Psychoneuroendocrinology 110 (2019) 104448

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Raloxifene recovers effects of prenatal immune activation on cognitive task-induced gamma power

Anna Schroeder[a,b,1], Jay P. Nakamura[a,b,1], Matthew Hudson[c,d], Nigel C. Jones[c,d], Xin Du[a,b], Suresh Sundram[b], Rachel A Hill[a,b,*]

[a] Psychoneuroendocrinology Laboratory, Florey Institute for Neuroscience and Mental Health, University of Melbourne, Parkville, VIC, 3010, Australia
[b] Behavioural Neuroscience Laboratory, Department of Psychiatry, School of Clinical Sciences, Monash University, 246 Clayton Rd, Clayton, VIC, 3168, Australia
[c] Department of Medicine, University of Melbourne, Parkville, VIC, 3010, Australia
[d] Department of Neuroscience, Monash University, Clayton, VIC, 3168, Australia

ARTICLE INFO

Keywords:
Raloxifene
Poly-I:C
Schizophrenia
Cognition
gamma oscillations
Sex differences

ABSTRACT

There is currently no treatment available for the cognitive symptoms of schizophrenia, but evidence suggests that selective estrogen receptor modulators (SERMs) may provide relief. Our recent animal model data showed that a lack of female sex hormones in mice impairs the ability of hippocampal neurons to synchronise and generate oscillations within the frequency range of 30–80 Hz (gamma power) leading to cognitive impairment, while both estradiol and the SERM, raloxifene, recovered this. Given that cognitive impairment is accompanied by abnormal gamma power in schizophrenia, this study aimed to determine the effects of raloxifene on gamma power during spatial memory tasks in the prenatal immune challenged (poly-I:C) mouse model with relevance to schizophrenia.

Pregnant dams received the viral mimetic poly-I:C (20 mg/kg, i.p.) at gestational day 17. Male and female offspring were treated with placebo or raloxifene implants at adulthood. Local field potentials from the CA1 hippocampus were simultaneously recorded during the Y-maze test of short term spatial memory and the cheeseboard maze test of long-term spatial learning and memory and cognitive flexibility.

In female but not male mice, poly I:C exposure reduced gamma power during decision making and prolonged the time spent in the centre (decision making phase) during the Y-maze task. Female poly-I:C exposed mice also showed increased gamma power during acquisition of the cheeseboard long term memory task and perseverative behaviour. Treatment with raloxifene recovered gamma power and decision making deficits in the Y-maze and restored gamma power changes during the cheeseboard maze task as well as perseverative behaviour. Male mice showed no electrophysiological or behavioural effects of poly-I:C or raloxifene treatment.

In summary, poly-I:C exposure induced female specific cognitive impairments accompanied by altered neural oscillations in the gamma frequency and raloxifene recovered these abnormalities.

1. Introduction

Cognitive impairments are now recognised as core features of schizophrenia, which emerge years before the first psychotic episode, but persist throughout the course of illness, and contribute significantly to functional disability in schizophrenia patients (Green et al., 2004). Although currently available antipsychotics can be effective at treating the positive symptoms of schizophrenia such as hallucinations and delusions, the cognitive symptom domains remain relatively unaffected by these drugs (Keefe et al., 2007; Young and Geyer, 2015) and new pharmacological treatment options are needed. Working memory – that is the ability to briefly hold and manipulate certain information – is consistently shown to be impaired in schizophrenia patients. Schizophrenia patients often show cognitive inflexibility, also known as perseverance, which can be described as a persistent repetitive behaviour, where the subjects would rather persist in achieving something despite obstacles rather than think of another possibility. This ability to shift cognitive set as well as spatial navigation is disrupted in people with schizophrenia and is believed to be subserved, at least partially, by the network mechanisms of the prefrontal cortex and its hippocampal

* Corresponding author at: Department of Psychiatry, School of Clinical Sciences at Monash Health, Monash University, Level 5, Block E, Monash Medical Centre, 246 Clayton Rd, Clayton, VIC, 3168, Australia.
E-mail address: Rachel.hill@monash.edu (R.A. Hill).
[1] These authors are joint first authors.

https://doi.org/10.1016/j.psyneuen.2019.104448
Received 30 July 2019; Received in revised form 4 September 2019; Accepted 13 September 2019

connections (Essizoglu et al., 2017; Folley et al., 2010; Morice, 1990).

Cognitive dysfunction in schizophrenia is associated with abnormal synchronisation of neuronal firing, predominantly in the gamma frequency range (30–80 Hz), also known as gamma power (Uhlhaas and Singer, 2010), but also in the theta frequency range (5–10 Hz), known as theta power (Schmiedt et al., 2005). While in healthy individuals' gamma power increases during cognitive tasks, patient's show an increased gamma power at baseline, which declines during cognitive tasks, inversely correlating with task performance (Senkowski and Gallinat, 2015; Uhlhaas and Singer, 2010). Gamma oscillations can be generated by a subtype of GABAergic interneurons expressing the calcium-binding protein parvalbumin (PV) (Sohal et al., 2009), which in turn is shown to be aberrant in schizophrenia post-mortem brains (Fung et al., 2010; Konradi et al., 2011b; Senitz, 1999; Zhang and Reynolds, 2002a). Many rodent studies have shown enhancements in both gamma and theta power during specific decision making stages of spatial memory tasks (Jones and Wilson, 2005; Yamamoto et al., 2014), suggesting decision-making requires co-ordinated activity of neural ensembles.

We have recently shown in mice that hippocampal gamma oscillations are impaired during a spatial memory task accompanied by behavioural deficits when there is a lack of estradiol, and both estradiol and the selective estrogen receptor modulator, raloxifene, can recover these deficits (Schroeder et al., 2017). The protective role of estrogens in schizophrenia has been supported by a number of clinical studies (Kulkarni et al., 2008a, 2015; Kulkarni et al., 2008b). Extended treatment with estrogens, however, is not feasible due to their unwanted peripheral effects (Lethaby et al., 2004). Raloxifene, a selective estrogen receptor modulator (SERM), has antagonistic effects in peripheral tissue such as the breast and uterus but is proposed to have agonistic effects in the brain, and hence may be a safer treatment option for schizophrenia. Raloxifene is currently used as treatment for osteoporosis in postmenopausal women and was recently shown to have beneficial effects on all symptom domains of schizophrenia, (Huerta-Ramos et al., 2014; Jacobsen et al., 2010; Kulkarni et al., 2008b, 2010; Usall et al., 2011; Weickert et al., 2015). Adjunctive raloxifene treatment not only improved cognitive performance in postmenopausal women with schizophrenia (Huerta-Ramos et al., 2014; Jacobsen et al., 2010; Kulkarni et al., 2008b), but also in young and middle aged women as well as men with schizophrenia (Weickert et al., 2015), showing its efficacy on cognitive function in a younger population and in both sexes. However, raloxifene is not yet accepted as a routine treatment for schizophrenia partly due to the limited understanding of its actions in the brain.

This study examined the effects of raloxifene on gamma oscillations and cognitive ability in an animal model that is highly relevant to schizophrenia – the prenatal immune activation model. This robust model is based on epidemiological evidence suggesting that maternal infection during pregnancy (particularly second trimester) greatly increases the offspring's risk of developing schizophrenia (Brown and Derkits, 2010). Prenatal immune activation (PIA) can be modelled in mice by administering the viral mimetic; polyinositic:polycitidylic acid (polyI:C) intraperitoneally at gestational day 17 – corresponding to the second trimester in humans. Offspring of poly I:C exposed mice in particular during this late stage of gestation have previously shown reduced PV expression, abnormal neuronal oscillations and cognitive dysfunction which tend to emerge at young adulthood (Connor et al., 2012; Dickerson et al., 2014; Meyer and Feldon, 2010; Meyer et al., 2006; Richetto et al., 2013), consistent with schizophrenia. In the current study prenatal immune activation was modelled using poly-I:C at GD17 and offspring were treated chronically with either placebo or raloxifene at young adulthood. To asses task-induced gamma and theta oscillations, local field potentials (LFP) were recorded from the CA1 region of the dorsal hippocampus during two spatial memory paradigms; the Y maze novelty preference task and the Cheeseboard maze, which tests for learning, long-term spatial memory and reversal memory. The CA1 region was chosen as the site for recording LFP's as it is specifically linked to the establishment of new place maps during reward-associated spatial learning (Dupret et al., 2010; Folley et al., 2010), and we have previously shown that estradiol and raloxifene alters gamma power recorded from this region (Schroeder et al., 2017). In this study we particularly focused on examining a) the decision making process during short-term spatial memory b) acquisition of long-term spatial memory and c) reversal learning (perseverance/cognitive flexibility) as these aspects were shown to be disrupted and accompanied by abnormal gamma-oscillations in schizophrenia patients.

2. Materials and methods

2.1. Animals

All experiments were conducted at the Florey Institute of Neuroscience and Mental Health (Parkville, VIC, Australia). All surgical and experimental procedures were conducted according to the guidelines in the Australian Code of Practise for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council of Australia, 8th edition 2013) and approved by the Animal Ethics Committee of the Florey Institute of Neuroscience and Mental Health University of Melbourne, Parkville, Victoria, Australia and adhered to the AARIVE guidelines in accordance with the NIH guide for care and use of Laboratory animals (NIH Publications No. 8023). For breeding purposes, all dams (C57BL/6J) were ordered from the Animal Resources Centre (Western Australia) to ascertain similar ages. Studs (C57BL/6J) were either obtained from the breeding colony at the Florey Institute of Neuroscience and Mental Health or ordered from the Animal Resources Centre (Western Australia) to ensure similar age. Breeding trios of two females and a stud were housed in individually ventilated cages. Pregnancy was ascertained by visual inspection of vaginal plugs in the morning and evening. The day a plug was seen was considered embryonic day 0.5. Offspring were weaned at 3 weeks of age and were group housed with same-sexed mice across different litters and treatment groups to minimise litter effects. In all cohorts, two to five mice per cage were housed in individually ventilated cages (391 × 199 x 160 mm) (Tecniplast, Sealsafe PLUS Mouse IVC Green Line) with ad libitum access to food (Specialty Feeds, Western Australia) and water in a 12/12 h light/dark cycle (lights on 7am-7pm). The temperature was maintained at approximately 22 °C. Cages were cleaned once a week. All behavioural experiments were performed during the day between 8am and 5 pm.

2.2. PolyI:C treatment

Pregnant dams were injected with either saline or poly-I:C (20 mg/kg) at gestation day (GD) 17.5. This time point was chosen as it was previously shown to induce cognitive deficits related to schizophrenia (Connor et al., 2012; Meyer et al., 2006; Richetto et al., 2013). The dose of 20 mg/kg (i.p) was chosen based on previous studies by our group and others (Nakamura et al., 2019; Smith et al., 2007). Here we previously showed that 20 mg/kg of poly-I:C delivered i.p. caused an increase in both maternal serum and foetal brain expression of the pro-inflammatory cytokine IL-6 and increased foetal brain expression of iba1 and CD68 (markers of microglia) (Nakamura et al., 2019). Pups were weaned at three weeks and group-housed with three to six mice per cage. Treatment groups consisted of male and female control + placebo, poly-I:C exposed + placebo and poly-IC exposed + raloxifene treated mice with 8–10 mice per treatment group, per sex. Groups were randomised between litters so that mice in each group were from different dams. Table 1 shows the spread of litters across treatment groups.

Table 1
Distribution of litters from dams injected with Poly (I:C) (grey) or Saline (white).

Prenatal Treatment	Poly-I:C									Saline			
Dam ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	S1	S2	S3	S4
Adult treatment: Placebo													
Males	1	2	1	1	0	4	0	0	0	3	2	3	0
Females	1	2	1	0	0	0	3	1	3	3	3	2	1
Adult treatment: Raloxifene													
Male	3	2	1	0	2	0	0	0	0	3	1	1	0
Female	0	2	1	1	0	1	3	0	2	2	3	0	0

2.3. Surgical procedures

At 8 weeks of age pups received a SC implant of either a 60-day-slow-release-raloxifene pellet (5 mg, Innovative Research of America, USA) or an empty silastic tube as placebo. Pellets designed by Innovative Research of America consist of a biodegradable matrix that allows effective and continuous release of the active product (in this case raloxifene) in the animal. A previous study showed that 21 day release raloxifene pellets (1 mg and 10 mg, Innovative Research of America) caused a steady activation of the estrogen response element (ERE) in selected peripheral tissues, including skeletal muscle and abdomen as measured using an ERE-luciferase tagged mouse model, confirming steady release of this drug (Rando et al., 2010).

At 10 weeks mice received a tripolar stainless-steel electrode implant (MS333-3-A-SPC, PlasticsOne, Roanoke, VA, USA) consisting of an active, reference and ground electrode, 0.01 in diameter each. Prior the surgery each electrode was cut and bent so that the active electrode could be inserted into the CA1 region of the dorsal hippocampus and reference electrodes could reside subdurally 2 mm left and right from lambda. The coordinates for the dorsal hippocampus with reference to Bregma (AP: −1.8 mm, ML: +1.7 mm, DV: +2 mm) were adopted from (Yamamoto et al., 2014). More details are provided in (Schroeder et al., 2017). Before each recording session, the mice were anaesthetised with isoflurane for 1 min and a 3 m long cable (PlasticsOne, 3 channel EEG cable 335–445) was attached to the electrode cap. Mice were left undisturbed for 30 min in their home cage to recover from anaesthesia and habituate to the cable. Behavioural tests were performed from 12 to 15 weeks.

2.4. Behavioural tests and electrophysiological recordings

2.4.1. Y-maze

Y-maze novelty preference test was performed to assess short-term spatial memory as described previously (Schroeder et al., 2015; Wu et al., 2015). The maze consisted of three arms (30 cm x 8 cm x 16 cm) at 120° angles to each other including geometric cues on the far end walls. Briefly, during the initial phase, the mouse was placed into the end of one arm (home arm) and was allowed to explore two arms for 10 min with one arm being closed (novel arm). After a one-hour retention time in the home cage, the mouse was placed into the same Y-maze with all arms open for 5 min. LFPs were recorded (1000 Hz) during the memory retrieval phase using LabChart7 (ID Instruments, NSW, Australia). The time spent in each arm and in the center area was analyzed with video tracking software (TopScan, CleverSys Inc., Reston, VA, USA). For details of LFP recording and analysis during Y-maze please refer to (Schroeder et al., 2017).

2.4.2. Cheeseboard maze

The cheeseboard task was constructed from a circular table (94 cm diameter) that was painted grey. Caps (Corning 50 ml polypropyl tube caps, 3.25 cm diameter, 1 cm depth) were painted the same colour and were superglued to the table to be used as reward wells. 32 wells were spread evenly in a radial pattern of 8 lines of 4 wells (Supplementary Fig. 1). The inner most well of each line was 14 cm from the center of the maze and the outer most well was 5 cm from the edge. Along each line, every well was separated from the next by 5 cm. A semitransparent cylindrical start cage (28 cm diameter) was attached to a pulley system and was placed in the center of the maze. High contrast spatial cues (A3 size) were attached to the interior of the faraday cage ("North", "South", "East", "West") (Fig. 1). This paradigm has been adapted from other studies (Karl et al., 2012; Lei et al., 2017) and was previously performed in our laboratory (Grech et al., 2019).

Baseline weights of the mice were obtained by taking the average weight over three days before commencement of food restriction. Food restriction was introduced gradually over 3 days prior to testing. Animals were fed approximately 80% of free feeding amount (1.5–2.5 g) (determined in a preliminary test on a separate cohort of mice) to obtain a total weight loss of 10%. The weights of the mice were recorded every day during the CB task and individually weighed food pellets were supplied to maintain their weights at 90%.

Mice were habituated to sweetened condensed milk (diluted in water 1:4) food reward for 2 days prior to testing. Testing for all mice across trials was conducted in the same room. All wells of the maze were brushed with condensed milk prior to testing to minimize the effect of odour cues. The maze was cleaned with diluted ethanol (approx. 10%) after each trial. The complete testing period spanned 14 consecutive days (Fig. 1); habituation: 2 days; acquisition learning: 7 days; reversal learning: 5 days. Two 2 min trials with an inter trial time of 20 min were conducted on each day, excluding day 14.

On the first 2 days, mice were habituated to the testing protocol and environment on an unbaited maze. During acquisition learning on days 1–7 after habituation, one well was baited with 100 µl of food reward. The location of the baited well was conserved across trials and days for each mouse, but the location of the baited well was different for each mouse. Electrophysiological recordings were taken on the first trial of acquisition day 6. A probe trial was then conducted on day 7, where no well was baited. Here time spent in each quadrant was assessed for the full 2 min of the trial. The original target well was re-baited for the second trial of day 7 to reinforce acquisition phase learning. Following the acquisition probe day, the maze was turned 180° to confirm distal spatial cues and not proximal (on the maze) cues were being utilized, then the location of the baited well was moved clockwise to the reversal target location (Fig. 1). This new location was conserved during reversal learning for 5 days after acquisition learning. The reversal phase tested for perseverative behaviour. Electrophysiological recordings were taken during day 3 of reversal learning. A probe test for the reversal phase was conducted on day 14 corresponding to day 5 of reversal learning, whereby no well was baited. Once again time spent in the new target quadrant was assessed across the 2 min trial.

Cheeseboard maze trials were analyzed using video tracking software (Topscan CleverSys, VA, USA). The maze was sectioned into zones between each line of wells, totaling 8 equal wedge-shaped zones. Two adjacent zones were combined to create quadrants, depending on the location of the baited well (Fig. 1). The centre zone (circular, reaching the inner-most well of each line of wells) was included for measurement of distance travelled but was excluded for quadrant based analysis. The software detected when the mice reached the baited well, distance travelled and time spent in specific zones.

2.4.3. Electrophysiology

All data analysis was conducted using custom-designed scripts written for MATLAB (v7.10.0, Natick, Massachusetts: The MathWorks Inc., 2010) (scripts available from NCJ on request). The recording consisted of 2 s epochs to measure gamma and theta power in the Y maze and cheeseboard tasks. Every epoch was inspected in MATLAB for signal distortion or movement artefact, which was manually marked and removed from the recorded data. Average power in the gamma frequency band (30–80 Hz) and theta frequency band (5–10 Hz) was

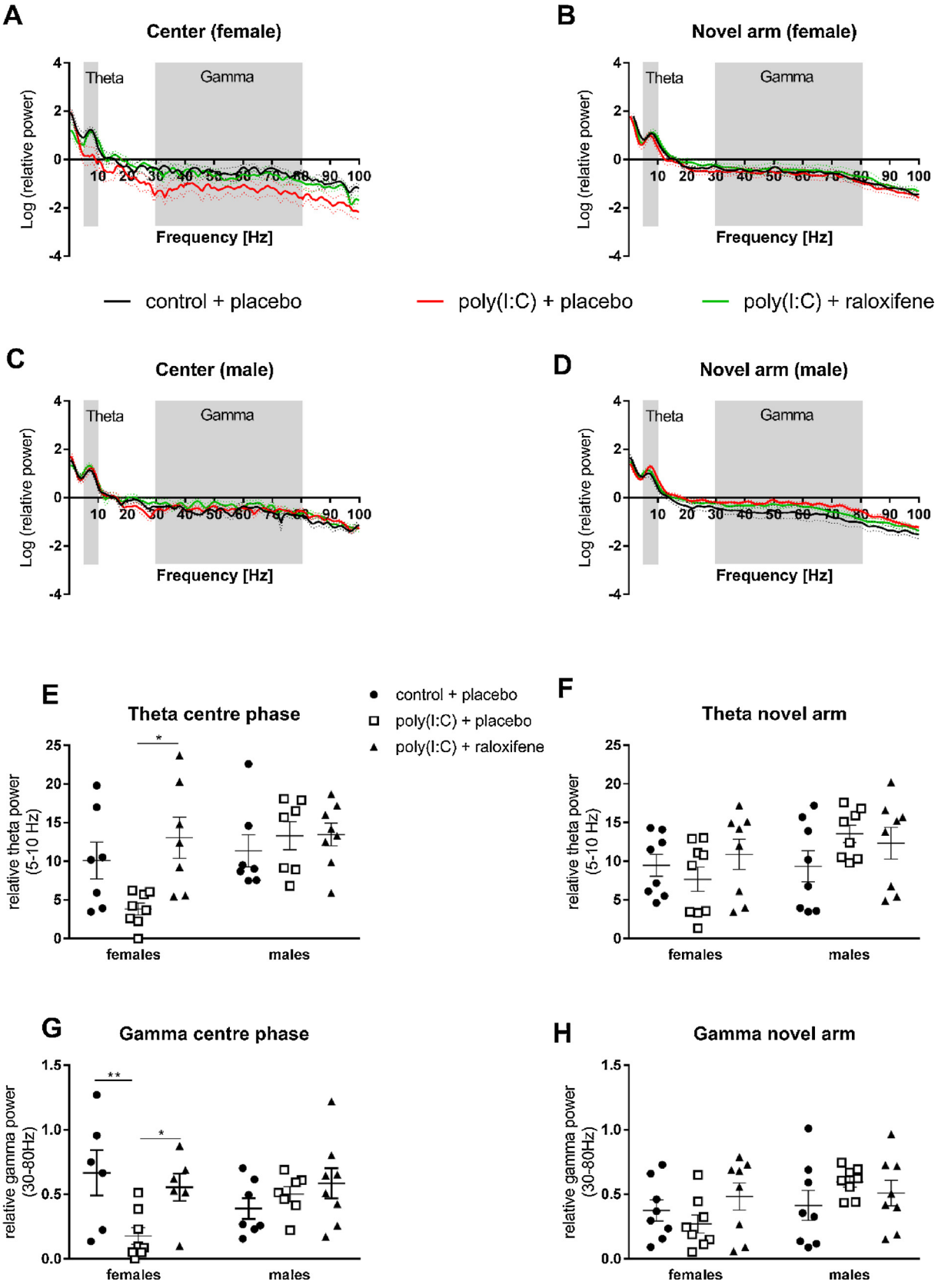

Fig. 1. Gamma and theta oscillations during the memory retrieval phase of the Y-maze novelty preference task in control (circles), poly-I:C exposed (squares) and poly-I:C exposed + raloxifene treated (triangles) male and female mice. Power spectrum densities during the first entry to the centre (A) and novel arm (B) in female mice and centre (C) and novel arm (D) in male mice. Quantification of theta power in the centre phase (E) and novel arm (F). Quantification of gamma power in the centre phase (G) and novel arm (H). Data are mean ± SEM. * = P < 0.05, ** = P < 0.01.

determined for each 2 s epoch during decision making by means of Fast Fourier Transformations (FFT). Relative gamma power was calculated as gamma power (30–80 Hz)/total power (1–200 Hz). Relative theta power was calculated as theta power (5–10 Hz)/total power (1–200 Hz). LFPs of each mouse were recorded during the Y-maze and cheeseboard maze tasks and synchronised with video recordings in order to match brain activity with behaviour.

2.4.3.1. Y-maze. For the Y-maze task, recordings were analysed during the probe test following the retention period. We previously found that raloxifene had an effect on gamma, but not theta power specifically on the first entry to the centre of the Y maze (decision making phase). Hence, in this study we analyzed gamma and theta power and time spent in the centre of the maze and compared it to the gamma power and time spent in the novel arm of the Y maze. This ranged from 2 to 10 secs for the centre phase and from 2 to 20 sec in each of the arms. For each mouse the average power from all 2 s epochs was calculated for each phase of the task. Power spectral density was represented as a function of log10[raw power/average of total power (1–200 Hz)).

2.4.3.2. Cheeseboard. Recordings were taken during the 6th day of acquisition and the 3rd day of reversal learning (Fig. 1). These time points were chosen as they give an indication of how gamma and theta oscillations may be involved in the "searching phase" after acquisition or reversal learning have been acquired. We chose not to record during the probe test so as to not interfere with the behaviour. Recordings were analysed from when the mouse first began to search for the baited well until the end of the test session, which lasted for 2 min for each mouse. Power spectral density was represented as a function of log10[raw power/average of total power (1–200 Hz)). Due to a background noise of around 50 Hz, all data points from 45 to 55 Hz were omitted from all groups.

2.5. Statistical analysis

All data are normally distributed, therefore 2-way ANOVAs were performed to analyse the effects of treatments (poly-I:C and raloxifene) and sex (male and female) on gamma/theta power as well as all behavioural outcomes on the Y-maze task. For the cheeseboard maze we had the added variable of time during acquisition of the task therefore the sexes were split and a two-way repeated measures ANOVA was performed with days (acquisition: 1–6 or reversal: 1–4) and treatments (control, poly-I:C placebo and poly-I:C raloxifene) as independent variables and latency (sec) as the dependent variable. For the probe test outcomes and the electrophysiology during cheeseboard testing two-way ANOVAs were performed with treatment and sex as the independent variables and time spent in the target quadrant or oscillatory power as the dependent variables respectively. Tukey's comparison was performed as post-hoc analysis. In addition, for the probe tests, single sample t-tests of each group (female and male; control; poly-I:C; and poly-I:C + ral.) comparing time spent in the target quadrant compared to chance level (25%) was performed. For the reversal probe test this included the analysis of time spent in the new target compared to chance (25%), and time spent in the old target compared to chance (25%). These tests were performed to determine whether each group effectively learned the task. In all cases, the significance level was set to $p \leq 0.05$.

3. Results

3.1. Gamma and theta oscillations during the memory retrieval phase of the y-maze

Power spectrum density (1–100 Hz) was determined during the first entry into centre of the Y maze (decision making phase) and into the novel arm in female (Fig. 1A and B) and male (Fig. 1C and D) mice. This is shown as log10 function of the relative power (power/average of total power within 1–200 Hz) as no differences were seen in absolute power between the groups (data not shown).

For quantification of theta power during the first entrance to the centre phase there was no main effect of treatment or sex but there was a significant sex x treatment interaction ($F(2, 38) = 3.493$, $p = 0.04$; Fig. 1E). Here, post-hoc analysis showed a significant difference between the poly-I:C + placebo and poly-I:C + raloxifene group in female mice ($p = 0.0038$) with raloxifene increasing theta power compared to poly-I:C + saline treated mice in female but not male mice (Fig. 1E). No effects of poly-I:C exposure or raloxifene treatment on theta power were found during the first entry to the novel arm in either sex (Fig. 1F), suggesting this effect in female mice is specific to the decision-making phase.

For gamma power during the first entrance to the centre phase there was no significant main effect of treatment or sex, but once again we found a significant sex x treatment interaction ($F(2, 36) = 4.192$, $p = 0.023$; Fig. 1G). Here, post-hoc analysis showed a significant difference between control and poly-I:C exposed female mice ($p = 0.0058$), with the poly-I:C group showing reduced gamma power, while raloxifene treatment in females increased gamma power compared to poly-I:C + placebo ($p = 0.0385$). Gamma power during exploration of the novel arm was unchanged by treatment or sex (Fig. 1H).

3.2. Behavioural performance in the Y-maze novelty preference task

Behavioural outcomes were time spent in the decision-making (centre) phase and time spent in the novel arm. For time spent in the centre phase, there was no effect of treatment or sex but a significant sex x treatment interaction ($F(2, 49) = 6.521$, $p = 0.0031$) (Fig. 2A). Here, post-hoc analysis showed significant differences between control and poly-I:C + placebo female mice ($p = 0.012$) and poly-I:C + placebo and poly-I:C + raloxifene treated female mice ($p = 0.01$) showing

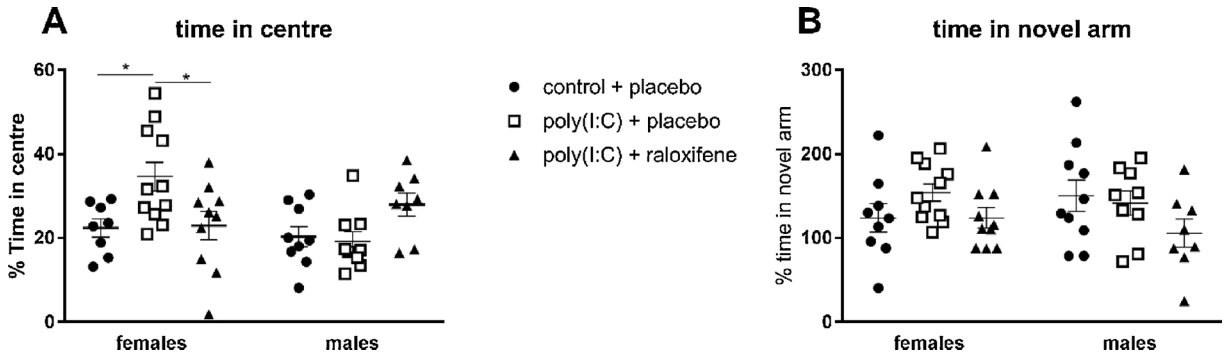

Fig. 2. Y maze behaviour in control, poly-I:C exposed and poly-I:C exposed + raloxifene treated female and male mice. **(A)** Time spent in the centre (decision making phase) of the Y maze **(B)** Time spent in the novel arm of the Y-maze Data are mean ± SEM. *P < 0.05.

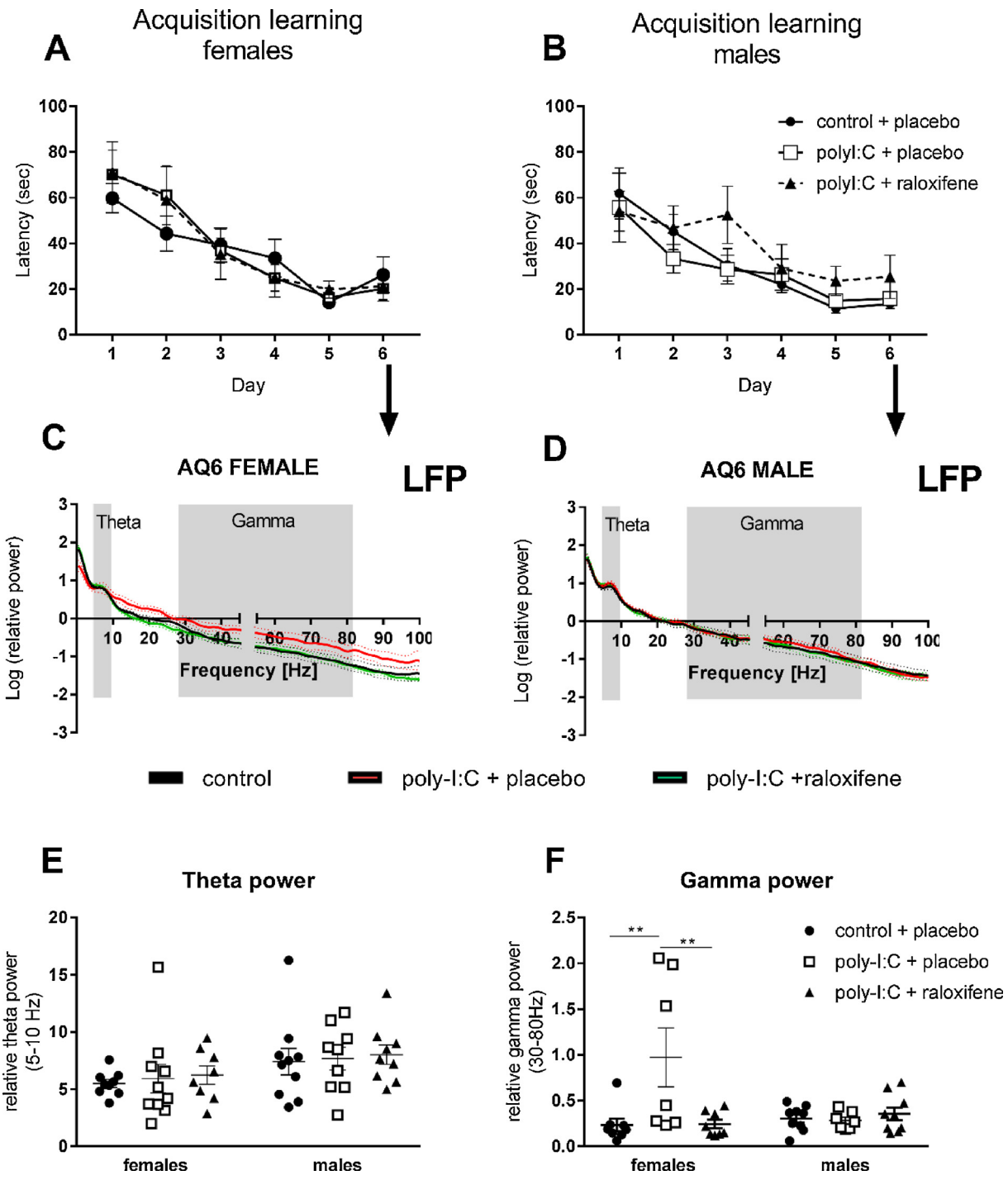

Fig. 3. Acquisition learning (AQ) (days 1–6) and oscillatory activity during final day of acquisition of the cheeseboard task. (A). Latency to find the baited well over 6 days in female mice, and **(B)** male mice. (**C**) Power spectral density relative to absolute average power (1–200 Hz) during AQ day 6 in female mice, and **(D)** male mice. (**E**) Relative theta power during AQ day 6 and **(F)** Relative gamma power during AQ day 6 in female and male mice. Data are represented as mean ± SEM. ** = $p < 0.01$.

that poly-I:C exposed female mice spent more time in the decision making phase of the task compared to controls and raloxifene treatment reduced time spent in this phase to levels similar to control. There was no effect of poly-I:C or raloxifene on time spent in the novel arm (Fig. 2B). No significant difference was observed in distance travelled (data not shown) suggesting similar locomotor function in all groups.

3.3. Neural oscillations during acquisition of the cheeseboard maze task

We observed no group differences in average speed during habituation to the cheeseboard maze (data not shown). Female mice were able to acquire the cheeseboard-maze task as demonstrated in Fig. 3A by a significant reduction in latency to find the baited well across the 6 days of trials (main effect of days: $F(5, 120) = 24.38$, $p < 0.0001$; Fig. 3A). No significant effects of treatment, and no treatment x day interactions were found during acquisition of the task. Male mice also successfully acquired the task with no group differences in latency to find the baited well (main effect of days: $F(5, 125) = 16.42$, $p < 0.0001$; Fig. 3B).

LFP recordings during the searching phase were taken on day 6 and

gamma as well as theta power were analysed. While no significant effects of treatment group or sex were observed in theta power (Fig. 3C), a significant effect of treatment group ($F(2, 42) = 4.634$, $p = 0.015$) and a significant treatment x sex interaction ($F(2, 42) = 6.062$, $p = 0.0049$) was found for gamma power (Fig. 3D). Here, post-hoc comparisons showed significant differences between control + placebo and poly-I:C + placebo females ($p = 0.0007$) indicating that poly-I:C exposed female mice showed an increase in gamma power while acquiring this task, while raloxifene treated poly-I:C exposed mice were significantly different from placebo treated poly-I:C exposed ($p = 0.008$), suggesting raloxifene recovers this increase in gamma power. Post-hoc comparisons showed no significant effects of poly-I:C or raloxifene in male mice.

3.4. Memory retrieval after acquisition learning in the cheeseboard maze task

During the probe trial on day 7 after acquisition learning, long-term spatial memory was measured by the % time spent in the target quadrant in the first minute of the trial. We chose to analyse just the first minute because when we assessed distance travelled across the full trial we found a significant drop in distance travelled during the second minute in both female ($F(1, 48) = 4.179$, $p = 0.04$; Fig. 4A) and male mice ($F(1, 48) = 6.796$, $p = 0.012$; Fig. 4B), with several data points showing 0 distance travelled. This indicated to us that by the second minute mice are not engaged with the task and are remaining stationary. Target quadrant preference and SEM for the full 2 min probe trials are presented in Table 2. During memory retrieval, no main effect of treatment or sex and no significant sex x treatment interaction was found for time spent in the target quadrant (Fig. 4C). Further analysis using single sample t-tests for each group comparing time spent in the target quadrant compared to chance (25% - represented as dotted line, Fig. 4C) found that all groups show a significant preference for the target quadrant (control females, $p = 0.003$; poly-I:C females, $p = 0.014$; poly-I:C + ral females, $p = 0.04$; control males, $p = 0.001$; poly-I:C males, $p = 0.013$; poly-I:C + ral males, $p = 0.006$) suggesting all mice showed intact long-term spatial memory.

Table 2
Duration (%) spent in target quadrant during full 2 min acquisition and probe trials.

	Control + placebo	Poly I:C + placebo	Poly I:C + raloxifene
	Acquisition		
Female	48.7 ± 8.3	51.4 ± 6.8	42.3 ± 7.6
Male	51.3 ± 8.3	36.6 ± 6.2	59.6 ± 10.7
	Reversal		
Female	34.3 ± 5.1	22.7 ± 4.6	21.2 ± 4.1
Male	38.7 ± 8.7	33.9 ± 6.4	46.8 ± 7.5

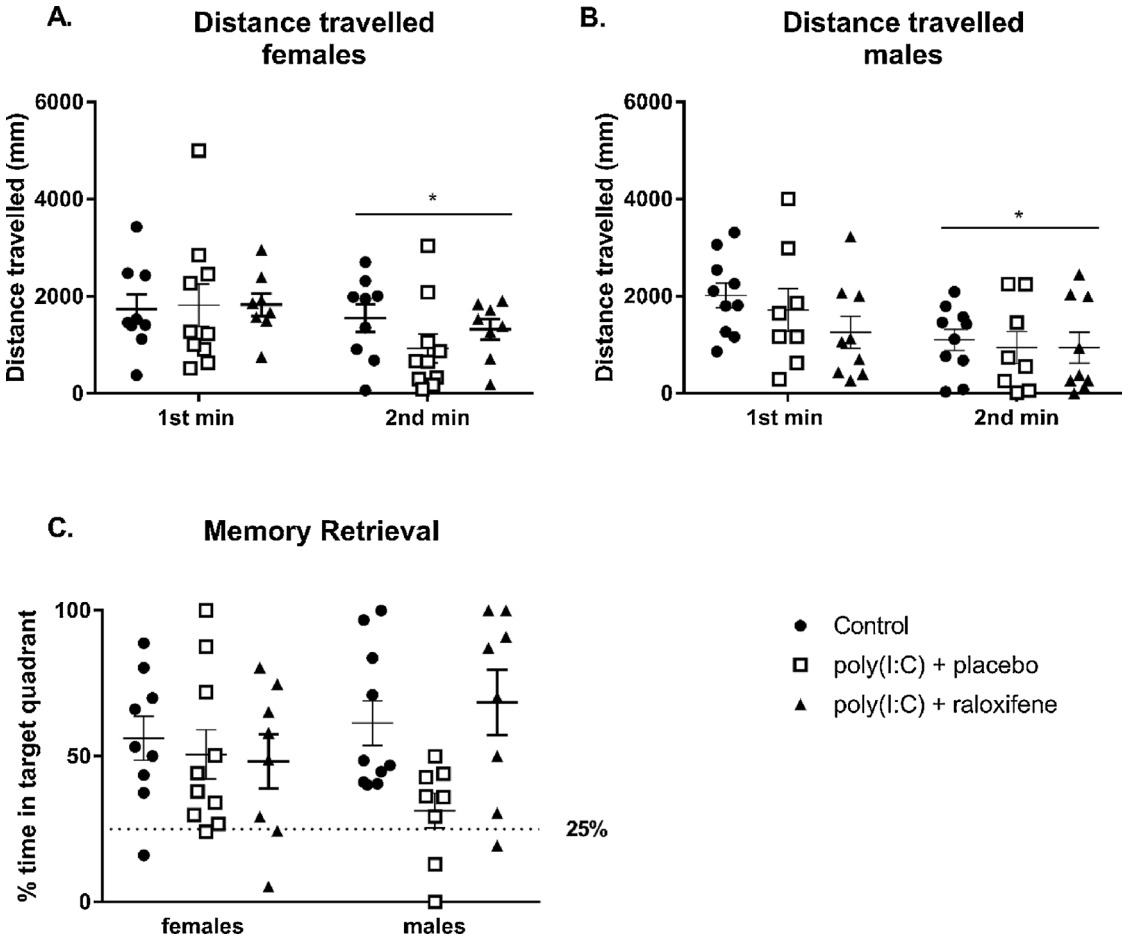

Fig. 4. Memory retrieval.
Distance travelled [mm] during the 2 min probe trial in (**A**) Female and (**B**) male mice is significantly reduced during the second minute, therefore memory performance was only analysed for the first minute. (**C**) Time spent [%] in the target quadrant of the cheeseboard maze during the 1 st min of the probe trial is unchanged by treatment group or sex. All groups spent significantly more time ($p < 0.05$) in the target quadrant compared to chance level (25% - dotted line), suggesting all groups show intact long term spatial memory. Data are represented as mean ± SEM.*$P < 0.005$.

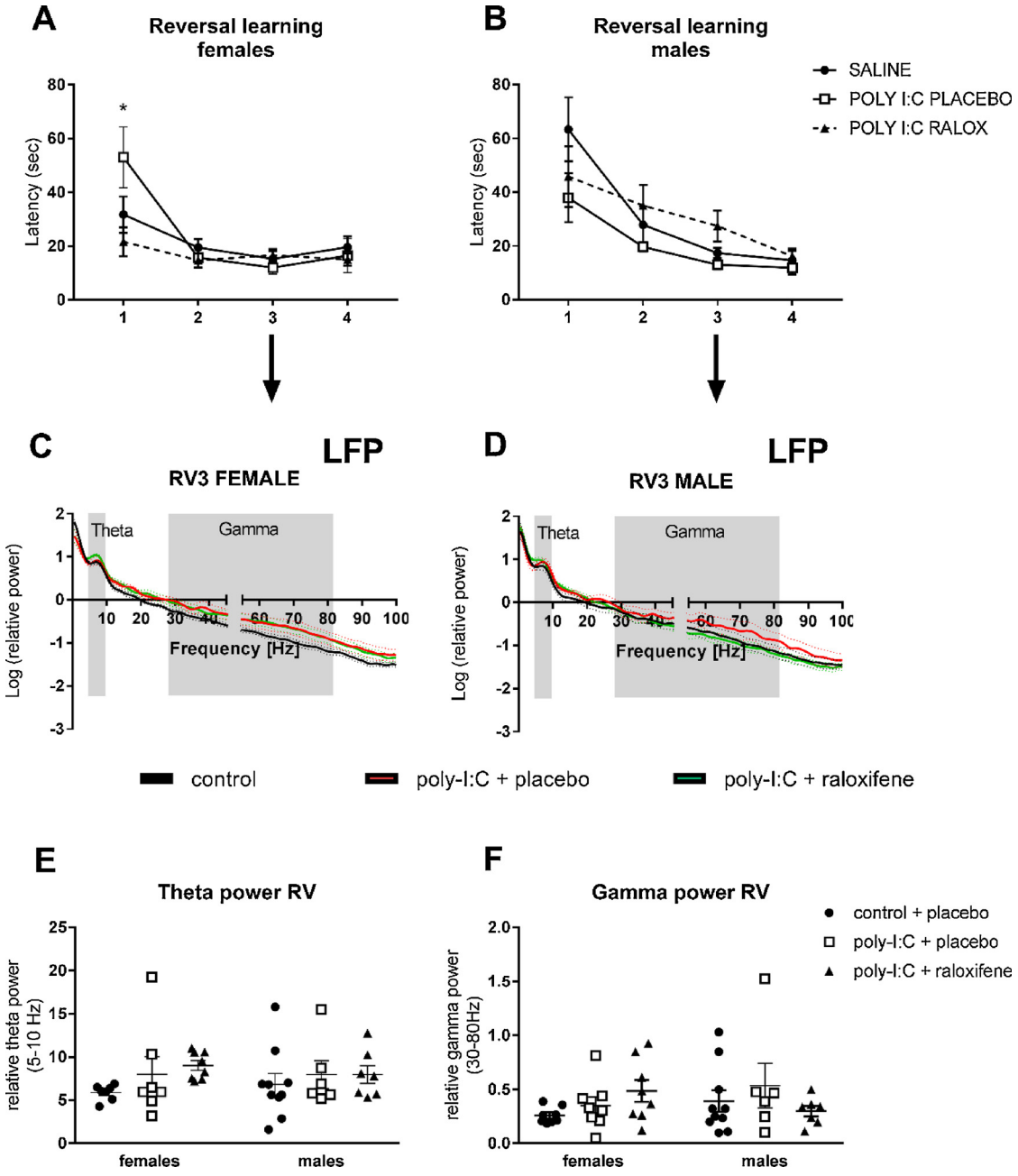

Fig. 5. Reversal learning (RV) (days 1–4) and oscillatory brain activity during cheeseboard task. (**A**) Latency to find the baited well over 4 days in female mice, and **(B)** male mice. **(C).** Power spectral density relative to absolute average power (1–200 Hz) during RV day 3 in female mice, and **(D)** male mice. **(E)** Relative theta power during RV day 3 in female and male mice, and **(F)** Relative gamma power during RV day 3 in female and male mice. Data are represented as mean ± SEM. * = $p < 0.05$.

3.5. Gamma and theta oscillations during reversal learning of the cheeseboard maze task

To test cognitive flexibility, the bait was relocated to a different location and the mice had to associate the new well with reward rather than the old one. Over the 4 day reversal training period, female mice showed a significant reduction in latency to find the baited well during reversal learning as reflected by a main effect of days ($F(3, 66) = 11.45$, $p < 0.0001$, Fig. 5A). No effect of treatment was found here, however, we did find a significant day x treatment interaction ($F(6, 66) = 3.052$, $p = 0.01$). Post-hoc analysis showed significant group differences on day 1 of reversal learning with poly-I:C + placebo mice showing increased latency to find the baited well when compared to controls ($p = 0.014$) and raloxifene treated poly-I:C exposed mice ($p = 0.0002$) (Fig. 5A). This demonstrates that female poly-I:C exposed mice may show reduced cognitive flexibility or increased perseverative behaviour. Male mice also showed reduced latency to find the baited well over the 4 day reversal period (main effect of days, $F(3, 75) = 20.61$, $p < 0.0001$, Fig. 5B), however, no significant effect of treatment and no day x treatment interaction was found.

LFPs were recorded on day 3 of reversal learning with relative spectral density shown in Figs. 5C (females) and 5D (males). No significant effects of treatment group or sex were observed in theta (Fig. 5E) or gamma power (Fig. 5F).

3.6. Cognitive flexibility

A probe test was conducted on day 5 of reversal learning

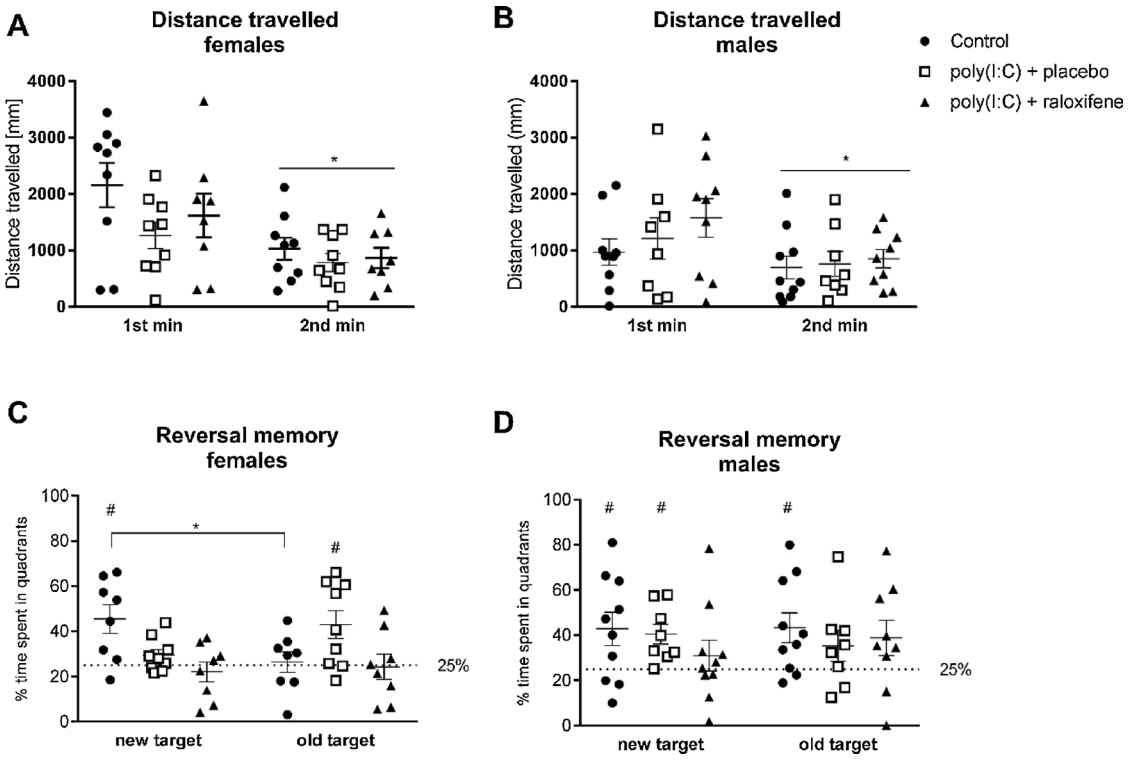

Fig. 6. Probe test of Reversal Memory (RV).
Distance travelled [mm] during the 1st and 2nd min of the probe trial in (**A)** female and **(B)** male mice. (**C**) Time spent [%] in the new and old target quadrants during the 1st min of the probe trial in female mice. Female control mice spend significantly more time in the new target quadrant compared to the old (connecting line * = $p < 0.05$). Female control mice show a significant preference for the new target quadrant (# = $p < 0.05$) over 25% chance level (dotted line), while female poly-I:C + placebo treated mice show a significant preference for the old target (# = $p < 0.05$) over chance level. (**D**) Time spent [%] in the new and old target quadrants during the 1st min of the probe trial in male mice. Male control and poly-I:C + placebo mice spend significantly more time in the new target quadrant compared to 25% chance (# = $p < 0.05$), and control mice also show a preference for the old target above chance level. Data are represented as mean ± SEM. *P < 0.05

corresponding to day 12 of the entire CB paradigm (Supplementary Fig. 1). This test is designed to assess cognitive flexibility anticipating healthy control mice to switch from old rules and adapt to new rules – in this case remembering the new target versus the old target.

All female and male mice travelled significantly less during the 2nd min as opposed to the 1st one (main effect of time: (female, Fig. 6A, $F(1,46) = 12.37; p = 0.001$) and (male, Fig. 6B, $F(1,47 = 5.1; p = 0.028)$). This again suggested that by the second minute mice are less engaged in the task. Hence, memory retrieval performance was only analyzed for the first minute and split by sex to assess preference for the new target over the old target in each treatment group. Only female control mice show a preference for the new target quadrant over the old one during the 1st min of the trial. This was shown by a significant treatment x target interaction ($F(1,30) = 10.25; p = 0.0032$; Fig. 6C) with further post-hoc analysis showing a significant difference between the new target and old target in the control + placebo group ($p = 0.03$) but not in poly-I:C + placebo or poly-I:C + raloxifene treated groups. Thus, only female control mice show a preference for the new target compared to the old, suggesting successful reversal. This effect was further confirmed by single sample *t*-test analyses of each group comparing percentage time spent in the new target quadrant compared to chance or old target quadrant compared to chance. Here, female control mice spend significantly more time in the new target compared to 25% chance ($p = 0.027$), while female poly-I:C and poly-I:C + raloxifene treated mice do not. However, only poly-I:C exposed female mice show significantly more time spent in the old target compared to 25% chance, suggesting the poly-I:C exposed female mice persevere in the old target zone (Fig. 6C).

In male mice, there was no effect of treatment or target and no treatment x target interaction, suggesting all groups spent a similar amount of time in the new target compared to the old (Fig. 6D).

Further single sample t-tests showed that male control ($p = 0.03$) and poly-I:C exposed ($p = 0.009$) mice show a significant preference for the new target compared to chance, while control mice also show a preference for the old target compared to chance ($p = 0.02$). However, poly-I:C + raloxifene treated male mice do not show a preference for the new or old target compared to chance.

4. Discussion

Here we investigated the effect of prenatal immune activation (PIA) as mimicked by the poly I:C injection in pregnant dams on hippocampus-dependent memory and respective hippocampal brain oscillations in the adult offspring, and the effect of raloxifene on PIA-induced changes. We found female specific effects of both PIA and adult raloxifene treatment on spatial working memory and hippocampal gamma and theta power.

4.1. Y-maze

Spatial working memory is an essential feature of goal-directed action. This requires a cached representation of relevant spatial features that must be continuously updated, preserved and applied as needed to the execution of adaptive behaviours (Baddeley and Hitch, 1974). Y-maze is a behavioural task that is able to measure such behaviour, as it requires recognition, consolidation and retrieval of cues within a spatial context to remember and decide where to go. Thus, this paradigm can be used to test for aspects of recognition memory, consolidation, and decision making. We show here that all mice show a preference for the novel arm, thus spatial memory is intact. Importantly, our test design

includes cues within the walls of the maze, thus this task may also tap into aspects of recognition memory, which again seem intact across all groups as evidenced by their preference for the novel arm (recognition that the arm is novel). However, female poly-I:C exposed mice spend significantly more time in the centre phase, which may suggest that they take longer to make a decision as to which arm to explore.

Females exposed to poly-I:C show impaired hippocampal gamma power upon first entrance to the centre of the Y-maze – a phase we have previously deemed as being indicative of decision making (Schroeder et al., 2017). Raloxifene recovers this deficit in gamma power and also increases theta power and simultaneously reduces time spent in the decision making phase of the task. This may suggest that improved power, particularly in the gamma frequency range, during this phase is related to the reduced time spent deciding which arm to investigate. In a similar paradigm to our Y-maze, the T-maze, Yamamoto et al. (2014) demonstrated that gamma oscillations were particularly elevated during the centre of the T maze during the retrieval task rather than the sample task as compared to other segments of the T-maze. This suggests that specifically during time in the centre of the maze rodents are making an active decision where to go next. In agreement with this, Montgomery and Buzsaki (2007) demonstrated that gamma power was enhanced in the CA1 region of the hippocampus when rats entered the centre of the T-maze as compared to entering the arms. This effect was specifically related to decision-making processes rather than the motor aspect of the task and correlated with higher CA3-CA1 coherence. The same study showed that this decision-making related gamma activity was generated locally in the CA1 region – the same region our LFP's were recorded from.

Spellman et al. (2015) showed that hippocampal-prefrontal afferents are critical for encoding, but not for maintenance or retrieval, of spatial cues in a T maze in mice and this encoding of cues was mediated by gamma-frequency synchrony between the hippocampus and the prefrontal cortex. This may suggest that reduced gamma oscillations in the poly-I:C female mice may be indicative of difficulties to encode spatial cues and hence the longer time spent in the centre of the Y maze deciding where to go. Sigurdsson et al. (2010) showed that in a genetic mouse model relevant to schizophrenia impaired spatial memory was accompanied by a reduction in theta power, measured both by phase-locking of prefrontal cells to hippocampal theta oscillations and by coherence of prefrontal and hippocampal LFPs. A significant sex by treatment interaction for theta power during the first entrance to the centre phase was identified in our study, with reduced theta power in female poly-I:C mice as compared to poly-I:C + raloxifene treated mice. The fact that we could not detect a significant difference between the control and poly-I:C mice may be due to high variability in the control mice. This could indicate that poly-I:C may impair hippocampal oscillations in females in these particular frequencies which appear to be crucial for spatial memory decision making processes, however, further studies are needed to confirm this result.

We did not find differences between the groups when looking at distance travelled within the centre of the Y-maze (data not shown) suggesting that observed changes in power were not due to locomotor behaviour. While poly-I:C exposure reduced gamma oscillations and decision-making time in adult females, mice were able to show preference for the novel arm in a similar manner to controls. This subtle decision-making phenotype that we show in this model aligns with the mild cognitive disturbances found in schizophrenia patients (Fond et al., 2013), rather than a severe memory deficit, such as those found in dementia. We have previously shown that raloxifene is able to recover gamma power and spatial memory deficits in ovariecomtized mice (Schroeder et al., 2017). The current study is the first to suggest that chronic raloxifene treatment may modify the decision-making process in poly-I:C exposed mice. Estrogen receptors are highly expressed in fast-spiking parvalbumin (PV) interneurons (Wu et al., 2014), which are cells with the capability of driving gamma oscillations (Sohal et al., 2009). Hence, raloxifene may protect the function or expression of these neurons, which were shown to be disrupted in the hippocampus after poly-I:C exposure (Piontkewitz et al., 2012) as well as in schizophrenia patients (Konradi et al., 2011a; Zhang and Reynolds, 2002b).

4.2. Cheeseboard task

During acquisition of the cheeseboard maze task, female, but not male mice exposed to poly-I:C showed an increase in gamma power, and raloxifene prevented this increase. Thus, while gamma power is reduced during the decision making process in the Y-maze short term spatial memory task, it is increased on the final day of the long-term spatial learning process in the cheeseboard maze. Here we show that raloxifene is not only able to prevent reduced gamma power during the short-term decision process (Y-maze) but it is also able to prevent abnormal increases during long-term memory formation (cheeseboard acquisition). Despite increased gamma power in female poly-I:C exposed mice, no differences were detected in their latency to find the baited well or remember the location of the baited well in the probe trial.

However, female poly-I:C exposed mice showed increased latency to find the *new* baited well compared to the controls and raloxifene treated mice on day 1 of the reversal task, suggesting that the poly-I:C exposed mice either show impaired cognitive flexibility or they may still harbour a preference for the old target (perseverance), while control and raloxifene treated mice, once finding no reward in the old target, have continued searching (cognitive flexibility) and consequently found the new well quicker. During the probe trial, we noted that the female poly-I:C exposed group showed a significant preference for the old target but not the new target above 25% chance level. Thus, these data suggest that female poly-I:C exposed mice show perseverative behaviour and this may be associated with the higher gamma power detected during the acquisition phase of the task. Increased gamma power while learning to find the baited well may have strengthened the association of this spatial location with reward, but in the face of reduced cognitive flexibility. Brown and Derkits (2010) showed that patients with schizophrenia who were exposed to serologically confirmed influenza or toxoplasmosis in utero showed greater deficits in set-shifting tests, showing perseverative behaviour. Perseverative behaviour seen in our female mice aligns with this observation and poly-I:C may indeed represent good face validity of PIA in people schizophrenia.

When comparing the time spent in the new target versus the old target, we found that only the female control group showed a significant preference for the new target over the old target in the first minute of the probe trial, suggesting that only the female control group was able to reverse their memory over 4 days of reversal training. Thus, raloxifene could not recover poly-I:C induced deficits in reversal memory. This speaks to the specificity of this compound and mechanistically may be explained by the variation in the regional distribution of estrogen receptors in hippocampal and prefrontal cortical regions involved in this type of memory retrieval process (Barker and Galea, 2009). In addition, in male mice, while control and poly-I:C exposed mice show a significant preference for the new target quadrant compared to 25% chance, raloxifene treated poly-I:C exposed mice do not. Thus, while there were no significant group differences, the raloxifene treated male group did not successfully reverse.

To the knowledge of the authors, this is the first published study to investigate long-term spatial learning and memory in the poly-I:C model using the cheeseboard maze task. A previous study by Bitanihirwe and colleagues (Bitanihirwe et al., 2010) used the same apparatus to investigate spatial working memory in the poly-I:C model. The location of the reward was moved across days and working memory performance was interpreted as the difference in latency and distance to the target between the first and second trial within each day. Multiple inter-trial times between the two trials were used to modulate working memory load. Prenatal exposure to poly-I:C reduced

performance on this task at high working memory load (ITI 15 min) (Bitanihirwe et al., 2010). We did not see this effect in the current study with an ITI of 20 min (data not shown), most likely due to multiple factors. Firstly, statistical power for this interpretation could be lacking because data could only be sourced from the two trials on the first day of acquisition training due to the location of the reward being maintained across days. Additionally, differences in the induction of PIC, housing conditions, and testing environments are also expected to influence this behaviour.

4.3. Sex differences in poly-I:C induced cognitive deficits

An interesting finding of this study was that only female poly-I:C exposed mice show cognitive deficits. This may be due to early organizational effects of sex hormones on the developing brain. Males receive a surge in androgens early in prenatal development which enter the brain and can be locally synthesized to estrogens (McCarthy, 2008), which may protect the male brain from an inflammatory insult. Sex differences also exist in the number of microglia and the levels of proinflammatory cytokines in the early developing brain (Nelson and Lenz, 2017). Cultured microglia from male and female mice show differential responses to lipopolysaccharide (LPS; bacterial mimetic) following estradiol treatment, with males treated with both LPS and estradiol showing reduced levels of interleukin 1β, while females treated with LPS and estradiol show an increase in IL-1β (Loram et al., 2012). Here further studies examining the sexually dimorphic response of microglia to poly-I:C at various developmental stages are needed to shed more light on this intriguing sex-specific result and how early sex hormone and immune cell interactions may dictate this long-term effect.

4.4. Limitations

A limitation of this study is that we did not include a control raloxifene treatment group. Our main hypothesis was that raloxifene would recover poly-I:C-induced deficits, which it did. However, here it is interesting to note that in the males, poly-I:C had no effect on gamma or theta power, nor on behaviour, and here raloxifene had no effect either. Thus, the male data here shows that without a deficit, raloxifene has no significant behavioural or electrophysiological effect, albeit this may be due to sex differences in the action of raloxifene in the brain. Here future studies should use a model whereby both male and female mice show deficits to assess the efficacy of raloxifene in both sexes. Another limitation is that LFP recordings were only taken from the dorsal hippocampus, thus further electrophysiological detail, such as phase coupling could not be assessed. Future studies should attempt to record from hippocampal and prefrontal cortex regions to obtain more insight into the network dysfunction caused by prenatal immune activation, and the capacity of raloxifene to recover this. In addition, we limited recordings during the cheeseboard task to specific days as to not interfere with performance outcomes. Future study designs may incorporate two cohorts here, one for behaviour only, and one with behaviour and electrophysiology to determine whether the recordings do in fact interfere with their behaviour.

5. Conclusions

We found that poly I:C exposure at GD17 caused long-term disruptions to adult gamma, but not theta oscillations in the dorsal hippocampus during short-term spatial learning and increased decision-making time in females but not males. Prenatal poly-I:C exposed female mice, also show in adulthood increased gamma power during acquisition of a long-term spatial memory task, which is accompanied by higher perseverative behaviour. Interestingly, raloxifene was able to rescue the aberrant gamma oscillations seen in female mice and the accompanied cognitive disturbances. This data strengthens our previous results that the SERM raloxifene can modify gamma oscillations and may explain the mechanism as to how it can rescue some aspects of cognitive behaviour relevant to schizophrenia. Importantly, both poly-I:C exposure and raloxifene treatment show female specific effects and hence, using both sexes within this research area is invaluable for deeper understanding of the mechanisms relevant to immunity, cognitive function and schizophrenia.

Funding and author contributions

This work was supported by a NARSAD Young Investigator Award from the Brain and Behavior Research Foundation for Dr Rachel Hill (22997) and a National Health and Medical Research Council (NHMRC) career development award for Dr Rachel Hill (1062826). The Behavioural Neuroscience Laboratory are supported by the philanthropic organisation, One in Five.

A.S. performed all Y-maze behavioural and LFP experiments as well as data analysis, and wrote the original draft, J.N. performed all cheeseboard maze behavioural and LFP experiments as well as data analysis and edited the draft. M.H. assisted with surgical implantation of electrodes and data analysis of LFP's and edited the manuscript. N.J. assisted with the LFP study design and edited the manuscript. X.D. assisted with electrode implantation and raloxifene implantation surgeries. S.S. assisted with the study design and edited the manuscript. R.H. designed and funded the study and co-wrote the manuscript with A.S. and supervised A.S. and J.N.

Declaration of Competing Interest

The authors declare no conflicts of interest

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.104448.

References

Baddeley, A.D., Hitch, G., 1974. Working memory. In: Bower, G.H. (Ed.), Psychology of Learning and Motivation. Academic Press, pp. 47–89.
Barker, J.M., Galea, L.A., 2009. Sex and regional differences in estradiol content in the prefrontal cortex, amygdala and hippocampus of adult male and female rats. Gen. Comp. Endocrinol. 164, 77–84.
Bitanihirwe, B.K., Peleg-Raibstein, D., Mouttet, F., Feldon, J., Meyer, U., 2010. Late prenatal immune activation in mice leads to behavioral and neurochemical abnormalities relevant to the negative symptoms of schizophrenia. Neuropsychopharmacol.: Off. Publ. Am. College Neuropsychopharmacol. 35, 2462–2478.
Brown, A.S., Derkits, E.J., 2010. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. Am. J. Psychiatry 167, 261–280.
Connor, C.M., Dincer, A., Straubhaar, J., Galler, J.R., Houston, I.B., Akbarian, S., 2012. Maternal immune activation alters behavior in adult offspring, with subtle changes in the cortical transcriptome and epigenome. Schizophr. Res. 140, 175–184.
Dickerson, D.D., Overeem, K.A., Wolff, A.R., Williams, J.M., Abraham, W.C., Bilkey, D.K., 2014. Association of aberrant neural synchrony and altered GAD67 expression following exposure to maternal immune activation, a risk factor for schizophrenia. Transl. Psychiatr. 4.
Dupret, D., O'Neill, J., Pleydell-Bouverie, B., Csicsvari, J., 2010. The reorganization and reactivation of hippocampal maps predict spatial memory performance. Nat. Neurosci. 13, 995–1002.
Essizoglu, A., Kosger, F., Akarsu, F.O., Ozaydin, O., Gulec, G., 2017. Theory of mind and selective attention, response inhibition, cognitive flexibility in patients with schizophrenia. Noro Psikiyatr. Ars. 54, 162–167.
Folley, B.S., Astur, R., Jagannathan, K., Calhoun, V.D., Pearlson, G.D., 2010. Anomalous neural circuit function in schizophrenia during a virtual Morris water task. NeuroImage 49, 3373–3384.
Fond, G., Bayard, S., Capdevielle, D., Del-Monte, J., Mimoun, N., Macgregor, A., Boulenger, J.P., Gely-Nargeot, M.C., Raffard, S., 2013. A further evaluation of decision-making under risk and under ambiguity in schizophrenia. Eur. Arch. Psychiatry Clin. Neurosci. 263, 249–257.
Fung, S.J., Webster, M.J., Sivagnanasundaram, S., Duncan, C., Elashoff, M., Weickert, C.S., 2010. Expression of interneuron markers in the dorsolateral prefrontal cortex of the developing human and in schizophrenia. Am. J. Psychiatry 167, 1479–1488.

Grech, A.M., Du, X., Murray, S.S., Xiao, J., Hill, R.A., 2019. Sex-specific spatial memory deficits in mice with a conditional TrkB deletion on parvalbumin interneurons. Behav. Brain Res. 372, 111984.
Green, M.F., Kern, R.S., Heaton, R.K., 2004. Longitudinal studies of cognition and functional outcome in schizophrenia: implications for MATRICS. Schizophr. Res. 72, 41–51.
Huerta-Ramos, E., Iniesta, R., Ochoa, S., Cobo, J., Miquel, E., Roca, M., Serrano-Blanco, A., Teba, F., Usall, J., 2014. Effects of raloxifene on cognition in postmenopausal women with schizophrenia: a double-blind, randomized, placebo-controlled trial. Eur. Neuropsychopharmacol. 24, 223–231.
Jacobsen, D.E., Samson, M.M., Emmelot-Vonk, M.H., Verhaar, H.J.J., 2010. Raloxifene improves verbal memory in late postmenopausal women: a randomized, double-blind, placebo-controlled trial. Menopause-J. N. Am. Menopause Soc. 17, 309–314.
Jones, M.W., Wilson, M.A., 2005. Theta rhythms coordinate hippocampal-prefrontal interactions in a spatial memory task. PLoS Biol. 3, e402.
Karl, T., Bhatia, S., Cheng, D., Kim, W.S., Garner, B., 2012. Cognitive phenotyping of amyloid precursor protein transgenic J20 mice. Behav. Brain Res. 228, 392–397.
Keefe, R.S.E., Bilder, R.M., Davis, S.M., Harvey, P.D., Palmer, B.W., Gold, J.M., Meltzer, H.Y., Green, M.F., Capuano, G., Stroup, T.S., McEvoy, J.P., Swartz, M.S., Roscnheck, R.A., Perkins, D.O., Davis, C.E., Hsiao, J.K., Lieberman, J.A., Neurocognitive, C.I., 2007. Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE trial. Arch. Gen. Psychiatry 64, 633–647.
Konradi, C., Yang, C.K., Zimmerman, E.I., Lohmann, K.M., Gresch, P., Pantazopoulos, H., Berretta, S., Heckers, S., 2011a. Hippocampal interneurons are abnormal in schizophrenia. Schizophr. Res. 131, 165–173.
Konradi, C., Yang, C.K., Zimmerman, E.I., Lohmann, K.M., Gresch, P., Pantazopoulos, H., Berretta, S., Heckers, S., 2011b. Hippocampal interneurons are abnormal in schizophrenia. Schizophr. Res. 131, 165–173.
Kulkarni, J., de Castella, A., Fitzgerald, P.B., Gurvich, C.T., Bailey, M., Bartholomeusz, C., Burger, H., 2008a. Estrogen in severe mental illness - A potential new treatment approach. Arch. Gen. Psychiatry 65, 955–960.
Kulkarni, J., Gavrilidis, E., Wang, W., Worsley, R., Fitzgerald, P.B., Gurvich, C., Van Rheenen, T., Berk, M., Burger, H., 2015. Estradiol for treatment-resistant schizophrenia: a large-scale randomized-controlled trial in women of child-bearing age. Mol. Psychiatry 20, 695–702.
Kulkarni, J., Gurvich, C., Gilbert, H., Mehmedbegovic, F., Mu, L., Marston, N., Gavrilidis, E., de Castella, A., 2008b. Hormone modulation: a novel therapeutic approach for women with severe mental illness. Aust. N. Z. J. Psych. 42, 83–88.
Kulkarni, J., Gurvich, C., Lee, S.J., Gilbert, H., Gavrilidis, E., de Castella, A., Berk, M., Dodd, S., Fitzgerald, P.B., Davis, S.R., 2010. Piloting the effective therapeutic dose of adjunctive selective estrogen receptor modulator treatment in postmenopausal women with schizophrenia. Psychoneuroendocrinology 35, 1142–1147.
Lei, M., Shafique, A., Shang, K., Couttas, T.A., Zhao, H., Don, A.S., Karl, T., 2017. Contextual fear conditioning is enhanced in mice lacking functional sphingosine kinase 2. Behav. Brain Res. 333, 9–16.
Lethaby, A., Suckling, J., Barlow, D., Farquhar, C.M., Jepson, R.G., Roberts, H., 2004. Hormone replacement therapy in postmenopausal women: endometrial hyperplasia and irregular bleeding. Cochrane Database Syst. Rev CD000402–CD000402.
Loram, L.C., Sholar, P.W., Taylor, F.R., Wiesler, J.L., Babb, J.A., Strand, K.A., Berkelhammer, D., Day, H.E., Maier, S.F., Watkins, L.R., 2012. Sex and estradiol influence glial pro-inflammatory responses to lipopolysaccharide in rats. Psychoneuroendocrinology 37, 1688–1699.
McCarthy, M.M., 2008. Estradiol and the developing brain. Physiol. Rev. 88, 91–124.
Meyer, U., Feldon, J., 2010. Epidemiology-driven neurodevelopmental animal models of schizophrenia. Prog. Neurobiol. 90, 285–326.
Meyer, U., Nyffeler, M., Engler, A., Urwyler, A., Schedlowski, M., Knuesel, I., Yee, B.K., Feldon, J., 2006. The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. J. Neurosci. 26, 4752–4762.
Montgomery, S.M., Buzsaki, G., 2007. Gamma oscillations dynamically couple hippocampal CA3 and CA1 regions during memory task performance. Proc. Natl. Acad. Sci. U.S.A. 104, 14495–14500.
Morice, R., 1990. Cognitive inflexibility and pre-frontal dysfunction in schizophrenia and mania. Br. J. Psychiatry 157, 50–54.
Nakamura, J.P., Schroeder, A., Hudson, M., Jones, N., Gillespie, B., Du, X., Notaras, M., Swaminathan, V., Reay, W.R., Atkins, J.R., Green, M.J., Carr, V.J., Cairns, M.J., Sundram, S., Hill, R.A., 2019. The maternal immune activation model uncovers a role for the Arx gene in GABAergic dysfunction in schizophrenia. Brain Behav. Immun.
Nelson, L.H., Lenz, K.M., 2017. The immune system as a novel regulator of sex differences in brain and behavioral development. J. Neurosci. Res. 95, 447–461.
Piontkewitz, Y., Arad, M., Weiner, I., 2012. Tracing the development of psychosis and its prevention: what can be learned from animal models. Neuropharmacology 62, 1273–1289.
Rando, G., Horner, D., Biserni, A., Ramachandran, B., Caruso, D., Ciana, P., Komm, B., Maggi, A., 2010. An innovative method to classify SERMs based on the dynamics of estrogen receptor transcriptional activity in living animals. Mol. Endocrinol. 24, 735–744.
Richetto, J., Calabrese, F., Meyer, U., Riva, M.A., 2013. Prenatal versus postnatal maternal factors in the development of infection-induced working memory impairments in mice. Brain Behav. Immun. 33, 190–200.
Schmiedt, C., Brand, A., Hildebrandt, H., Basar-Eroglu, C., 2005. Event-related theta oscillations during working memory tasks in patients with schizophrenia and healthy controls. Brain research. Cogn. Brain Res. 25, 936–947.
Schroeder, A., Buret, L., Hill, R.A., van den Buuse, M., 2015. Gene-environment interaction of reelin and stress in cognitive behaviours in mice: implications for schizophrenia. Behav. Brain Res. 287, 304–314.
Schroeder, A., Hudson, M., Du, X., Wu, Y.W., Nakamura, J., van den Buuse, M., Jones, N.C., Hill, R.A., 2017. Estradiol and raloxifene modulate hippocampal gamma oscillations during a spatial memory task. Psychoneuroendocrinology 78, 85–92.
Senitz, D., 1999. A reduction of nonpyramidal cells in sector CA2 of schizophrenics and manic depressives. Biol. Psychiatry 45, 1528–1529.
Senkowski, D., Gallinat, J., 2015. Dysfunctional prefrontal gamma-band oscillations reflect working memory and other cognitive deficits in schizophrenia. Biol. Psychiatry 77, 1010–1019.
Sigurdsson, T., Stark, K.L., Karayiorgou, M., Gogos, J.A., Gordon, J.A., 2010. Impaired hippocampal–prefrontal synchrony in a genetic mouse model of schizophrenia. Nature 464, 763.
Smith, S.E.P., Li, J., Garbett, K., Mirnics, K., Patterson, P.H., 2007. Maternal immune activation alters fetal brain development through interleukin-6. J. Neurosci. 27, 10695–10702.
Sohal, V.S., Zhang, F., Yizhar, O., Deisseroth, K., 2009. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature 459, 698–702.
Spellman, T., Rigotti, M., Ahmari, S.E., Fusi, S., Gogos, J.A., Gordon, J.A., 2015. Hippocampal-prefrontal input supports spatial encoding in working memory. Nature 522, 309–314.
Uhlhaas, P.J., Singer, W., 2010. Abnormal neural oscillations and synchrony in schizophrenia. Nat. Rev. Neurosci. 11, 100–113.
Usall, J., Huerta-Ramos, E., Iniesta, R., Cobo, J., Araya, S., Roca, M., Serrano-Blanco, A., Teba, F., Ochoa, S., 2011. Raloxifene as an adjunctive treatment for postmenopausal women with schizophrenia: a double-blind, randomized, placebo-controlled trial. J. Clin. Psychiatry 72, 1552–1557.
Weickert, T.W., Weinberg, D., Lenroot, R., Catts, S.V., Wells, R., Vercammen, A., O'Donnell, M., Galletly, C., Liu, D., Balzan, R., Short, B., Pellen, D., Curtis, J., Carr, V.J., Kulkarni, J., Schofield, P.R., Weickert, C.S., 2015. Adjunctive raloxifene treatment improves attention and memory in men and women with schizophrenia. Mol. Psychiatr. 20, 685–694.
Wu, Y.C., Du, X., van den Buuse, M., Hill, R.A., 2014. Sex differences in the adolescent developmental trajectory of parvalbumin interneurons in the hippocampus: a role for estradiol. Psychoneuroendocrinology 45, 167–178.
Wu, Y.W., Du, X., Hill, R.A., 2015. Analyzing the influence of BDNF heterozygosity on spatial memory response to 17beta-estradiol. Transl. Psychiatry 5, e498.
Yamamoto, J., Suh, J., Takeuchi, D., Tonegawa, S., 2014. Successful execution of working memory linked to synchronized high-frequency gamma oscillations. Cell 157, 845–857.
Young, J.W., Geyer, M.A., 2015. Developing treatments for cognitive deficits in schizophrenia: the challenge of translation. J. Psychopharmacol. 29, 178–196.
Zhang, Z.J., Reynolds, G.P., 2002a. A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. Schizophr. Res. 55, 1–10.
Zhang, Z.J., Reynolds, G.P., 2002b. A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. Schizophr. Res. 55, 1–10.