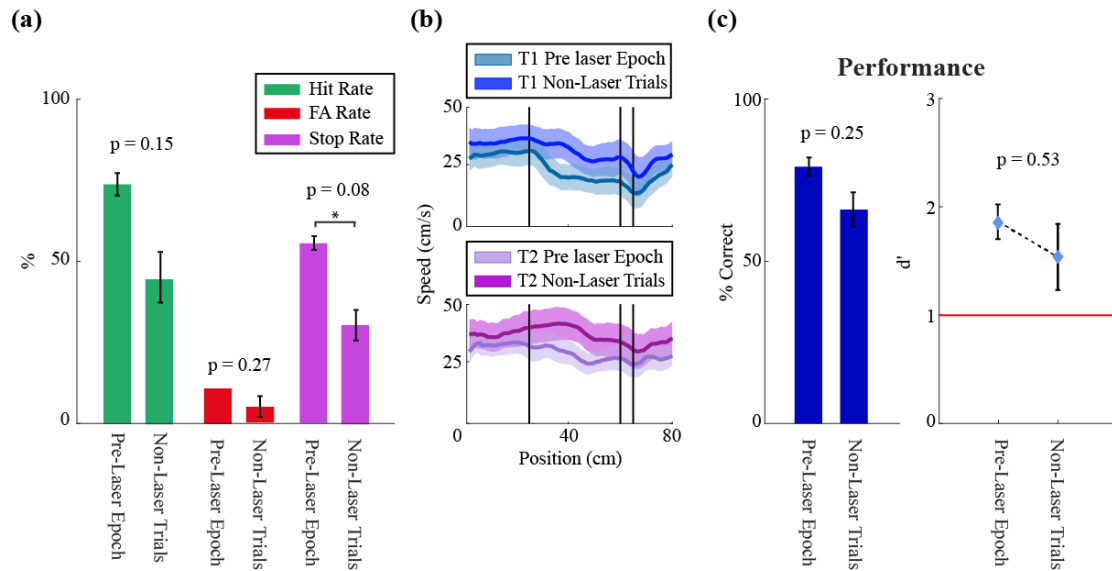


Figure 67: Effect of neuronal perturbation on mouse confidence (n = 3). (a) The Hit, FA and Stop rate between the pre-laser epoch and non-laser trials of the post-laser epoch. (b) The speed profiles of T1 and T2 trials between the pre-laser epoch and non-laser trials. (c) The percent correct and d' for the two trial types.

Perturbation effect on working memory neuronal responses

Laser stimulation disrupted WM responses of many neurons during the DR Task during trials in which the laser was presented (Figure 68). Given the higher proportion of pyramidal cells typically recorded from in extracellular recordings of this type, most neurons showed an inhibitory effect whilst the laser was activated. The excitatory and inhibitory effect of the stimulus can be seen in the examples of pv-interneurons and a putative pyramidal cell in Figure 69. An example of the effect of laser stimulation on neural activity can be seen in the comparison between panels (a) and (c) of Figure 70 and Figure 71. In both instances the laser interrupted the response profile of the neuron during the delay period. The neurons were unable to recover their response profile for the remainder of the trial i.e. mimic the remainder of the response profile shown in panel (a) for T1 and T2 trials. However, the effect of the laser on subsequent non-laser trials during the post-laser epoch differed. Some neurons successfully retained their response profiles (Figure 70 (d)), whilst for others, their response profile was eradicated entirely (Figure 71 (d)). The example shown in Figure 70 (a) shows the response of a pyramidal cell during the delay period of T2 trials in the pre-laser epoch. The differentiation ability between T1 and T2 trials is given in the profile of the AUROC value in Figure 70 (b). The response profile is no longer present in laser trials (Figure 70 (c)), however it recovers in non-laser trials Figure 70 (d)). Whilst it is worth noting that despite no simulation present in these trials, the latency of the response profile is longer than in the pre-laser epoch, and furthermore the magnitude of the response is much lower. The example pyramidal cell shown in Figure 71 (a) shows a response profile during the delay period of the pre-laser epoch. Whilst the response is strongest during the beginning of the delay period, the differentiation capacity between T1 and T2 trials is retained for the remainder of the trial (Figure 71 (b)). However, this response is eradicated throughout the post-laser epoch as shown by the response profiles in panels (c) and (d) of Figure 71. Interestingly, Figure 71 (d) appears to reflect the decrease in performance in non-laser trials of the post-laser epoch. This may be an indication that the perturbation added sufficient noise to the network to abolish the response profile of some neurons throughout the post-laser epoch.

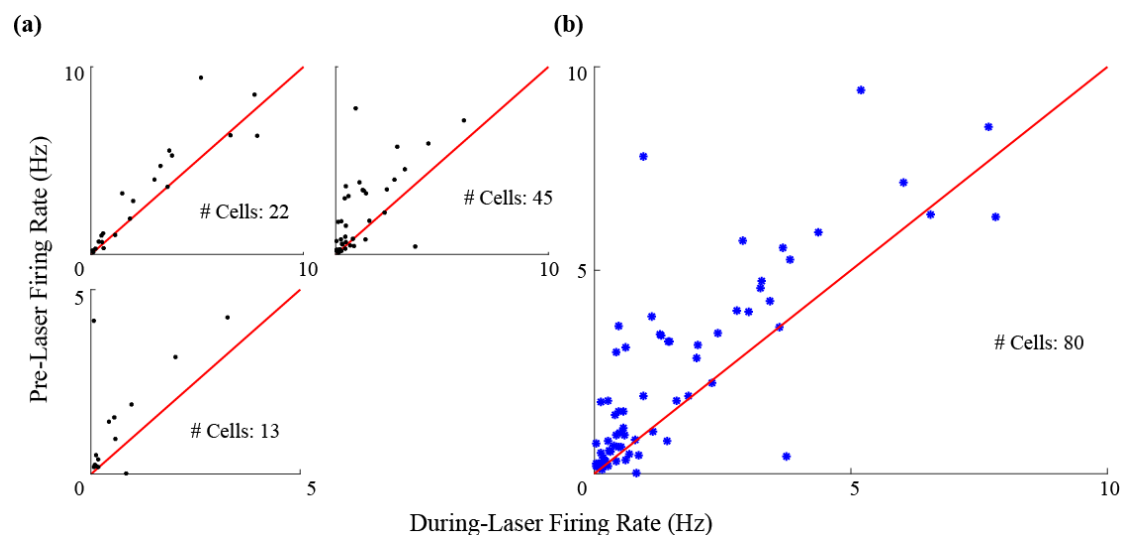


Figure 68: The effect of laser stimulation on neural firing rates. (a) The firing rate of cell populations of three experiments in which laser stimulation was performed during the delay period of a working memory task is shown whereby the pre-laser firing rate is compared with the firing rate of the cell during laser stimulation. Most cells appear to be inhibited by the stimulation (putative pyramidal cells). Of note, not all cells showed a change in firing rate, as shown by the neurons that lay close to the unity line in the plot. (b) As in (a) for all cells in all experiments.

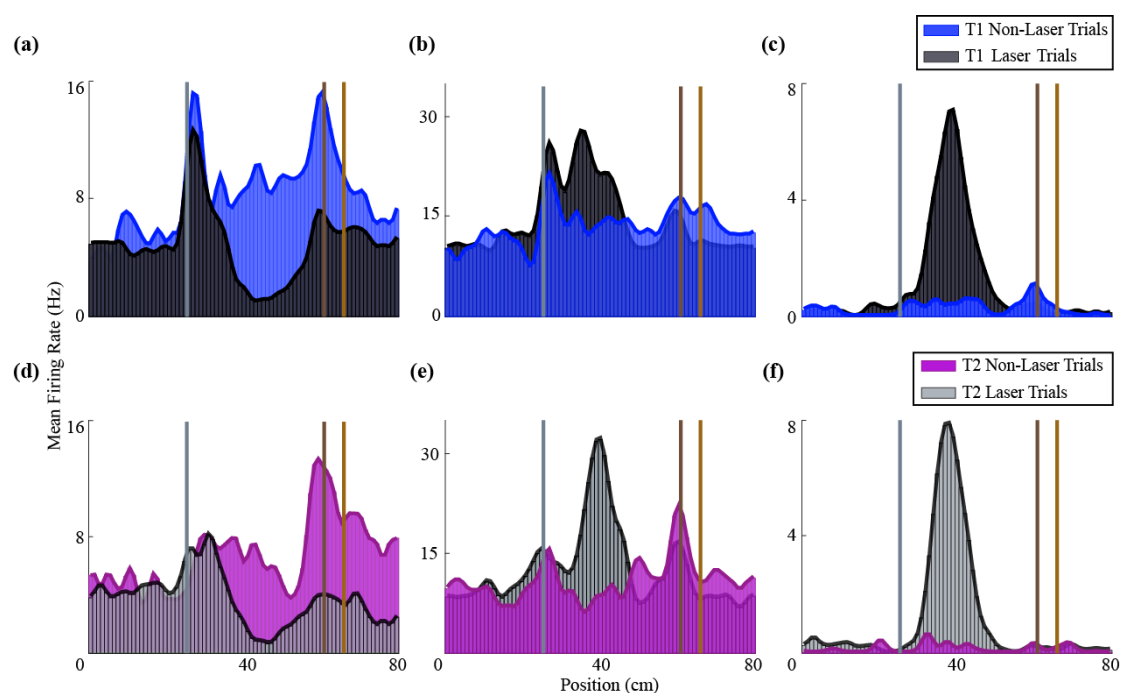
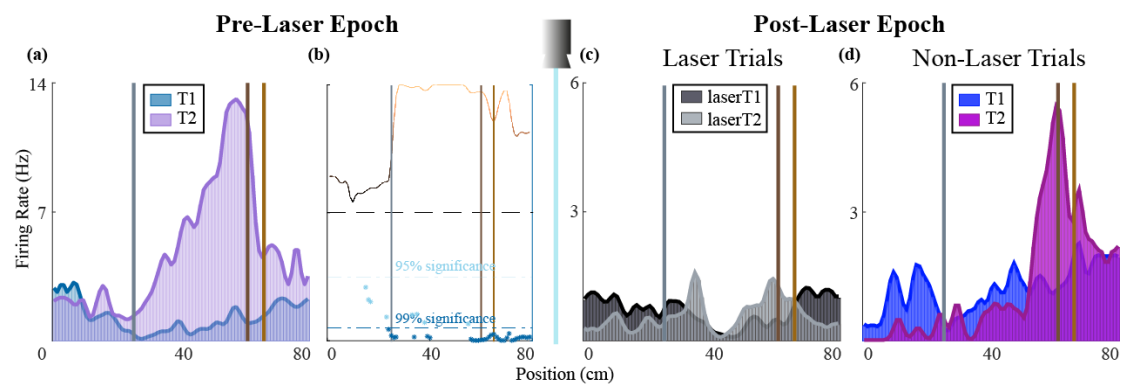
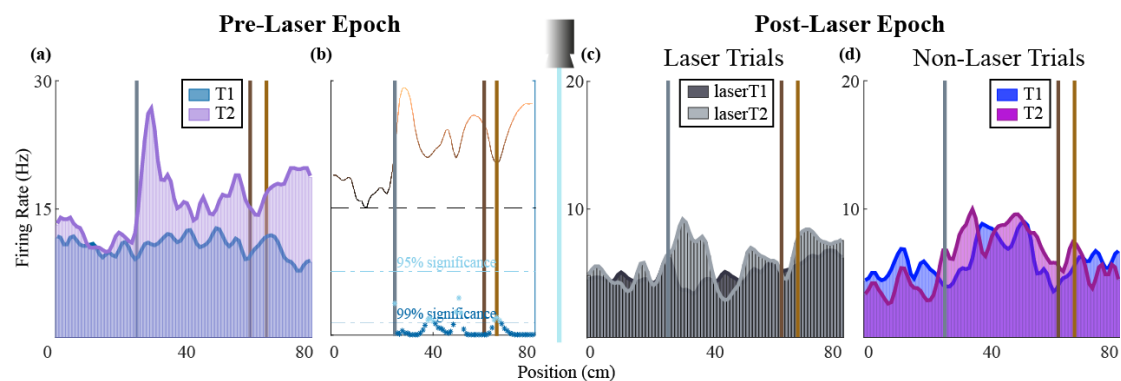


Figure 69: The effect of laser stimulation on neuronal responses in T1 and T2 trials. (a) The neuronal response of a putative pyramidal cell is inhibited in T1 laser trials, and (d) in T2 laser trials. (b) The neuronal response of a pv-interneuron is activated in T1 laser trials, and (e) in T2 laser trials. (c) As in (b) for a more quiescent pv-interneuron.



Discussion

Working memory is an intriguing form of memory that facilitates memory retention for goal-directed tasks, whilst maintaining the balance between being robust to noise, and being flexible to update to new relevant inputs. Over four decades of research has revealed many potential mechanisms behind this phenomenon, including persistent activity, transient activation, synaptic theory and attractor systems. Whilst evidence for each of these hypothesis exist, we are still obtaining puzzle pieces of the encoding system of working memory in the PFC, which is also reliant on gaining a stronger

understanding of how the neurons of the PFC integrate information. The experiments presented here provide datasets of population activity in the ACC of awake mice performing a delayed response task. The results show evidence for the coarse representation of low-order stimulus information, in conjunction with high-level information such as upcoming choice. The data also shows strong evidence for population encoding of mnemonic activity, through transiently active neurons in the delay period, sequential activation depending on trial type, and the ability of a binary classifier to predict behavioural outcome based on characteristics of population activity. Furthermore, the neural information was able to predict the behavioural outcome of the mouse more rapidly than the locomotion classifier. The neural network was then perturbed by introducing optogenetic activation of pv-interneurons using ChR2. These experiments show the ability to perturb the network on a fine temporal scale, and the versatility of this technique to examine the encoding patterns of the ACC at different points in the delay period. There is also evidence presented to show the potential for a behavioural disturbance to be introduced via this form of perturbation, once the perturbation parameters were more finely tuned. This forms an exciting method to test the hypotheses found in the previous experiments, and help future experiments determine the mechanism behind the encoding of working memory in the neural circuits of the ACC.

Delayed response task as a working memory paradigm

The delayed response task implemented as numerous advantages as outlined in section Delayed-response working memory task. However, as with all working memory paradigms there are disadvantages to this task, each of which are addressed below:

- 1) Mice can use postural mediation strategies such as a specific locomotion pattern from tone onset, to reduce the working memory load in the task (202).
- 2) Task difficulty results in a relatively long training period for mice, with multiple cut-off points from which mice are removed from training (see section Behavioural training hurdles).

The results of the training data shown in Figure 8 preclude disadvantage (1). The training data show a drop in performance as the delay period is lengthened. If an alternative behavioural cue was being used after tone presentation, the duration of the delay period would be independent from the performance. Regarding disadvantage (2), the yield in the number of mice per batch that successfully performed the full expression of the task, improved to a 70% yield in the final protocol. Given that it is necessary to

engage the mice in a task that has sufficient cognitive load to engage the PFC, and be resistant to over-training, it is somewhat expected that the yield would be less than 100% in any given batch. The cognitive demand that the task imposed on the mice was estimated by increasing the working memory load through lengthening the delay period until the performance of the mice began to decrease. At this time, the working memory load would be fixed at this point, and thereby ensure that the cognitive demand in the task was high, and thus engage PFC activity.

Response properties of single unit activity during a delayed response task

The response properties of single neurons in the PFC across species (94,95,146) have shown tuning properties to specific epochs of a working memory task, namely cue-selective neurons, delay period selective neurons and response selective neurons. Indeed, neurons of the PFC are known for their versatility in encoding information of different sensory modalities and different cognitive levels (i.e. low level information such as the colour of a stimulus, or high level information such as reward anticipation). For example, Mante et al., found evidence for the encoding of upcoming choice, context, strength of motion and colour (103). The findings presented here show that in a sample population of the ACC, a similar array of response properties are seen amongst recorded cells (Figure 32-36). Of note, the classification of response epochs as cue, delay period and reward zone, are not discrete in the context of this experiment. For example, the cue period is continuous with the beginning of the delay period. As such, the segregation of task epochs were subjective to the criteria set forth in this experiment (see section Single unit activity). Many neurons showed intrigued mixed selectivity for different task parameters depending on trial type, as seen in the tone echo responses of Figure 32 (b-c). This neuron shows a bimodal response in T1 trials, which may be evidence of a tone echo response i.e. a recall of the stimulus immediately preceding the reward zone to prime the upcoming response. This bimodal response was absent in the T2 trials whereby only a single peak in firing rate was found after the tone was presented. This example shows the ability of a single neuron to robustly encode distinct neuronal responses depending on context i.e. trial type, and rapidly change between the representations of the two as the inter-trial period was <1s.

The tone responsive neurons showed an interesting array of both low and high level encoding. An example of a tone responsive cell can be seen in Figure 32 (a). This cell shows a shift in response

latency depending on trial type, with the corresponding peak AUROC value occurring shortly after tone presentation Figure 32 (d). Upon closer examination, some tone-modulated cells could be divided into stimulus-specific neurons (Figure 36), and predictive neurons (Figure 37). The stimulus specific neurons represent low-level information encoding as the response property of the neuron had a strong fidelity with the type of tone that was presented, regardless of behavioural outcome. This is shown in Figure 36 (a,c) as the response profiles for Hit and Miss trials are approximately equal, as tone T1 was presented in both instances, and CR and FA trials are similar, when tone T2 was presented in both instances Figure 36 (b,d). Of note, all response profiles in relation to FA trials are less reliable than their Hit/Miss/CR counterparts due to the low number of FA trials present. On the contrary, some tone-modulated neurons functioned as early stage predictors of upcoming behaviour (Figure 37). Despite different tones being present in Hit and FA trials, the response profiles are remarkably similar Figure 37 (a). A similar effect is seen for CR and Miss trials. The strong discriminability is seen in Figure 37 (b). Despite the presentation of tone T1 in both Hit and Miss trials, the neural response of this neuron can predict the upcoming behavioural response of the mouse within ~120ms of cue presentation. This is an example of high-level information in the form of predictive properties being encoded on a single neuron level.

Neurons that responded in the delay period showed a mixture of persistent activity (Figure 33 (a)) and transiently active neurons that had a peak response during different parts of the delay period (Figure 33 (b-c)). Similar to the previously reported findings (139), the results presented here show a mixture of the two proposed encoding mechanisms of mnemonic information. In this study, the transient activation appeared to account for a larger proportion of delay period responsive cells than cells that displayed persistent activity. Moreover, the transient activity often shifted location depending on trial type (Figure 30). It may be that the dominance of transiently active neurons may point to a dynamic encoding of memory information during the delay period, as opposed to the more static model found in neurons that display persistent activity. Indeed, even on a single neuron basis, the versatility of the neuron increases within a task if the response profile is restricted to a short temporal window i.e. a small portion of the delay period, as this temporal window can be shifted depending on context. Using this method the same neuronal population can encode different contexts/rules and dynamically switch between representations by changing the location/timing of their temporal output.

Overall, the single unit responses represented a diverse response profile over different task parameters. Their response profiles were also measured by their ability to discriminate between T1 and T2 trials. It can be seen in the diversity of AUROC values in Figure 35, that this differentiation was very pronounced in some neurons and much more subtle in others. For instance, those that display high AUROC values throughout the delay period may be representative of the presence of persistent activity for a particular trial type, whereas a neuron that showed a transiently active response profile during the delay period which shifted depending on trial type, is more likely to show the more nuanced, discrete peaks in AUROC values seen in other cells.

A note on the SNR of neurons of the prefrontal cortex

Neurons of the PFC pose a statistical challenge to neuroscientists. A large number of cells display a baseline firing rate of 0-5Hz (>40% in cross modal WM tasks (582 cells), and > 70% spatial delayed response tasks (149 cells) (122)). From a physiological perspective, such sparse firing can be computationally efficient, from an analysis perspective, it renders many current methods, such as assessing differences in firing rates during task epochs, vulnerable to statistical fluctuations. Furthermore, the relative activation/inhibition of delay period responsive cells is moderate; large changes in firing rate defined as a change > 10Hz are rare, with most changes in firing rate lying within a window of <5Hz (122). A comprehensive study of single trial statistics in cross modal WM and spatial delayed response tasks found that in both instances the relative change in firing rate between baseline and delay period was <4Hz for the majority of cells (~60-93% of cells) (122). There is also a large degree of cross- and within-trial variability in neuronal responses in WM tasks, which has been characterised by Shafi et al., (122). As per the hypothesis put forward by Shafi et al., (122), it is possible that the low firing rate and high variability of PFC neurons contributes to an overall low SNR which may have a key functionality in enabling the PFC neurons to be very versatile in encoding task parameters. It may enable single cells to encode information that is sufficiently robust for recall at a later point in the task (e.g. tone echo cells – see Figure 32 (b-c)), and yet be sufficiently vulnerable to perturbation to switch response profiles for a new, more relevant task.

Population encoding findings

Whilst single neuron responses contain a multitude of information, there has been evidence to show that further information is to be gained by analysing the population as a whole, as opposed to the culmination of discrete elements (104), i.e. neuron response profiles. This has been shown in Mante et al., (103), whereby the discrimination between the sensory inputs and the upcoming choice was more difficult to decipher on a single neuron level, but when the population was analysed, the identification of the two types of information became clearer. As previously mentioned, this may be in part due to the higher dimensionality of information contained in the population dataset, as one is also examining co-variability, and other interactions. On a single neuron level, the dataset presented here shows numerous neurons that show transient activation during the delay period, which may indicate evidence for a pattern of activity between neurons during the delay period to encode the memory, similar to the relay-race activity shown by Batuev et al., (123). Moreover, the location of the peak response is seen to shift in some neurons between different trial types (Figure 30). As such, the population of ACC neurons was examined for evidence of a sequence of activation that may provide a phenotype for neuronal activity in a particular trial type. Figure 43 shows evidence for a stereotyped sequence of activation within the neuronal population that is robust in T1 trials ($p = 0.96$, $p = 0.00$). The sequence was much less robust in T2 trials ($p = 0.48$, $p = 0.14$) (Figure 44), although this may be due to a temporal dependence of neuronal responses in T2 trials, which will be discussed further in section The temporal influence on neuronal response profiles in the delay period. Of note, the parameter used to estimate the sequence is the location of the peak firing rate of each neuron, which is one of many potential parameters that may provide more/less robust sequence generation in T1/T2 trials. The “best” sequence estimation was estimated by taking all the sequences generated during cross validation, and from this dataset, calculating the probability of a cell being positioned at a particular index in the sequence. The cells were then sorted according to highest probability and placed in the “best sequence” in that order i.e. the cells with the highest probability for being located in a particular position were given first priority and so forth. The best sequence was then plotted in Figure 43 (a-c) and Figure 44 (a-c). This approach produces the “optimal” sequence by viewing the position of cells individually. The sequence may be better estimated if one were to account for the relationship between cells, i.e. focus on the probability of a collection of cells being adjacent to each other in the sequence, in addition to their overall location in the sequence.

The context-dependent maps shown here echo the trial-type dependent sequential activity shown in Harvey et al., (146). Moreover, the similarity in spatial information between trial types in the results presented here, may indicate similarities to the context-dependent stimulus response mappings of Buschman et al., (21), whereby the mappings were thought to represent task rules. One of the intriguing aspects of the behavioural paradigm implemented here is that on each trial type the spatial information is consistent, as opposed to a T-maze in which the rodent enters a different environment i.e. another arm of a maze, to complete a different trial type. In this respect, the only two elements that have changed between a T1 and a T2 trial are the tone and the trial rule, i.e. to stop or not stop. If the difference in the sequence of activation and the neuronal response profiles between T1 and T2 trials was solely due to different tones being presented, then the Hit and FA trials, and the CR and Miss trials should bear no resemblance to each other, as in both instances different tones are being presented. However, as shown by the predictive neuron in Figure 37, this is certainly not the case. Hence, the evidence would point towards the change in context map shown between T1 and T2 trials, being evidence of rule encoding between the two trial types. This is further emphasised by the performance of the neural classifier that relies on the similarity between Hit and FA, and CR and Miss trials in order to correctly classify all four trial outcomes despite being trained on Hit and CR trials alone. This will be discussed further in section PCA classifier for outcome prediction.

The change in neuronal response patterns shown here between T1 and T2 trials occurs on a very fast timescale (inter-trial period $<1s$). This rapid shift has yet to be shown in an environment that maintains the spatial information between trial types and that changes on a short timescale. This is evidence for the cognitive flexibility within PFC neurons during a WM task. Moreover, given that the population of cells is the same for different representations, it is possible that the network is performing similar to the attractor network proposed by Brody et al., (106). It is possible that the two patterns of activation represent two attractor basins of activity. A proposed mechanism by which neuronal ensembles can switch between representations is via neuronal synchronisation with neural oscillatory bands. It has been shown that synchronisation with neural frequency bands can activate specific neuronal ensembles (15). This could allow specific patterns within a neuronal ensemble to be activated in a fluid, versatile manner, which would facilitate the rapid context switch that is seen here.

PCA classifier for outcome prediction

Based on the evidence for population encoding dynamics between T1 and T2 trials, and the predictive nature of some single units, a neuronal classifier based on PCA was built to determine if the neuronal activity would be a faster, more accurate classifier than behaviour parameters, namely speed. PCA has been performed on cell populations in many occasions to determine patterns of activation in PC state space (146,203-205). Dimensionality reduction is an attractive statistical approach to determining response patterns of neuronal networks. When recording from a neural population, it follows from the highly interconnected nature of the brain that the individual neuronal responses are likely to have a large degree of dependence, and as such can be represented by a much lower dimensional system than the dimensionality taken by the number of neurons within the population. However, the disadvantage of a technique such as PCA, is that the descriptive variables of the lower dimensional space are latent variables, hence they cannot be interpreted into physiological parameters. Furthermore, once a dimensionality, D , is chosen, any variance within the data set that is not explained by the D -latent variables, is assumed to be noise. In addition, PCA examines all variability (not just shared variability as in factor analysis), hence the PCs are influenced more strongly by neurons with higher firing rates (206), due to the Poisson nature of neuronal firing whereby the variance increases with the firing rate (207). However, given the low firing rates of PFC neurons (see section A note on the SNR of neurons of the prefrontal cortex), the bias of neurons with high firing rates should not have strong impact on classifier performance.

The PCA classifier was trained on correct trials only [Hit, CR], despite being tested on the full test dataset containing Hit, Miss, CR and FA trials. Hence the ability for the classifier to correctly predict an incorrect trial was reliant on a Hit trial having a similar neuronal trajectory in state space as an FA, and likewise CR and Miss trials sharing similar trajectories. When the mice perform error trials, this may have been due to one of two reasons; 1) The mouse no longer knew how to perform the task and hence disengaged, or 2) the mouse thought that it was the opposing trial type. The ability of the classifier to predict the outcome of incorrect trials despite only being trained on Hit and CR trials, would indicate that the latter is occurring, and so the mouse is performing the task incorrectly because

he/she thinks that the opposite tone has been presented. Evidence of this on a single neuron level can be seen in Figure 37.

The overall performance of the PCA classifier was moderate (67% correct for the four trial outcomes). The classification performance peaked shortly after tone presentation which may be indicative of a high weighting of prediction neurons, such as the example shown in Figure 37, for the PCs used to construct the state space trajectory. However, the scores for each neuron for each PC used shown in Figure 48, would indicate that a number of neurons were influential in determining each PC, hence it is unlikely that it was only restricted to the tone-modulated neurons that show predictive capacities. Interestingly, of all trial subtypes shown in Figure 50, the classifier had the poorest performance in Hit trials, despite being one of the trial types that the classifier was trained on. This may be due a combination of a low discriminability between Hit and Miss trials (Figure 52 (b), see Figure 51 (b) for a comparison with trials that show a high discriminability), and a strong similarity between CR and Miss trials (Figure 53 (b)). If a Hit trial is miscategorised, it is classed as a Miss trial. Hence, a low discrimination between Hit and Miss trials would facilitate this scenario. However, Miss trials would still be able to be correctly classified if they had a strong resemblance to a CR trial, hence the categorisation of a Miss trial would be more robust. However, the exact reasoning would require further analysis and would be greatly aided by constructing a classifier using a method such as factor analysis whereby the identity of the latent variables could be seen.

Whilst the PCA classifier can correctly predict trial outcome in the majority of cases, an enhanced classifier could be constructed by including two factors:

1. Bayesian inference to account for past information.
2. Utilising a weighted decision boundary based on population firing rate variability.

The current classifier computes the behavioural outcome at each position by treating each position bin as discrete, independent points. The inclusion of a Bayesian prior such that the outcome predicted by a given position bin is influenced by the outcome predicted by the previous n position bins would be highly likely to produce a more effective classifier. Furthermore, the decision boundary could be enhanced by setting the magnitude of the decision line to be weighted according to the variability of the firing rate of the cell population of that point. Given that the variability of the population firing rate

will directly influence the accuracy with which the classifier can determine the outcome, it follows that a more conservative boundary in positions whereby the variability is high, should lead to enhanced classifier performance.

Locomotion classifier for outcome prediction

The classifier built on behavioural data was estimated by computing the mean speed trajectories for different trial types. This classifier outperformed the PCA classifier in terms of overall classification, reaching ~78% during the delay period. However, the PCA classifier was much superior in terms of the speed of which it could make a classification. The locomotion classifier could not make a correct classification until a later point in the delay period. It was expected that the locomotion classifier would be able to correctly classify task outcome given that there were clear speed profiles for each trial type as shown in Figure 27 (b), and whilst the speed profiles between T1 and T2 trials were distinct, the speed profiles between Hit and FA trials, and CR and Miss trials showed strong similarities. As previously discussed, this would enable this type of binary classifier to predict four outcomes. Moreover, this has been shown previously by Cowen et al., (208). In this study, the behavioural data was found to predict the chosen path of the mouse 200ms before the neural data (recorded from the ACC). In the data presented here the significance lies in the speed at which the neural data can predict the outcome, which surpasses the locomotion classifier.

The temporal influence on neuronal response profiles in the delay period

The implication of a population code in the delay period of a working memory task, in combination with the strong presence of transiently active neurons that appear to have a sequential relationship, gives rise to interesting questions as to how this pattern of activation is encoded. In the behavioural paradigm presented here, the delay period length is variable as it is dependent on the speed of the mouse. The advantage of this system over other paradigms that use a fixed delay period, is that it facilitates the examination of the temporal influence on the encoding mechanism, by comparing the response profiles of neurons between trials with short and long delay periods. This has been examined previously by Brody et al., (96), who found that neurons retained their response profiles over a 3 and 6 second delay period. The neuronal response profiles were simply elongated in the 6 second delay

period. The findings here show a mixture of temporally dependent and temporally independent neurons in T1 trials and a strong presence of temporally dependent neurons in T2 trials. This may form part of the reason behind the unstable nature of the context-dependent sequential activation in T2 trials compared with T1 trials, and the higher NRMSE in the PCA classifier in T2 trials (~43%) versus T1 trials (~18%). The input matrices into both sets of analysis were the spatially binned mean firing rates of each cell. As previously mentioned (see section Data analysis), this was performed in order to average over trials without excluding spikes, as the trials were consistent in the spatial domain and variable in the temporal domain. However, if many response profiles in T2 trials have a temporal influence, then this method of firing rate estimation will blur the response profile across the trial, and thus introduce more variability. An example of this can be seen in the neuron in Figure 33 (a), that displayed persistent firing during the delay period, although the response appears more graduated given that the onset time of the activity was blurred through spatial binning of the firing rate. In order to examine this hypothesis further, it would be interesting to build a PCA classifier based on temporally binned firing rates for the first half of the delay period (based on the mean delay period length) and examine the change in the NRMSE of the T2 state space trajectory with the PCA classifier that is built from spatially binned information from the first half of the delay period.

Optogenetic perturbation

Optogenetic perturbation of neural circuits is very a useful method to probe hypotheses regarding the principles of neural circuit encoding (209). The results presented here show the reliability with which pv-interneurons can be activated using this technique, and that pyramidal cells can be indirectly silenced. The neuronal responses shown in Figure 65 show the fast timescale within which the stimulation can induce an effect on the circuit. Neurons responded within 20ms, with the rebound effect of the stimulus lasting ~100ms after stimulus cessation. This enables this technique to be very useful in probing the network mechanisms underlying mnemonic activity in working memory tasks. Many of the results presented in this chapter show evidence for population encoding during the delay period (for example the sequential activation shown in Figure 42), and evidence of transiently active cells that show a peak response at various points in the delay period. The experimental approach presented here provides an opportunity to examine perturbation to the circuit on a fine timescale at multiple points in the delay period. The strength, timing and duration of the stimulus are highly

versatile. However, there are some disadvantages to this technique. Firstly, the strength of perturbation is limited by the scattering of light through neural tissue when trying to activate an entire brain region such as the ACC. Moreover, the strength of stimulation will not be uniform due to the scattering of photons with increased distance from the light source. Secondly, the transfection of the opsin to the target neural tissue contains some variability. One can only make an estimation of the spread of a volume of liquid (~500nL in the case of the injection of an AAV) once injected *in vivo* into the cortex. The transfection of the virus can be verified later via histology, however, the case remains that one cannot accurately control the dissipation of the virus once it is injected. As such, this can lead to variable results such as too little virus resulting in reduced stimulation strength (as fewer neurons will be activated), or non-target brain tissue being infected and subsequently activated when the laser pulse is delivered. However, these disadvantages are fundamental to most optogenetic experiments and so once appropriate precautions are made in terms of verifying the transfection of the virus, and restricting the experimental criteria to examining minor perturbations to a given neural circuit, this is a very useful technique for examining mechanisms such as the encoding principles underlying working memory.

Effects of perturbation on working memory performance

The effect of unilateral perturbation on the behavioural performance of the mouse was minor. Whilst the mice displayed behavioural phenotypes in laser trials, that appeared to be similar across both T1 and T2 laser trials (as seen in the speed profiles of Figure 66 (c)), this was not sufficient to produce a significant decrease in mouse performance. Although, the disruption appeared to induce a sufficient number of mistakes in the laser trials to affect the confidence of the mouse in non-laser trials of the post-laser epoch. The findings of Li et al., (162), show that in motor planning, unilateral perturbation only produces a minor decrease in performance, despite an increase in volume of neural tissue disrupted. However, once bilateral perturbation was performed, the behavioural performance significantly decreased. In the context of the experiments performed here, it may be the case that sufficient redundancy exists between hemispheres of the ACC to allow the execution of working memory to remain intact despite disruption to a single hemisphere. It is also possible that the perturbation was not strong enough to disrupt the network. In comparison with a technique such as the injection of muscimol to silence the neural activity, the activation of a small population of pv-interneurons may not have sufficed to inhibit the entire network of the ACC. In future work, both

hypotheses could be examined by performing bilateral laser stimulation and altering the length of time within the delay period for which the network is silenced. Indeed, if required, muscimol may be injected into the ACC to examine the effects of a strong perturbation of the network. However, this would yield less interesting results on a neural network encoding level, hence if a sufficient perturbation could be obtained through laser stimulation, that would be the most desired approach. Furthermore, it is important to consider the potential for the ACC to not be the primary integration centre of working memory encoding. In the scenario whereby the ACC receives downstream working memory information from elsewhere, then disruption to the network activity would not necessarily yield a behavioural impairment in a working memory task. This eventuality may be determined in the future by large-scale inactivation of the ACC bi-hemispherically using muscimol.

Effects of perturbation on neural populations of the anterior cingulate cortex

As expected, the perturbation introduced inhibition in the ACC network (Figure 68). Moreover, the firing rate of many neurons showed a decrease that was maintained throughout the post-laser epoch. This may be due to the activation of a recurrent inhibitory circuit from the pulse of inhibition introduced by the stimulation. One could point to the possibility of the cell signal depleting as the experiment progressed, however this depletion would be highly unlikely to be repeatable across three experiments, and reliably occur following the 61st trial in each experiment. Hence, this effect is likely to be physiological and not an artefact of recording. Given the onset time of the stimulation (400ms after tone onset), the duration and rebound time of the stimulation (~200ms in total – see Figure 65), and the average length of the delay period (mean duration = 1.9s), it was expected that the response profile of many neurons would be strongly negated by this stimulation. Particularly given that the response of some neurons during the delay period is that of a discrete peak, such as that shown in Figure 32 (a). Whilst this did occur for some neurons, such as the neuron in Figure 71, interestingly, other neurons were capable of partially recovering their response profile before the end of the trial. This may in part explain the minor reduction in behavioural performance, as the mnemonic activity, or higher order activity such as the upcoming decision may not have been abolished in all cells.

As previously mentioned, the reduction in behavioural performance was not limited to the laser trials, but also in the non-laser trials of the post-laser epoch. This appears to be echoed on a neuronal level.

For example the response profile shown in panel (a) of Figure 71 is eradicated in the non-laser trials (d) in addition to the laser trials (c). Hence, the impact on the confidence of the mouse in non-laser trials may be reflective of some neurons being perturbed from a semi-stable attractor state of activity and thus can no longer encode the relevant information for the working memory task. This hypothesis could be probed further by designing the experimental protocol such that discrete periods of stimulation versus non-stimulation were interleaved. For example, an experiment similar to that described in section Experiment outline, whereby the pre-laser epoch of 1:60 trials, would be followed by a short post-laser epoch of 61:120 trials, following by a recovery period of trials 121:180 that would be identical to the pre-laser epoch in terms of stimulation protocol i.e. no laser stimulation. Using this approach, one could examine the extent to which the stimulation introduces inhibition/noise into the network that extends beyond a laser trial, and whether the robustness of the network could be regained during the recovery epoch. This highlights the versatility, and utility of this technique as a mechanism of probing the hypotheses presented in the former portion of this chapter.

Chapter 5: Summary and Concluding Remarks

Neural dynamics underlying working memory in the anterior cingulate cortex

Working memory is a complex phenomenon requiring the integration of the both retrospective encoding of stimulus properties, and the prospective encoding of the upcoming choice, and the relevancy of the stimuli received. The work presented here was conducted with the aim of examining the encoding properties of the ACC during working memory. In particular, the nature of mnemonic encoding during the delay period was investigated for evidence of persistent activity and transiently active neurons. Furthermore, the nature of the information encoded was examined for evidence of low-level stimulus encoding and high-level encoding of the upcoming choice. The saliency of this encoding was examined on a single cell and population level. The predictive properties of the neural information were also compared to that of the behavioural information. A series of experiments incorporating optogenetic perturbation during the delay period of the working memory task was conducted to determine the stability of neural response profiles to a brief perturbation, and to examine whether unilateral perturbation of the network would be sufficient to decrease behavioural performance.

The work presented here shows evidence for strong stimulus-response properties on a single neuron and population level in the ACC to a working memory task. As found in previous studies (94), each neural population showed a diverse set of responses to cue, delay, and response epochs of the task. The neural responses to delay period activity showed a mixture of cells displaying persistent activity, and those that displayed transient activation. Those that displayed transient activation often showed a shift in latency in their response profiles between T1 and T2 trials (see Figure 33 (b-c), Figure 30). Evidence for low-level information encoding was shown by the strong stimulus-response mappings of neurons such as Figure 36, and high level encoding has been in predictive neurons such as that seen in Figure 37. The versatility of PFC neurons was shown by the rapid switch in neural responses between T1 and T2 trials (<1s inter-trial period) (see Figure 30, and Figure 32 (a)). This versatility was also seen on a population level. The results in Figure 42 indicate that there was a repeatable population dynamic in T1 trials. The context-dependent sequence of activation in T1 trials was robust despite T1 and T2 trials being randomly presented during the delayed response task. This illustrates the ability of the network to switch information representation on a fast timescale. Furthermore, it may be evidence of rule-

encoding such as that shown by Buschman et al. (210), as the spatial information between T1 and T2 was maintained, however the tone and the rule changed. If the shift in activation pattern was due to a difference in tone presentation, then one would expect the same neural responses to be found for Hit and Miss trials, and CR and FA trials. However, as shown by Figure 37, this is not the case in all neurons. Hence, it is likely that the pattern of activation shown here is encoding the rule of a particular trial type. The temporal influence on the encoding properties of delay period cells was examined by comparing the response profiles between trials with short and long delay periods. There has been previous evidence to show that delay period responsive neurons elongate their response profile in a long delay period (96), hence the response profile shows a temporal independence. The results presented here show a similar temporal independence in neuronal responses to T2 trials (Figure 63 (b)), but a mixture of temporally dependent and temporally independent response profiles in T1 trials (Figure 63 (a)). This may represent encoding mechanisms that are context-dependent, as opposed to intrinsic properties of the neurons, as the same neurons alter their encoding mechanism based on trial type.

The predictive properties of the population dynamic were examined by building a PCA classifier using the normalised mean firing rates of each neuron. Given the characteristic speed profiles displayed in T1 and T2 trials, the predictive abilities of the behavioural data were assessed by building a binary classifier using locomotion information. Whilst the accuracy of the locomotion classifier surpassed that of the PCA classifier, the neural data had superior performance in the speed at which it could predict a correct trial outcome.

Optogenetics is known to be a very useful tool to perturb neural networks in an effort to uncover the mechanism behind information encoding patterns in the brain (209). The results presented here show how this tool can be used to introduce inhibition into a neural population by activating pv-interneurons through the stimulation of ChR2. The perturbation produced a minor reduction in behavioural performance, which may be indicative of the robustness of the ACC network to unilateral perturbation, as found in the results of Li et al. (162). Interestingly, the perturbation appeared to introduce sufficient noise into the network to reduce the behavioural performance of mice in non-laser trials in the post-trial epoch. This was reflected in the neural response profiles of some cells in which the response profile

shown in the pre-laser epoch (Figure 71 (a)), was eradicated in the non-laser trials in addition to the laser trials of the post laser epoch (Figure 71 (c)). However, this was not the case for all cells (see Figure 70). This may indicate that the perturbation was not sufficient to perturb the response profiles of all cells, and thus, only a moderate reduction in behavioural performance was found.

Future investigations on the neural dynamics underlying working memory in the anterior cingulate cortex

There are many different phenomena that have yet to be investigated in the working memory datasets presented here. Whilst single neurons, neural trajectories of population activity in PC state space, and locomotive patterns have been shown to be predictive of trial outcome, it would be interesting to examine the most predictive parameters of the upcoming response including different behavioural parameters, and different response profiles of neural populations, that are not restricted to mean firing rate. This may reveal more in depth knowledge regarding the encoding properties of ACC cells during a working memory task. This could be aided further by constructing a neural classifier with the incorporation of a Bayesian prior, and a more sensitive decision boundary to the population variability, as discussed in the section PCA classifier for outcome prediction. Furthermore, it would be interesting to examine the relationship between the power of neural oscillatory bands and the change in neural representations between trials types seen on both a single neuron level (Figure 30), and a population level (Figure 42). Previous evidence has shown that there is a strong overlap within neuronal ensembles in the representation of different rule information (19,20). It has been shown that different ensembles can be activated in a context dependent manner via increases in synchrony with specific neural frequency bands. Namely that beta frequency synchronisation selects the relevant neuronal ensemble and that alpha deselects the newly irrelevant ensemble (21). It would be interesting to assess the level of synchrony of cells to beta and alpha frequency bands during different task epochs e.g. during cue presentation to examine the transition to a new rule, and during the delay period, to examine the maintenance of this rule information. The behavioural paradigm presented here would be particularly amenable to this investigation as it would highlight the potential for neural synchrony with LFP oscillatory bands to switch the activation of neural ensembles on a second timescale, as the inter-trial period is $<1s$.

The experiments presented here incorporating optogenetic perturbation provide a highly versatile method of probing working memory circuits, which is likely to be very useful in future studies of the encoding mechanisms of working memory in the PFC.

A closing note on the role of the prefrontal cortex in working memory

Numerous studies in addition to the work presented here have implicated the PFC in working memory function (2,87,89,94,95,109-112). Indeed, lesioning studies have found the area to be crucial for the execution of this task (89,109,110,115,116). The often times higher-order nature of the information encoded in the PFC during a working memory task gives credence to the theories proposed by Fuster (214), and Miller and Cohen (2) that the PFC acts as a top-down control centre for WM function. In my opinion, this theory marries with the Multicompartment Model of Baddeley and Hitch (88) such that it presents the PFC top-down control system (211-213), with the storage capacity being secondary, and simply present as part of the highly diverse data that is stored in this brain region. Hence, in my opinion, the current evidence would point to the PFC taking the role of the Central Executive in this model. However, whether the PFC is the puppeteer or the puppet of working memory remains unknown. A puppeteer cannot execute any action without a puppet and vice versa. Hence, in the absence of a full connectome, with all functional neuronal dynamics elucidated, one can merely speculate.

The effects of psilocybin on the anterior cingulate cortex

Psilocybin is a hallucinogenic compound that can alter states of consciousness via activation of the serotonergic system. Our knowledge of the effects of psilocybin on areas of cognition such as the prefrontal cortex is mostly restricted to the fMRI domain. The results presented in this thesis are the first known population recordings of the ACC in awake mice under the influence of psilocybin. The results show an overall increase in excitation in the circuit, with sub-populations of inhibition and null effects. This medley of responses follows expectation given the heterogeneity in the distribution of 5-HT_{1A} and 5-HT_{2A} receptors across both pyramidal cells and interneurons in the ACC. There was a significant decrease in power across low frequency bands of the LFP (delta, theta, and alpha neural frequency bands). The gamma band showed a moderate increase in power, whilst the beta band remained unaffected. The network overall became less phase-modulated with delta, theta, alpha and

beta neural frequency bands. The phase modulation with the gamma band was unchanged. Neurons in the network showed a spread of phase-modulation levels in the spontaneous cortical state (pre-condition). The distribution of phase modulation shifted in response to psilocybin as phase modulated cells become un-modulated, whilst phase un-modulated cells remained unchanged. The reduction in power of low frequency neural frequency bands, coupled with a moderate increase in power in the gamma band, and an overall reduction in phase modulation of cells with all neural frequency bands (except gamma), may indicate a transition of the cortex to a desynchronised state. This state is associated with two functions; REM sleep and awake attentiveness. The behaviour of the mice transitioned from an alert, locomotive state on the treadmill setup, to a flat-body posture, low locomotive state, following psilocybin administration. Taken together, these findings may indicate that psilocybin induced a transition into a desynchronised cortical state similar to REM sleep.

Future investigations of the neural effects of psilocybin

The experiments outlined in section Intra-cortical injection of a serotonergic agonist, have the potential to reveal interesting dynamics of psilocybin when applied locally to the ACC. With the acquisition of further experiments to create a full dataset (without the aforementioned caveat associated with injecting a volume of liquid into the cortex), one would be able to examine the degree to which the dynamics shown here are as a results of intrinsic stimulation of serotonergic receptors within the ACC, or a result of being a downstream target of stimulation in another brain region. Furthermore, the sub-threshold effects of psilocybin could be examined through patching cells *in vitro*. This may yield further information as to why neural populations show such heterogeneous responses.

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Appendix

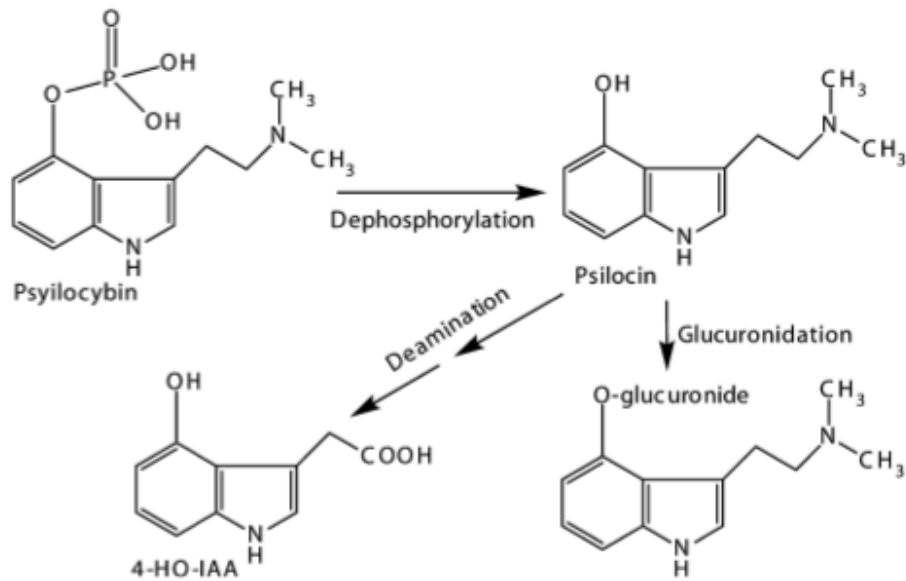


Fig. 7. Biotransformations of psilocybin and psilocin. Psilocybin is dephosphorylated to produce psilocin that undergoes deamination and glucuronidation

Figure 72: The dephosphorylation process in the conversion of psilocybin to psilocin (adapted from (35)).

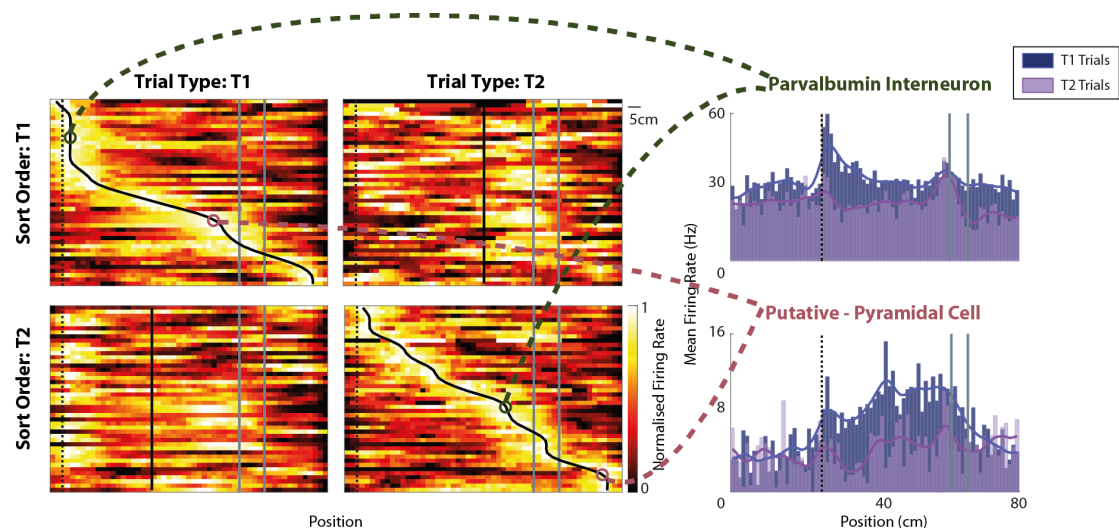


Figure 73: The shift in peak response of transiently active putative pyramidal cells and interneurons (identified via opto-tagging) between context dependent sequence maps. This illustrates the versatility of PFC neurons in the type of information they can represent, and the speed at which these response profiles can switch.

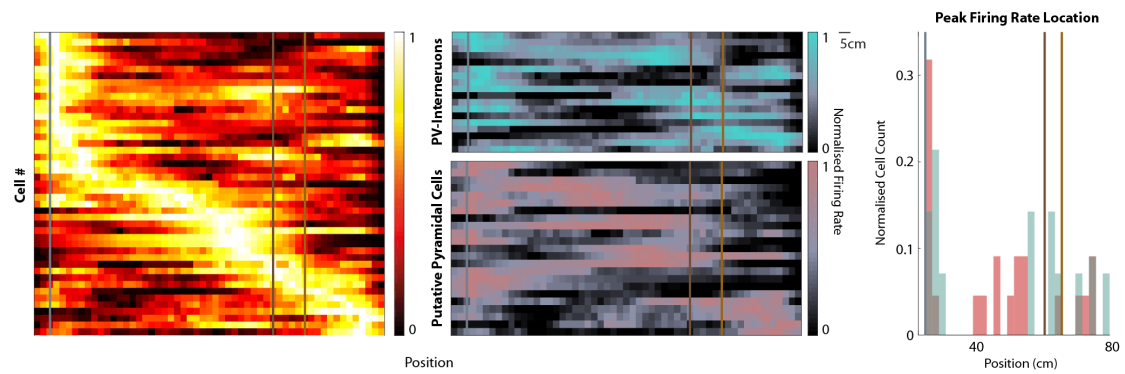


Figure 74: A trend in functional roles between putative pyramidal cells and interneurons can be seen when examining where the peak response of each neural sub-population falls in relation to belt position. The pyramidal cells and interneurons from the sequence map in the first panel, are displayed separately in the second panel. The location of the peak responses of each cell type are shown in the third panel. There is a minor tendency for pyramidal cells to be active during the delay period, while the interneurons appear to preferentially encode the tone and the reward area.

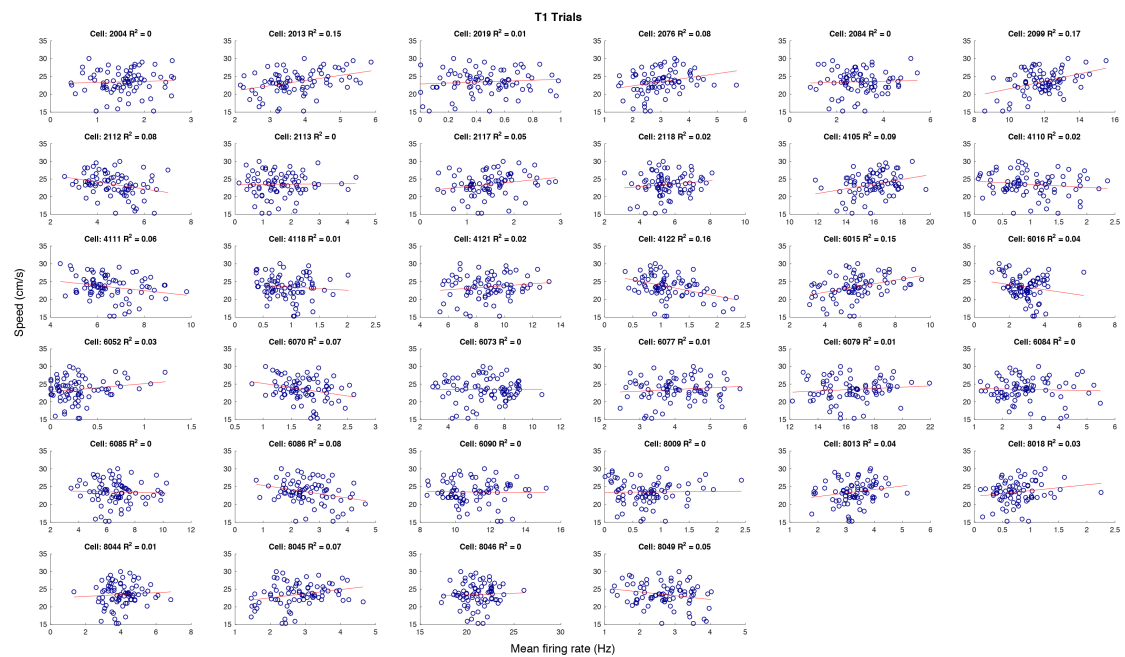


Figure 75: Linear regression between the mean speed profile in T1 trials and the mean firing rate of each neuron in T1 trials for another neuronal population. The raw data is shown in blue, and the linear regression is shown in by the red line. The coefficient of determination (R^2) is quoted in the title of each plot.

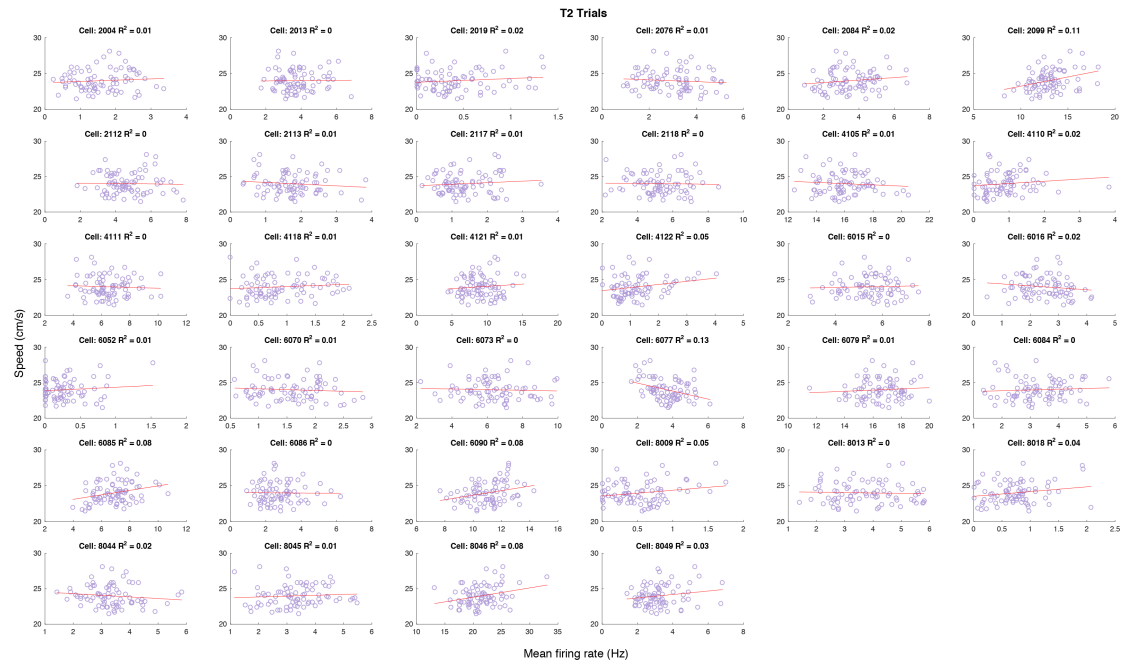


Figure 76: Linear regression between the mean speed profile in T2 trials and the mean firing rate of each neuron in T2 trials for another neuronal population. The raw data is shown in blue, and the linear regression is shown in by the red line. The coefficient of determination (R^2) is quoted in the title of each plot.

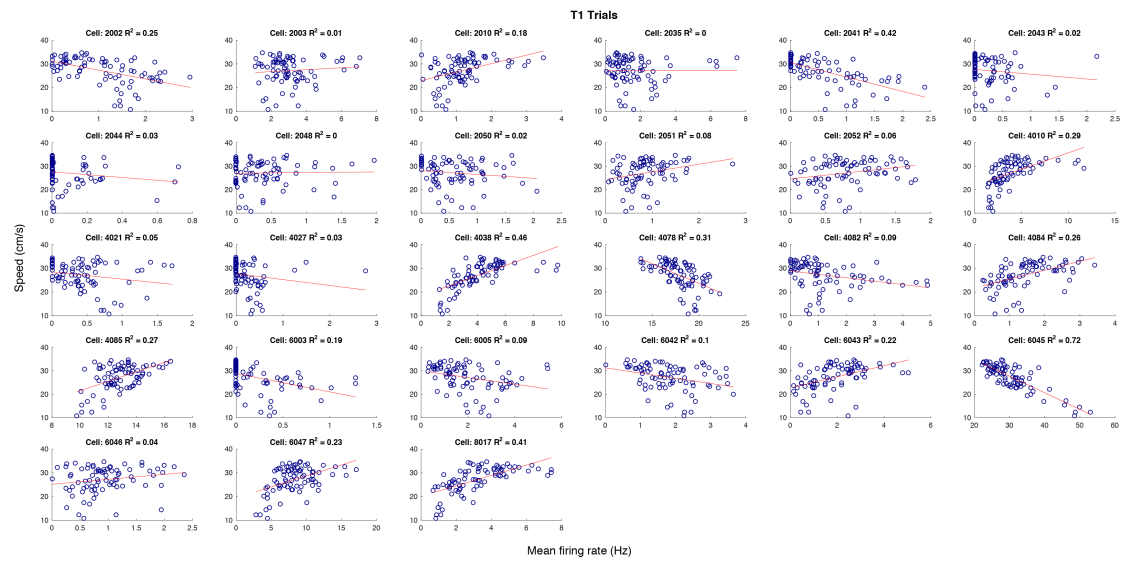


Figure 77: Linear regression between the mean speed profile in T1 trials and the mean firing rate of each neuron in T1 trials for another neuronal population. The raw data is shown in blue, and the linear regression is shown in by the red line. The coefficient of determination (R^2) is quoted in the title of each plot.

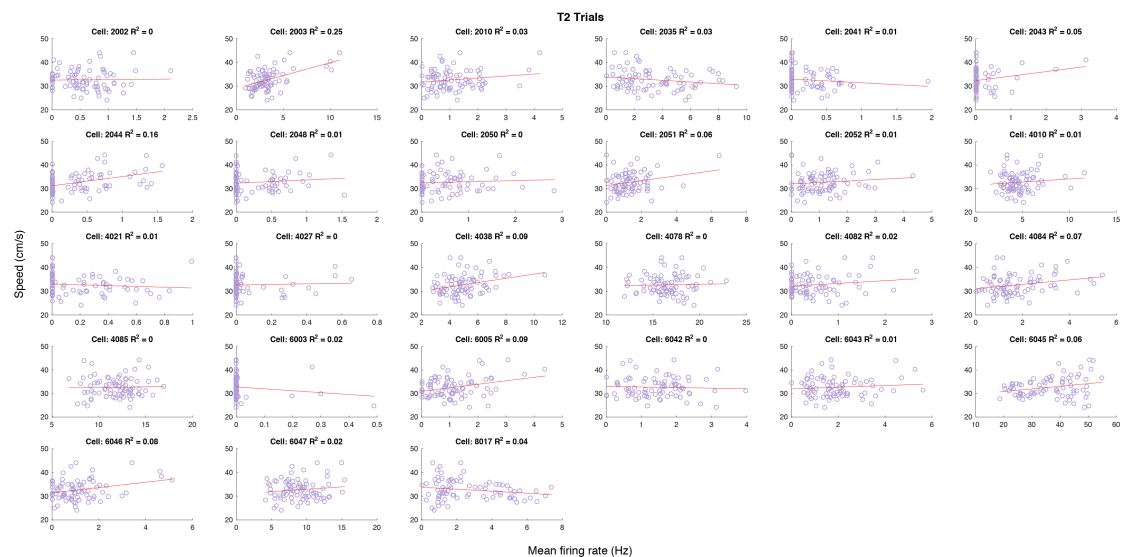


Figure 78: Linear regression between the mean speed profile in T2 trials and the mean firing rate of each neuron in T2 trials for another neuronal population. The raw data is shown in blue, and the linear regression is shown in by the red line. The coefficient of determination (R^2) is quoted in the title of each plot.