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Hippocampal sequencing mechanisms are disrupted in a maternal immune activation model of schizophrenia risk

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Abstract

Episodic memory requires information to be stored and recalled in sequential order, and these processes are disrupted in schizophrenia. Hippocampal phase precession and theta sequences are thought to provide a biological mechanism for sequential ordering of experience at timescales suitable for plasticity. These phenomena have not previously been examined in any models of schizophrenia risk. Here, we examine these phenomena in a maternal immune activation (MIA) rodent model. We show that while individual pyramidal cells in the CA1 region continue to precess normally in MIA animals, the starting phase of precession as an animal enters a new place field is considerably more variable in MIA animals than in controls. A critical consequence of this change is a disorganization of the ordered representation of experience via theta sequences. These results provide the first evidence of a biological-level mechanism that, if it occurs in schizophrenia, may explain aspects of disorganized sequential processing that contribute to the cognitive symptoms of the disorder.

Significance statement

Hippocampal phase precession and theta sequences have been proposed as biophysical mechanisms by which the sequential structure of cognition might be ordered. Disturbances of sequential processing have frequently been observed in schizophrenia. Here we show for the first time that phase precession and theta sequences are disrupted in a maternal immune activation model of schizophrenia risk. This is a result of greater variability in the starting phase of precession, indicating that the mechanisms that coordinate precession at the assembly level are disrupted. We propose that this disturbance in phase precession underlies some of the disorganized cognitive symptoms that occur in schizophrenia. These findings could have important preclinical significance for the identification and treatment of schizophrenia risk factors.

The hippocampus is known to be involved in memory-related processes where information is encoded, stored and recalled sequentially, including spatial navigation (O'keefe & Nadel, 1978), episodic memory (Tulving & Markowitsch, 1998), and thinking about the future (Schacter et al., 2007). Hippocampal phase precession has been proposed to underlie the sequential organization of information (Buzsáki & Tingley, 2018; Dragoi & Buzsáki, 2006; Jaramillo & Kempter, 2017; Wikenheiser & Redish, 2015). Phase precession describes the phenomenon whereby the firing of a hippocampal 'place cell' precesses systematically from later to earlier phases of the underlying local field potential (LFP) theta oscillation as the animal advances across the cell's 'place field' (Huxter et al., 2003; Tingley & Buzsáki, 2018) (O'Keefe & Recce, 1993; Skaggs et al., 1996). Phase precession has also recently been confirmed in humans (Qasim et al., 2020).

At the network level, an emergent property of phase precession occurs when a population of cells with overlapping place fields are co-active (Figure 1). Within a single theta cycle (~120ms), these cells tend to fire in a 'theta sequence' (Foster & Wilson, 2007), thereby reproducing the ordered spatial arrangement of their place fields within a timescale that is appropriate for the induction of synaptic plasticity (Dan & Poo, 2004; Skaggs et al., 1996). Theta sequences may therefore provide a biological mechanism for sequential memory encoding and storage (Dragoi & Buzsáki, 2006; Jaramillo & Kempter, 2017), as well as event prediction (Foster & Wilson, 2007; Lisman & Redish, 2009; Wikenheiser & Redish, 2015). Coherent theta sequences depend on the coordinated activity of cell assemblies (Dragoi & Buzsáki, 2006; Itskov et al., 2008; Middleton & McHugh, 2016), so that co-active cells have both a similar degree of precession over space and time, and a similar starting phase as the animal enters a new firing field (Feng et al., 2015; Schmidt et al., 2009). A failure of this coordination could disrupt theta sequences, thereby disturbing some of the cellular mechanisms that underlie sequential memory and predictive processes.

……Figure 1 here……

Structural, biochemical, and functional abnormalities of the hippocampus have previously been observed in several neurodevelopmental disorders, including schizophrenia (Harrison, 2004; Heckers & Konradi, 2002; Li et al., 2019). Such disturbances likely contribute to several cognitive symptoms associated with schizophrenia, including the disorganization of sensory and contextual information, memory and imagination (D'Argembeau et al., 2008; Hardy-Baylé et al., 2003). Furthermore, sequential processing deficits that may have a hippocampal component are well documented in schizophrenia (Eichenbaum, 2017; Lisman & Buzsáki, 2008; Meck et al., 2013), including disturbed judgement of temporal order and duration (Ciullo et al., 2016; Thoenes & Oberfeld, 2017), and impaired sequence learning (Pedersen et al., 2008; Siegert et al., 2008). Similar deficits have also been observed in first-degree relatives and other at-risk individuals during the prodromal phase (Dickinson et al., 2007), and they are independent of other cognitive impairments (Ciullo et al., 2016), suggesting that they may be a primary feature of the disorder. It is possible, therefore, that dysfunction in hippocampal phase precession and theta sequences could underlie some of the sequencing and disorganization symptoms observed in schizophrenia (Lisman & Buzsáki, 2008). To our knowledge however, no previous studies have systematically investigated theta sequences and phase precession in any models of the disorder.

Here we examined phase precession and theta sequences in the maternal immune activation (MIA) model of schizophrenia risk. The MIA model is built on epidemiological evidence suggesting that exposure to viruses or other pathogens during the gestation period is an etiological risk factor for the development of schizophrenia or autism spectrum disorder

(Adams et al., 1993; Brown & Meyer, 2018). Several studies have confirmed that MIA offspring manifest many of the neurobiological, cognitive and behavioural symptoms of schizophrenia, including irregularities of hippocampal structure and neural transmission, reduced sensorimotor gating, decreased behavioural flexibility, and memory deficits (Brown & Meyer, 2018; Meyer et al., 2005; Wolff & Bilkey, 2010; Zuckerman & Weiner, 2005).

Materials and methods

ANIMALS AND EXPERIMENTAL DESIGN

All subjects were generated using the MIA intervention as we have described previously (Dickerson et al., 2010; Wolff & Bilkey, 2015). Female Sprague Dawley rats (~3 months old) were time-mated with GD1 considered to be the first day after copulation. On GD 15, pregnant rats were briefly anesthetized with isoflurane (5%; Bayer) and administered either a single injection of polyinosinic:polycytidylic acid (poly I:C; Sigma-Aldrich) 4.0 mg/kg, i.v. dissolved in 0.9% saline (Baxter), or an equivalent saline injection 1 ml/kg. Poly I:C and saline treatments were always performed in pairs on the same day. All dams and pups were housed in open cages prior to weaning. After birth, litters were culled to a maximum of 6 male pups and, post-weaning, were housed in littermate groups of 2-3 in individually ventilated cages (IVC). Only male offspring were used for experimental purposes due to resource limitations. At this stage all pups were randomly allocated a litter number. The housing room was maintained at a normal 12-h light/dark cycle, and temperature controlled to 20-22ºC. Immature rats were provided with access to food and water ad libitum, and after 3 months were food deprived to no less than 85% of their free-feeding weight in preparation for the experimental procedure. Water was available *ad libitum* throughout the entire experimental procedure. All rats weighed between 400 and 650 grams at the time of surgery.

APPARATUS AND TRAINING

The apparatus consisted of a rectangular wooden circuit measuring 900 by 800mm (Figure 2a). All arms were 100mm wide with 270mm high side walls. The entire apparatus was painted in matte black and was devoid of visual cues. A video camera was mounted to the ceiling of the recording room to track the animals' position, which was captured from 3 infrared LED lights attached to the acquisition system's head stage. All experiments were performed in a darkened environment with some ambient light from the recording computer and a small lamp aimed away from the apparatus into one corner of the room.

The mature male offspring (3-12 months) were trained over a period of 5 to 15 days. Animals were randomly selected according to their litter number, with a maximum of two rats per litter. On days 1-5 rats were habituated to the recording room, apparatus and food reward, and were allowed to free-forage for Coco Pops (Kellogg Company) scattered throughout the apparatus. Following successful habituation, whereby rats actively explored the maze and consumed the food reward, the placement of Coco Pops was gradually restricted, first to the top 2 corners of the track and in the centre of the reward arm, and then to the reward arm only. During this period, rats were trained to run in a clockwise direction and were turned back to the correct direction with a paddle when necessary. Coco-pops (approx. 6 per reward delivery) were delivered manually by the experimenter. Training was considered completed when rats consistently ran in a clockwise direction for the food reward over a twenty-minute session.

SURGICAL PROCEDURES

All experimental protocols were approved by the Otago University Animal Ethics Committee and conducted in accordance with New Zealand animal welfare legislation. Following successful training, animals were anesthetized with 5% isoflurane (Merial New

Zealand) in oxygen and maintained at 1.5 to 2.5% throughout surgery. After animals were deeply anesthetized, they were given a subcutaneous injection of Atropine (1mg/kg) to ease their breathing, as well as the analgesics Carprofen (1mg/kg) and Temgesic (buprenorphine; 0.1mL), and a prophylactic antibiotic, Amphoprim (trimethoprim and sulfamethazine, 0.2mL). Rats were then mounted on a stereotaxic apparatus (David Kopf Instruments) above a heating pad, and a lubricating eye gel (Visine) was applied. The scalp was shaved and sterilized with Betadine (Povidone-iodine), followed by a subcutaneous injection in the scalp of the local anesthetic Lopaine (lignocaine hydrochloride 20mg mL^{-1}; 0.1mL diluted in 0.4mL of saline). After exposing the skull, an opening was drilled above the left hemisphere dorsal hippocampus, and a custom built, 8 channel microdrive containing 2 moveable tetrode bundles of equal length was targeted to the CA1 subregion at -3.8mm AP from bregma, -2.5mm ML from the midline, and lowered just above the pyramidal cell layer (1.8mm from dura; Figure 2b). Electrodes consisted of 25µm nichrome, heavy formvar insulated wire (Stablohm 675 HFV NATRL; California Fine Wire Company), and had been gold electroplated until impedences were reduced to ~ 200 – 300 kΩ (NanoZ, Neuralynx). Microdrives were secured to the skull with jewellers' screws and dental cement, and a ground wire was secured to an additional screw placed above the right hemisphere. Post-surgery rats received a secondary dose of Amphoprim immediately upon waking, and then an additional dose of Carprofen 24 hours later. Rats were provided with ad libitum food and water post-surgery and were given 8 days to recover.

EXPERIMENTAL PROCEDURE AND ELECTROPHYSIOLOGICAL RECORDINGS

Following recovery, rats were again food deprived to no less than 85% of their free-feeding weight. Post-operative training was carried out to ensure that the animals could still

perform the task adequately. Rats were then attached to a multichannel data acquisition system (DacqUSB; Axona Ltd), and single unit data was closely monitored as tetrodes were slowly lowered (~40µm per day) towards the dorsal CA1 pyramidal cell layer until well-isolated single units were identified. Extracellular unit activity was first passed through an AC-coupled unity gain amplifier before passing through to the recording system. Single unit data was bandpass filtered between 600 and 6000 Hz, and sampled at a rate of 48 kHz with 24-bit resolution. For each tetrode, one electrode with minimal spiking activity was selected as a reference. Action potential thresholds were set at a minimum of 70 – 80 µV and recorded for a 1 ms window whenever the spiking amplitude met this threshold. All spike events were time-stamped relative to the beginning of the recording. LFP data was simultaneously recorded from electrodes that has active place cells and were referenced to ground. LFP data was filtered at 500 Hz (with notch filtering selective for activity at 50 Hz) with a gain of ~500, and sampled at 48 kHz. The animal's location was determined from 3 infrared LEDs mounted on the animal's headstage and recorded by a camera located above the chamber. Positional data was analysed with a sampling rate of 50 Hz and then converted into x and y coordinates by the recording system. Once well-isolated single units were identified, tetrodes were not lowered any further for the duration of the experiment. Rats ran no more than one session per day, for ~60 – 80 laps per session. Single unit, position and LFP data was saved for later analysis. All recordings with at least 1 putative place cell were included in the final dataset on the condition that there was a minimum of 4 separate recordings from that particular animal.

ISOLATION OF SINGLE UNITS

For each recording, single units were identified manually offline using purpose designed cluster cutting software (Plexon Offline Sorter, Version 3), primarily via the peak-

to-valley distance and principal components analysis of the waveforms. Putative place cells were isolated if they had an average firing rate <5 Hz, a peak to trough spike width of ~400 µs, and a complex pattern of bursting activity identified from the autocorrelation of spike times (Figure 2c). All cells that did not meet these criteria were excluded from further analysis. Sorted data was then exported to MATLAB (version R2019a, MathWorks), and analysis of single unit, position and LFP data was carried out in MATLAB with custom-written scripts.

ANALYSIS OF PLACE CELL PROPERTIES

The rectangular track was linearized so that the starting location was the lower left corner (figure 2a). Place fields were identified by dividing the track floor into 1 cm long bins and creating an occupancy map from the position tracking data based on the amount of time the rat spent in each bin. Spikes were binned similarly for each single unit by identifying the number of spikes that occurred within each bin. Element-wise division was then used between the spike map and the occupancy map to create a firing mate map where each bin contained the firing rate for a cell. Firing rate maps were smoothed with a 10 cm wide moving window. Place fields were then detected automatically by detecting regions of at least 10cm in length that had a firing rate of at least twice the mean firing rate for the cell (Porter et al., 2018). If more than two place fields were detected for a cell, only the largest was analysed. Following this, each place field map was analysed separately to determine place field length and mean infield firing rate. Where place fields wrapped around the start-end position of the linearized maze they were linearly shifted prior to firing rate analysis.

Spatial information content provides a measure of how informative a spike from a cell is regarding the animal's current location within an environment. Place cells with a higher information value therefore provide a more reliable prediction of current location than cells

with a lower information value (Skaggs et al., 1993). The formula for information content, measured in bits per spike is:

$$Information = \sum_{i=1}^{N} p_i \frac{\lambda_i}{\lambda} log2 \frac{\lambda_i}{\lambda}$$

where the environment is divided into N distinct bins ($i = 1, \ldots, N$), p_I denotes the occupancy probability of bin i, λ_i is the mean firing rate for bin i, and λ is the overall mean firing rate of the cell.

Correlations of theta frequency and speed were generated for each recording from every tetrode that had single unit activity. This process involved estimating instantaneous values for theta frequency from the Hilbert transform of LFP filtered between 6 and 10 Hz. Estimates of instantaneous speed were determined by monitoring the animals change in position over 500 ms time windows. Speed and theta frequency data were then sampled at one second intervals and correlated. Samples where speed was below 5 cm/s were excluded from the analysis.

ANALYSIS OF LFP PROPERTIES

Sampling of local field potentials (LFPs) occurred from either the same electrode from which unit data was detected or one in the same bundle. LFP activity was sampled at 4800Hz. To determine theta waveform shape, the LFP was bandpass filtered between 6-10Hz and a phase profile was determined using the Hilbert transform. A sample waveform of 200 ms duration was subsequently captured whenever the phase data indicated a trough had been reached. These samples were then averaged, as were the phase profiles.

PHASE PRECESSION ANALYSIS

For all phase precession analyses, the phase reference was always to the LFP signal taken from the CA1 pyramidal cell layer, and 0º corresponds to the trough of the oscillation. An EEG amplitude threshold was also applied to discard spikes recorded where theta was of very low amplitude. This threshold was set at 0.25 of one standard deviation of the amplitude envelope generated by the Hilbert transform from the theta-filtered LFP of each recording. On average it removed approximately 8% of spikes from the dataset. For all spikes that occurred within a place field, spike phase was determined by matching the animal's position within the place field to the instantaneous phase of the 6-10 Hz theta rhythm, and then analysed using procedures described previously (Kempter et al., 2012). This involves using circular-linear regression to provide a robust estimate of the slope and phase offset of the regression line, and a correlation coefficient for circular-linear data analogous to the Pearson product-moment correlation coefficient for linear-linear data. The fits were constrained to have a slope of no more than -2 and +1 theta cycles per place field transverse. Phase precession analysis was conducted by pooling spiking data from all passes through the place field within a given recording session. Because theta states are associated with locomotion (Vanderwolf, 1969), phase precession analysis was only performed on data where the animal was running at least 5 cm/s.

ANALYSIS OF THETA SEQUENCES

For this analysis recordings where at least three cells had been recorded simultaneously were identified. The place fields of these cells were then displayed on a 20 by 20 pixel matrix and the centre marked manually. Here the centre was defined as the bin with the highest firing rate that was closest to the centre of place field mass. For cells with multiple place fields, the largest place field was always selected except in cases where this cell was situated directly in the reward region. In such cases, the next largest place field located in a non-

277 reward location was selected. Cells with a single place field located in the reward area were
278 included if the place field covered regions adjacent to reward, but were excluded if they were
279 restricted to reward area only (such cells had place fields that were smaller than average, and
280 were not common). This was to ensure that analysis of cell spiking unrelated to the theta
281 rhythm, such as that which occurs during epochs of consummation, when theta oscillations
282 are absent or weak, was minimized. Place field location around the track was then converted
283 to polar coordinates so that the distance between place fields could be represented in angular
284 format. Ripple events (140-200 Hz) were detected in the LFP and any spikes that occurred
285 during these events were discarded. The time between every spike generated by each of the
286 cells in the recording was determined, and where that time interval was within a set window
287 (e.g. 40 ms), the data were correlated with the angular distance between the place fields of the
288 two cells using a circular-linear correlation. The use of a circular representation helped to
289 resolve the difficulty of determining whether a place field is ahead of or behind another in a
290 topologically circular apparatus.
291
292 HISTOLOGY
293 Following completion of experiments, rats were anaesthetised with 5% isoflurane in
294 oxygen, and a 2mA direct current was passed through each electrode for approximately 1
295 second to lesion the site of the electrode tip. Rats were then euthanized with an overdose of
296 isoflurane and transcardially perfused, first with 120 ml of 0.9% saline, and then 120 ml of
297 10% formalin in saline. Brains were then carefully extracted from the skull after removal of
298 the Microdrive, and stored in 10 % formalin in saline. One week prior to sectioning, brains
299 were transferred first to 10% formalin in H_2O for 24 hours, and then to a 10% formalin/30%
300 sucrose solution for approximately 3-7 days, until the brain sunk to the bottom of the sucrose
301 solution. Dehydrated brains were then sectioned into 60 µm coronal slices with a cryostat

(Leica CM1950). Sections were then mounted on slides and stained with a thionine acetate Nissl stain (Santa Cruz Biotechnology, Inc. After slides were dry (min. 24 hours) electrode placement was imaged with a local power (1.5x) digital microscope (Leica Biosystems, LLC) to verify electrode placement (Figure 2b).

STATISTICAL ANALYSES

For all statistical analyses, we performed the following procedure. First, raw data were transformed to a lognormal distribution if appropriate. All data (either in raw form or the log transform) were then checked for assumptions of normality and equality of variances. These checks were performed in GraphPad Prism 8.1.1 (GraphPad Software, Inc., San Diego, CA, USA), using the d'Agostino & Pearson test for normality, and the F test to compare variances. If data did not meet the assumptions for normality based on the d'Agostino & Pearson test, visual inspection of histograms and QQ plots was performed, and extreme outliers were removed using the Graphpad function for removal of outliers. All data that failed to meet assumptions of normality based on this procedure were then analysed using the appropriate non-parametric test. Details about the specific tests used are provided in the results section, and in Table 1. For normally distributed data with unequal variances, Welch's t-test was used instead of a student's t-test. All t-tests were two-tailed. Data with a normal distribution are presented as mean ± SEM unless explicitly stated otherwise in the figure legends. For all data that did not meet normality assumptions, the median is depicted instead. Significance levels were defined as $p < .05$. Additional information about significance levels is provided in the figures as: * $p < .05$, ** $p < .01$, *** $p < .001$.

Additional circular statistics (to compare group differences in the intercept of the circular correlation of phase and position, and to generate the MVL for animal by animal and litter by litter analyses) were performed in Oriana 4 (Kovach Computing Services, Inc.,

Anglesey, UK). Group differences for angular variance (defined as 1-MVL) were performed using the variance ratio F-test, found at https://www.statskingdom.com/220VarF2.html

……Figure 2 here……

Results

MIA results in increased firing rates, but basic place field properties and theta dynamics are largely unchanged

A total of 327 place cells from 9 MIA animals and 222 place cells from 8 CTL animals were recorded from the dorsal CA1 region of the hippocampus as animals ran around a rectangular track for a food reward (refer to methods). The firing rate of cells recorded from MIA animals was significantly higher than for control cells, including both the mean firing rate (Mann Whitney U = 26557, $p < .001$) and the infield firing rate (U = 30118, $p < .001$; Figure 3a). The increased firing rates in the MIA group did not appear to be due to differences in running speed, as mean speed through the non-reward arms was not significantly different between groups (Mann Whitney U = 76689, p = .283; Figure 3b). The MIA intervention had no significant effect on place field length ($t(547) = 1.79$, p = .075), although there was a trend towards slightly larger place fields among MIA cells Figure 3c). There was also no significant group difference for the information content measure of place field specificity (Mann Whitney U = 76475, p = .314, Figure 3d). A comparison of the local field potential (LFP) activity, recorded from the electrodes at which place cells were located, showed that the frequency of theta oscillations was marginally, but significantly, lower in the CTL group (Welch's $t(131.1) = 4.09$, $p < .001$; Figure 3f). Inspection of LFP waveforms revealed that CTL and MIA theta oscillations were of near identical shape, although CTL waves have a marginally, but significantly, higher amplitude (Mann Whitney U =64494, p

<.001; Figure 3h). Importantly, the phase profile of the theta waveform, as generated by the Hilbert transform, was virtually identical across the two groups (Figure 3i). However, only CTL recordings demonstrated evidence of theta frequency fluctuations that were significantly correlated with speed, as measured by a comparison of r values generated for each recording (Mann Whitney U + 2669, p <.001; figure 3j).

……Figure 3 here……

Individual cells continue to precess normally following the MIA manipulation

Phase precession was characterised as animals moved through the place fields of putative pyramidal cells. When data from all cells from each of the groups were examined, there was no significant difference between the MIA and CTL cells for the majority of phase precession properties. This included the r value of the circular-linear correlation of phase and position, the p value of that correlation, and the slope of the regression line (Table 1). These results indicated that the MIA intervention did not alter the ability of individual cells to precess, and that this precession had a similar structure and slope across the place field when compared to phase precession in CTL cells (see Figure 4 for example plots). Since these data included all cells, regardless of whether they had significant phase precession (circular-linear correlation $p < 0.05$) or not, we also examined these characteristics in the subset of cells that had a significant p-value for the circular-linear correlation (s = significant subset). This subset accounted for 50% of all CTL cells (n = 112) and 44% of MIA cells (n = 145). These proportions were not significantly different to expected values (chi-square, p = .159). Again, we found no significant differences for any of the phase precession measures described above, confirming that individual cells continue to precess normally following an MIA

intervention (Table 1, lower half), irrespective of whether the whole population of cells, or just the significantly precessing cells were analysed.

……Figure 4 here……

Starting phase of precession is more variable in the MIA group

While individual MIA cells appeared to display unimpaired phase precession, a clear between-group difference was observed for the intercept of the regression line of the circular-linear correlation, a measure that quantifies the starting phase of precession as the animal enters the place field (Figure 5a). While the mean intercepts were similar between groups (CLT = 56.54º, MIA = 60.58º, where 0º denotes the trough of the theta oscillation at the cell layer, Watson Williams $F(1, 547) = 0.15$, $p = .694$), the variance in starting phase angle was considerably lower for CTL cells, as evidenced by a longer mean vector length (MVL: CTL = .30, MIA = .13) and higher concentration (CTL = .62, MIA = .26). This difference between groups was confirmed in three separate analyses: First, we performed a Mardia Watson Wheeler test (a nonparametric test for circular data that considers differences in either the mean or variance), which returned a significant result ($W = 7.27$, $p = .026$). As further verification that this difference emerges specifically from the variance, we then performed a variance ratio F-test on angular variance, which returned a significant result ($F = .66$, $p < .001$). We also computed the difference from the group mean angle for each individual cell and then compared between groups. Again the result was significant (Mann Whitney $U = 31516$, $p = .009$), indicating that MIA cells have a higher variance in starting phase as they begin to precess through a place field (Figure 5b). Although the starting phase variance was significantly greater in the MIA group, both groups had a non-uniform distribution of this

measure that was significantly different from zero, indicating that, despite the variance, the MIA group still demonstrated a preferred starting phase (CTL Rayleigh Z = 19.44 $p < .001$; MIA Z = 5.59, p = .004). As before, we re-examined these differences using the subset of cells with significant phase-location correlations (p-value < .05). Again, MIA cells had a smaller MVL (CTL = .42, MIA = .24) and a lower concentration (CTL = .92, MIA = .49; Figure 5c), although again, the intercept distribution was significantly different from zero in both groups, (CTL Rayleigh Z = 19.44, p = .001; MIA Z = 8.11, $p < .001$). The result of the Mardia-Watson Wheeler test was significant (W =7.09, p = .029), as was the variance ratio F-test (F = .58, p = .003) and the group difference from mean angle (U = 6785, p = .024; Figure 5d), confirming that circular variance remained higher in the MIA group. These results were not dependant on either the increased firing rates observed in MIA cells, as correlations between the difference from mean intercept angle and either infield firing rate or mean firing rate were non-significant for both groups (infield firing rates: CTL r = .00, p = .95; MIA r = .03, p = .591; mean firing rates: CTL r = -.01, p = .892; MIA r = .06, p = .28).

To ensure that these results were not driven by aberrant recordings from a small proportion of the MIA animals, we also tested for starting phase variance on a between-animal basis (Figure 5e). The mean vector length (MVL) of the starting phase for CTL cells, calculated on a per animal basis was .57, much greater than that for MIA animals (.29, t (15) = 3.43, p = .004). Again, these results were upheld following the removal of cells with weak (p>0.05) phase precession (MVL CTL = .63; MIA = .37, t (15) = 3.01, p = .009; Figure 5f). Similarly, when calculated across the litters from which each animal came from, the mean vector length of starting phase in MIA litters (.28, n = 8) was significantly smaller than that of control litters (.59, n = 5, t (11) = 4.13, p = .002; Figure 5g), indicating that these results are consistent across both individual animals and litter groups.

The finding that cells from MIA animals show greater variability in phase precession starting phase, while other measures of the phenomenon, particularly the regression fit, remain intact, indicates that the variability does not occur on a moment-to-moment basis. One possibility is that individual MIA cells have particular preferred starting phases, which are stable over time for that cell and can co-occur simultaneously alongside other cells with widely different starting phases. An alternative possibility is that the population of MIA cells could all have a similar starting phase at a particular time period, but this starting phase could shift coherently for the population between one recording session and the next, which should not disrupt theta sequences. To examine this possibility, we determined the MVL across cells for each individual recording that had a minimum of three simultaneously recorded cells. The results showed a shorter MVL for MIA cells (.53) compared to CTL cells (.65; $t(82) = 2.41$, $p = .018$), indicating that starting phase was significantly more variable across simultaneously recorded MIA cells. This demonstrated that the phenomenon was not a result of recording to recording phase shifts across a coherent population (Figure 5h).

……Figure 5 here……

Theta sequences are disrupted in the MIA group

One outcome of coordinated phase precession across a population of cells is a theta sequence, the phenomenon by which the firing of several place cells recapitulates their relative locations during a single theta cycle (Foster & Wilson, 2007; Skaggs et al., 1996). A possible effect of the increased variance of starting phase in MIA cells is a disturbance of the ordered temporal/spatial structure of theta sequences (Feng et al., 2015; Schmidt et al., 2009). To test for this, we examined the correlations between the spike time difference of simultaneously recorded cell pairs and the distance between their respective place fields. If

449 theta sequences are intact, this correlation should be positive, indicating that the difference in
450 firing time between individual place cells tends to be greater within the theta cycle when the
451 individual cell's place fields are further apart. Because theta sequences are a circular
452 phenomenon and because the running track was topologically circular it becomes difficult to
453 determine lead/lag relationships at greater times and distances. For these reasons we limited
454 our analysis to spike pairs from different cells that occurred within a portion of the theta cycle
455 over a time window of 40 ms. This time period reflects the upper limits at which spike-time
456 dependent plasticity (STDP) occurs (Dan & Poo, 2004), a plasticity phenomenon that has
457 been linked theoretically to theta sequences (Mehta et al., 2002). It is also within the temporal
458 window of sharp-wave ripple replay/preplay events and so spikes that occurred during ripples
459 in the LFP were discarded to exclude this phenomenon. As predicted, we found a significant
460 positive circular-linear correlation between the time difference between spike pairs and the
461 distance between place fields in CTL cells ($r = .11$, $p < .001$; figure 6). In contrast, for MIA
462 cells there was no correlation ($r = .01$, $p = .447$). The difference between the MIA and CTL
463 correlation coefficients was significant when tested using Fisher's r to z transformation ($z =$
464 5.94, $p < .001$), confirming that theta sequences are disrupted in the MIA group. These results
465 cannot be explained by group differences in place field distance, as the mean distance of CTL
466 fields (86 ± 5.6^{o}) was not statistically different to the MIA mean distance (82.7 ± 4.4, $t(208)$
467 $= .59$, $p = .553$). With a 40 ms analysis window the slope of the time/distance relationship in
468 CTL cells was 44 degrees across the period, which corresponds to around 130 degrees in a
469 120ms theta cycle. This is lower than might be expected, as on average precession occurred
470 over 220 degrees of the theta cycle in our data, however when the duration of the analysis
471 window was reduced to 30 ms the CTL slope was 56 degrees (224 degrees across a 120 ms
472 theta cycle). In contrast the slope for the MIA data fit was virtually flat (-1.3 degrees/30ms
473 and -3.8 degrees/40ms; -5 degrees/cycle and -11 degrees/cycle respectively (figure 6).

474 **……Figure 6 here……**

475

476 **Discussion**

477 Our control data are consistent with previous studies showing that as an animal enters a
478 CA1 place field, the cell's firing will initially occur just after the trough of the local theta
479 cycle (Dragoi & Buzsáki, 2006; O'Keefe & Recce, 1993; Skaggs et al., 1996). In contrast,
480 the MIA intervention produces greater variance in this starting phase, such that individual
481 cells are more likely to begin firing at different phases of the theta cycle. This effect occurs
482 without changes to other phase precession properties of individual cells, such as the
483 robustness of precession and the phase/distance relationship (slope). This MIA-induced
484 change was observed both at the level of the pooled data set, and after filtering for the subset
485 of cells that showed the strongest phase precession. Incoherent starting phase was also
486 observed in the MIA group when data were analysed on either an animal by animal or litter
487 by litter basis. These disturbances also occurred independently of any group differences in
488 place field size, information content, or mean speed through the non-reward arms.
489 Furthermore, it was not related to differences in firing rate. There was no also evidence to
490 suggest the small between-group differences we observed in theta amplitude and frequency
491 were a factor, as the phase profile of theta was maintained across time and phase-location
492 correlations were preserved.

493 Positive correlations of theta frequency and speed have been observed previously
494 (Geisler et al., 2007), and our CTL data are consistent with these findings. This relationship
495 was not apparent in the MIA group. This disruption is unlikely to account for the increased
496 variability in starting phase observed in this group as phase precession does not appear to be
497 affected by running speed (Huxter et al., 2003). Rather, given that theta frequency has been
498 shown to predict speed via a hippocampal-lateral septum pathway (Bender et al., 2015), this

may reflect disturbed transmission of theta sequences to downstream structures (Tingley & Buzsáki, 2018).

One potential explanation for the increased variance in starting phase is that electrode depth was more variable in the MIA group, as previous reports have demonstrated that theta phase varies with electrode depth across the *stratum radiatum* of CA1 (Buzsáki et al., 1985; Lubenov & Siapas, 2009). This explanation is unlikely however given that we observed no systematic differences in electrode placement after histology, and both cells and LFPs were always recorded from the same depth. Furthermore, starting phase variation in MIA animals could be observed across cells that were simultaneously recorded from the same tetrode bundle.

Theta sequences are phase precession-related phenomena whereby individual cells contribute to a population-based representation of the local ordering of place fields within a theta cycle. One predicted consequence of increased variability in the starting phase of precession is that theta sequences will be disrupted, such that the organised structure of spatial locations or experience will be reconstructed in a disorganised manner during the theta sequence (Figure 7). Our data support this prediction, showing that in MIA animals there is a disruption in the temporal relationship between cell pair firing during a theta cycle and the spatial distance between the place fields of the cell pairs.

……Figure 7 here……

While it was initially assumed that theta sequences were an inevitable consequence of phase precession (Skaggs et al., 1996), recent findings suggest that they can be dissociated (Dragoi & Buzsáki, 2006; Feng et al., 2015; Foster & Wilson, 2007; Itskov et al., 2008). Our data provide additional evidence that theta sequences require additional network coherence

above and beyond the precession of individual cells, including a consistent starting phase as animals enter a new place field. Inhibitory interneurons may play an important role in this process (Chadwick et al., 2016; Kamondi et al., 1998; Losonczy et al., 2010; Magee, 2001; Maurer et al., 2006; Nicola & Clopath, 2019; Royer et al., 2012). GABAergic systems are known to be disturbed in both schizophrenia patients (Akbarian & Huang, 2006), and in MIA animal models of the disorder (Corradini et al., 2018; Dickerson et al., 2014), including specific disruptions to PV expressing interneurons (Gonzalez-Burgos et al., 2015; Lodge et al., 2009; Steullet et al., 2017). These changes could underlie the increased starting phase variability demonstrated in this study, and are also consistent with the elevated firing rates observed in the MIA group.

In rodents, theta sequences are necessary for maintaining internally generated place fields when external cues are held constant (Wang et al., 2015), and for non-spatial event sequencing (Terada et al., 2017), suggesting that they may play a larger role in sequential processing beyond spatial cognition. Theta sequences have also been associated with goal planning and prediction (Gupta et al., 2012; Wikenheiser & Redish, 2015) and phase precession has been associated with non-spatial forms of sequential processing in both rodents (Pastalkova et al., 2008; Royer et al., 2012) and humans (Heusser et al., 2016; Qasim et al., 2020). In humans the hippocampus is involved in both episodic memory processes and thinking about the future (Schacter et al., 2007), and theta sequences may therefore have a fundamental role in these processes (Buzsáki & Tingley, 2018; Jaramillo & Kempter, 2017; Terada et al., 2017; Wang et al., 2015). In support of this hypothesis, the developmental emergence of theta sequences coincides with the maturation of hippocampal memory (Muessig et al., 2019), and increases in hippocampal theta power have been observed in humans undertaking sequential planning tasks (Kaplan et al., 2020).

548 The disrupted theta sequences observed after a MIA intervention could therefore
549 contribute to episodic and relational memory impairments. Although MIA-induced memory
550 deficits are not always apparent in simple memory tasks, they frequently emerge as task
551 complexity increases, including tasks that involve a working memory or reversal component
552 (Bitanihirwe et al., 2010; Savanthrapadian et al., 2013; Wolff et al., 2011). To date no MIA
553 study has explicitly examined sequential memory, although a recent study has demonstrated
554 that MIA animals display deficits in temporal perception (Deane et al., 2017). In contrast,
555 impairments in temporal processing and sequential ordering are well documented in
556 individuals with schizophrenia, their first-degree relatives, and other at-risk individuals
557 (Ciullo et al., 2016; Thoenes & Oberfeld, 2017). For example, a recent meta-analysis has
558 confirmed that patients with schizophrenia have more variable, and therefore less precise,
559 judgement of temporal order than healthy control participants (Thoenes & Oberfeld, 2017).
560 This lack of precision when making temporal judgements may reflect a fundamental
561 disorganization in the encoding and storage of events as they occur across time and space,
562 such that the sequential order of information becomes scrambled or unstable. Disordered
563 sequential processing could also have additional effects on a wide range of cognitive
564 processes that require sequential ordering, including episodic memory, speech production,
565 goal planning, and flexible decision-making processes. These cognitive processes are all
566 disturbed in schizophrenia (Barch & Ceaser, 2012). It has also been suggested that
567 disorganized temporal processing may be a potential trait marker of the disorder, with
568 underlying relevance to several classic symptoms (Andreasen et al., 1999). For example,
569 disordered sequences could contribute to erroneous connections being made between
570 externally generated stimuli and internally generated thoughts and actions, resulting in
571 misattributions of agency and control, as well as other forms of delusion and paranoia
572 (Andreasen et al., 1999; Thoenes & Oberfeld, 2017).

One further consequence of increased variability in the starting phase of precession is that sequential spiking would not be clustered as tightly within each individual theta cycle, particularly if individual cells precess less than 360° (Schmidt et al., 2009). Thus, the clustering of spikes within a single theta cycle is likely to be more dispersed following an MIA intervention, potentially contributing to the erroneous association of spikes across successive theta cycles. For example, spikes which represent the end of an earlier sequence might be misattributed as early spikes in the next cycle (see Figure 7). This may have important consequences in terms of how experience is segmented into "chunks," (Gupta et al., 2012), and as a result MIA animals may have difficulty processing discrete units of information that are updated continuously across successive theta cycles. One proposed analogy for this phenomenon is a series of sentences without punctuation marks, and this lack of "punctuation" may contribute to the confused order of thoughts that occurs in schizophrenia (Lisman & Buzsáki, 2008). Furthermore, such blurring of event boundaries is likely to have profound implications for memory and learning (Lisman & Buzsáki, 2008), as current evidence suggests that the ability to successfully parse events into meaningful segments predicts performance on numerous tasks (Richmond et al., 2017). Consistent with these findings, individuals with schizophrenia appear to have event segmentation deficits at both perceptual (Coffman et al., 2016) and high-order levels (Zalla et al., 2004).

In summary, our results demonstrate that the coherent activity of individually precessing cells is compromised following an MIA intervention, resulting in disordered theta sequences. This finding provides a potential biological-level mechanism that may explain some aspects of disorganized temporal processing in schizophrenia and underlie some of the core features of the disorder, particularly the disruption of episodic memory and planning processes.

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Figure 1. Schematic diagram of place cells, phase precession and theta sequences. (a) Canonical place field defined by firing rate. (b) Example of a place field defined by its phase code. Note that cell spiking begins at a later phase of the theta cycle and precesses to an earlier phase as the animal moves through the field. The range of phase precession across the place field is also lower than 360º. (c) Illustration of how the phase of firing of a single cell advances across several theta cycles as an animal moves from left to right through a place field as in b. (d) When an animal is in a particular location along a track (top) there will be a number of place cells active, all with overlapping place fields (A-E; middle). Vertical black lines delineate the position of the animal in each of the place fields and the resultant phase of the theta cycle that the cell fires in. As a result, cells A-E fire in an ordered sequence across a theta cycle where the order matches their relative spatial relationship (bottom). Note that the generation of this ordered sequence depends on the starting phase and the slope of precession being coherent across all cells A-E.

Figure 2. Experimental procedures. (a) Diagram of the rectangular track. Rats were pre-trained to run in a clockwise direction for a food reward delivered at the centre of the bottom arm. (b) Diagram of the hippocampus showing the target area for surgical implantation, and an example photograph of histology demonstrating electrode placement in the pyramidal cell layer of CA1 (top). Below is a diagram of tetrode recording locations for both groups, with CTL locations shown in black, and MIA locations in red. Modified image is originally from Paxinos and Watson (2006). (c) Examples of cluster cutting of three separate cells for CTL and MIA recordings (on left), and an example of a place field (shown here before linearization of the track). The X denotes the reward area.

Figure 3. Basic properties of single units and LFP oscillations in MIA and CTL animals. (a) Mean firing rates of single units across the entire track and inside the place field. Black bars denote the median. (b) Mean speed across the 3 non-reward arms. (c) Place field length. Bars denote mean and standard error. (d) Information content in bits per spike. Bars denote median. (e) Example of filtered and raw EEG recordings. (f) Theta frequency in Hz. (g) Average (± sem) waveform shape for all LFP data, bandpass filtered between 6-10Hz and with samples triggered from the trough. (h) LFP amplitude. Bars denote median. (i) Average phase profile of the theta waveform as in g, as determined from Hilbert transform. (j) Median r value of the linear correlation of theta frequency and speed for each recording. Significance levels for all images: * $p < .05$, ** $p < .01$, *** $p < .001$.

Figure 4. Example plots of phase precession showing (a) cell firing phase relative to position across the 3 non-reward arms of the track. Track is linearized, and zero on the x-axis corresponds to the bottom left corner in figure 2A. Animal is moving left to right in these and other diagrams. Firing phase is duplicated across two theta cycles on the y axis for clarity. (b) Data as displayed in b, normalised to each place field for analysis. The red line denotes the line of best-fit for the circular-linear regression.

Figure 5. Precession starting phase is more variable in MIA cells. (a) Circular histograms of intercept values for both groups, where all cells with a place field are included in the analysis. Red bars denote the mean angle with 95% confidence intervals. Between group differences are based on the variance ratio F test. (b) Violin plots showing the distance from the mean angle for each individual cell. (c and d) As for a and b respectively, except only cells with significant phase precession (circular linear correlation $p < .05$) are included

837 (s = significant subset). (e) Mean vector length on an animal-by-animal basis. (f) As for e, but
838 only including cells that have significant phase precession. (g) Mean vector length on a litter-
839 by-litter basis. (h) Mean vector length calculated across cells recorded simultaneously in a
840 single recording (three cells minimum).

841

842

843 **Figure 6.** Significant positive circular-linear correlations occur between angular
844 distance between place fields and the time between spikes in the CTL group, demonstrating
845 that theta sequences are intact. In contrast, no such relationship was observed for the MIA
846 group. The distance between place field centres in a clockwise direction (cw) is shown on the
847 y axis as a circular measure, repeated for two cycles for clarity. Time between spike pairs is
848 shown on the x axis for three different maximum time windows. In each example, the line of
849 best fit, is projected out to 120 ms (~ 1 theta cycle) to demonstrate how much phase shift
850 might occur across a full cycle. Dashed blue lines are included to aid visualization of this
851 phase shift.

852

853 **Figure 7.** The effect of the MIA manipulation on starting phase and theta sequences. A
854 cartoon of CTL data is displayed on the left as for figure 1b and d where coherent starting
855 phase and slope allow cells A-E to fire in an ordered sequence within a theta cycle that
856 matches the relative spatial ordering of the place cells' fields (bottom). Variance in starting
857 phase, as occurs in the MIA animals (right), would, however, disrupt this replay sequence
858 despite MIA cells still displaying phase precession. Note that as a consequence of the
859 variable starting phase the disorganized theta sequence (bottom) is more dispersed across the
860 theta cycle in MIA animals.

861

862 **Table 1.** Measures of phase precession (circular-linear correlation) in hippocampal place
863 cells following an MIA intervention. Upper data includes all cells. Lower data is from the
864 subset of cells that displayed significant ($p<0.05$) phase precession.

	Mean ± SEM		Test (df)	Result	Sig.
All cells	CTL (n = 222)	MIA (n = 327)			
Correlation	-.10 ± .02	-.10 ± .02	t test (547)	t = .36	p = .717
p-value	.22 ± .02	.24 ± .02	Mann Whitney	U = 34438	p = .308
Slope	-219.30 ± 15.24	222.62 ± 14.35	Welch's t test (513.1)	t =.16	p = .876
Cells (s) for p-value < 0.05	CTL (n = 112)	MIA (n = 145)			
Correlation (s)	-.15 ± 0.03	-.18 ± 0.02	Mann Whitney	U = 7992	p = .829
p-value (s)	.01 ± .00	.007 ± .00	Mann Whitney	U = 7671	p = .447
Slope (s)	-267.44 ± 20.65	-307.687 ± 19.61	t-test (255)	t = 1.41	p = .161

865