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SHORT COMMUNICATION

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Microbial memories: Sex-dependent impact of the gut microbiome on hippocampal plasticity

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Abstract

Germ-free rodents, raised in the absence of a measurable gut microbiome, have been a key model to study the microbiome-gut-brain axis. Germ-free mice exhibit marked behavioural and neurochemical differences to their conventionally raised counterparts. It is as yet unclear how these neurochemical differences lead to the behavioural differences. Here, we test the electrophysiological properties of hippocampal plasticity in adult germ-free mice and compare them to conventionally raised counterparts. Whilst basal synaptic efficacy and pre-synaptic short-term plasticity appear normal, we find a striking alteration of hippocampal long-term potentiation specifically in male germ-free slices. However, the spike output of these neurons remains normal along with altered input-output coupling, potentially indicating homeostatic compensatory mechanisms, or an altered excitation/inhibition balance. To our knowledge this is the first time the electrophysiological properties of the hippocampus have been assessed in a microbiome deficient animal. Our data indicate that the absence of a microbiome alters integration of dendritic signalling in the CA1 region in mice.

KEYWORDS

electrophysiology, gut-brain axis, microbiome

1 | INTRODUCTION

Growing evidence suggests that the commensal gut microbiota modulate our behaviours and may contribute to certain disease processes (Cryan et al., 2019). One of the principle animal models in the field has been the germ-free (GF) rodent (Spichak et al., 2018). In this model, animals are born into a sterile environment, depriving them of a microbiome, thereby allowing us to observe how they develop in the absence of microbial influence, and make inferences about what role the microbiome plays in normal physiology.

GF animals have been shown to exhibit altered central neurochemistry and behaviours (Bercik et al., 2011; Clarke

Abbreviations: aCSF, artificial cerebrospinal fluid; Conv., conventionally raised; E-S coupling, excitatory post-synaptic potential – spike coupling; fEPSP, field excitatory post-synaptic potential; GF, germ-free; LTP, long-term potentiation; pop. Spike, population spike; PPP, paired-pulse potentiation; TBS, theta-burst stimulation.

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et al., 2013; De Palma et al., 2015; Neufeld et al., 2011). Both cortical and hippocampal BDNF expression has been reported lower than normal in GF mice (Clarke et al., 2013; Diaz Heijtz et al., 2011). GF mice exhibit an altered transcriptional profile, combined with an increase in immediate-early gene (Fos, Egr2, Fosb, Arc) expression in the amygdala (Hoban et al., 2018), enhanced expression of gene splicing factors and exon usage (Stilling et al., 2018), and altered expression of genes implicated in neurophysiology in the amygdala (Stilling et al., 2015) and hippocampus (Chen et al., 2017). Recent evidence has also implicated Lactobacilli in the expression of GABA in the hippocampus (Mao et al., 2020). Hippocampal Fos expression following a water avoidance stress paradigm was lower in GF mice compared to colonized counterparts (Gareau et al., 2011). Swiss-Webster GF mice also exhibit altered hippocampal morphology with increased hippocampal volume (10%) and altered pyramidal neuron morphology when compared to conventionally raised controls (Luczynski et al., 2016).

Furthermore, GF mice have been shown to exhibit altered anxiety-like behaviours in both the open-field and elevated plus maze, and that this is mediated via microbial regulation of NMDA receptors (Neufeld et al., 2011), important in brain development and neural plasticity (Bercik et al., 2010; O'Sullivan et al., 2011; Sudo et al., 2004). GF mice exhibit other cognitive deficits including hippocampus dependent learning and memory processes (Gareau et al., 2011; Hoban et al., 2018; Lu et al., 2018; Luk et al., 2018; Pan et al., 2019), as well as social behaviours (Buffington et al., 2016; Desbonnet et al., 2014; Sarkar et al., 2020; Sgritta et al., 2019). Together, this evidence implies that the microbiome has the potential to alter a host's neuronal activity, either directly or indirectly.

There are limited published studies directly measuring neuronal activity in a model of microbiome disruption, and the functional consequence to neuronal activity has yet to be shown. However, long-term dietary supplementation of a lactobacillus probiotic rescued diabetes induced CA3-CA1 LTP deficits in rats (Davari et al., 2013), chronic treatment with *Lactobacillus/Bifidobacteria* probiotic mix enhanced CA3-CA1 LTP in a rat model of Alzheimers' disease and control animals (Rezaei Asl et al., 2019), and a probiotic mixture of *Actinobacter* and *Bacteroidetes* ameliorated age-related LTP deficits (Distrutti et al., 2014). Furthermore, *L.reuteri* restored social interaction induced plasticity in the ventral tegmental area of a mouse model of autism, through an oxytocin dependent mechanism (Sgritta et al., 2019). These provide evidence that the microbiome can indeed modify functional neuronal activity.

Here, we have tested whether the absence of a microbiome alters basal synaptic efficacy, and inducibility of plasticity, in the adult mouse hippocampus.

2 | METHODS

2.1 | Animals

C57/Bl6 mice, purchased from Taconic (NY, USA), were bred either as GF ($n = 14$, 6 female), or conventionally raised ($n = 13$, 6 female) in University College Cork. Breeding was performed according to supplier guidelines, and we used animals ranging from F8-F11. GF animals were housed 4–5 per cage in individually ventilated cages (area: 420cm^2. Arrowmight, UK), conventionally raised animals were raised 4–5 per cage in standard cages (area:330cm^2. NKP isotech, UK). Both had paper bedding/nesting material and were housed in identical controlled conditions (20–21°C, 55%–60% humidity) on a 12-hr light/dark cycle, with access ad libitum to autoclaved chow and water. On each recording day, one animal (8–12 weeks old) was separated into its own cage and brought to the laboratory for use in the experiments. Experiments were undertaken in accordance with National and European legislation (Directive 2010/63/EU and Irish SI No 543 of 2012).

2.2 | Slice preparation

Hippocampal slice electrophysiology is a well-used technique and our methods followed common practices (Papouin & Haydon, 2018). Fresh artificial cerebrospinal fluid (aCSF) was made (see below) and bubbled with carbogen (95% O_2/5% CO_2) for at least 40 min prior to use, and continually throughout recordings.

Mice were rapidly decapitated, and the brain removed and cooled with ice cold 'cutting' aCSF (see protocol below). The brain was quickly hemisected, the cerebellum and midbrain removed, and the remaining tissue affixed to the stage of a Leica VT1200 vibratome to enable 300 μm transverse slices of the hippocampus and attached cortex to be cut, under ice-cold cutting aCSF. It is important to note that GF mice exhibit altered acute stress responses and HPA axis function (Sudo et al., 2004; Vagnerova et al., 2019). To minimize any effect of differential stress response, all animals were left to acclimatize to the experimental room for at least 20 min and were all observed to be in a non-stressed state.

Slices were transferred to a chilled glass petri-dish, filled with cutting aCSF, and the cortex were removed from the hippocampal slices. A selection of slices spanning the central portion of the dorso-ventral axis (approximately 1.5–2.1 mm from dorsal end) were transferred to mesh holding wells in a bath of standard aCSF held at 32°C for 20 min, and then at room temperature for a further hour.

After this recovery period, slices were transferred to a multi-electrode array (MEA) chip (Multichannel Systems,

Germany), perfused with aCSF. Slices were viewed under an inverted microscope (4x magnification, Olympus IX70) and compared for structural integrity and general slice health. The best candidate slice was used for electrophysiological testing.

2.3 | Electrophysiology recording

The slice was placed onto an MEA chip (Menigoz et al., 2016; Shaban et al., 2017) and aligned so that a stimulating electrode was located over the Schaffer collateral path leaving the CA3, heading towards the CA1, and a recording electrode was situated in the middle of the CA1 striatum radiatum (200 μm 'downstream' of the stimulus electrode) (O'Dell et al., 1991). The slice was held with a nylon mesh anchor. A benefit of using an MEA chip is that it allowed for an additional recording electrode to be sited in the striatum oriens, to record the somatic population spike of the stimulated neurons.

Perfusion rates of the aCSF (heated to 31–32°C) were 2.5 ml/min in, 4 ml/min out, which optimally maintained fluid volume while minimizing turbulent flow forces.

After testing the presence of field excitatory post-synaptic potential (fEPSP) responses with a small bipolar current (10–20 μA), the slice was left to recover in the MEA chip for another hour.

Once fEPSP slope measurements were stable (±15%) for approximately 15 min (typically 1-hr after placement), an input/output curve was computed by increasing the stimulation current from 1 μA to 100 μA and measuring the slopes of the resultant fEPSPs, taking an average of three sequential stimulation ramps. All subsequent stimulation was performed at an intensity eliciting approximately 40% of the maximum response of each slice. Subsequently, a test of short-term synaptic plasticity was performed with a paired-pulse stimulation protocol (PPP). Inter-pulse intervals were; 25, 50, 75, 100, 150 and 200 ms. A 30 s interval between each of these paired pulse events was maintained, and this train of 6 paired pulses was repeated three times.

Once both input/output, and PPP tests were performed, the slice was again stimulated using single bipolar pulses every 30s. After a minimum period of 30 min in which the fEPSP slope did not exceed ± 15% of itself (defined here to be the baseline period), the Schaffer collateral pathway was stimulated with a theta burst (TBS) protocol to induce long-term potentiation (LTP). Each TBS consisted of four 100Hz pulses, repeated 10 times at 5Hz. Three theta bursts (3xTBS) were delivered with an inter-burst interval of 30 s. After this induction protocol, the stimulator reverted back to a single pulse every 30 s as in baseline.

All stimulation protocols were designed in MCStimulusII (Multichannel Systems, Germany) and played during recordings via MCRack (Multichannel Systems, Germany) software.

2.4 | aCSF content

The aCSF was made up of (in mM): NaCl (124), KCl (2.7), NaH2PO4 (1.25), $CaCl_2$ (2), $MgSO_4$ (1.3), D-Glucose (18) and Ascorbic acid (2).

Cutting aCSF was made up of the same aCSF, with excess $MgSO_4$ (8.3 mM).

2.5 | Data analysis and statistical methods

Data files were converted to '.abf' files and the slope (capturing 80%–90% of the inward current slope) of fEPSPs was extracted using semi-automated procedures within Clampfit software. To extract the amplitude of the population spike, we subtracted the trough of the spike from an average of the adjacent peaks. For input/output relationships and PPP, the average of three responses at each stimulus intensity/interval was used. For the test of LTP, data values of fEPSP slopes were expressed as a percentage of the average of the baseline period fEPSPs (30 min prior to TBS), and an average of 10 min (20 stimuli) were used for statistical analysis.

Parametric tests were utilised for all statistical analyses after appropriate checks for data normality. Specific test details are given in the results. Individual slices (each originating from an individual animal) were treated as subjects, sex and GF status of the donor animals was treated as fixed factors. Data originating from individual slices were treated as repeated measures (e.g. pre and post TBS data).

3 | RESULTS

3.1 | Germ-free mice display normal basal synaptic excitability

To test basal synaptic efficacy of GF hippocampal slices, we computed 'input-output' characteristics of the fibre volley and resultant fEPSP at a variety of stimulus intensities (Figure 1b and c). We summarized each slice's data by calculating the linear slope of this relationship (Kim et al., 2005; Woo et al., 2005). ANOVA revealed no significant difference between GF and Conv groups ($F_{1,23} = 1.326$, $p = .261$), and no interaction between GF status and sex ($F_{1,23} = 0.644$, $p = .431$). Nor was there a main effect of sex ($F_{1,23} = 0.004$, $p = .953$). This suggests that GF and Conv slices exhibit the same basal synaptic excitability, although Figure 1d reveals that male GF slices had a tendency for lower values

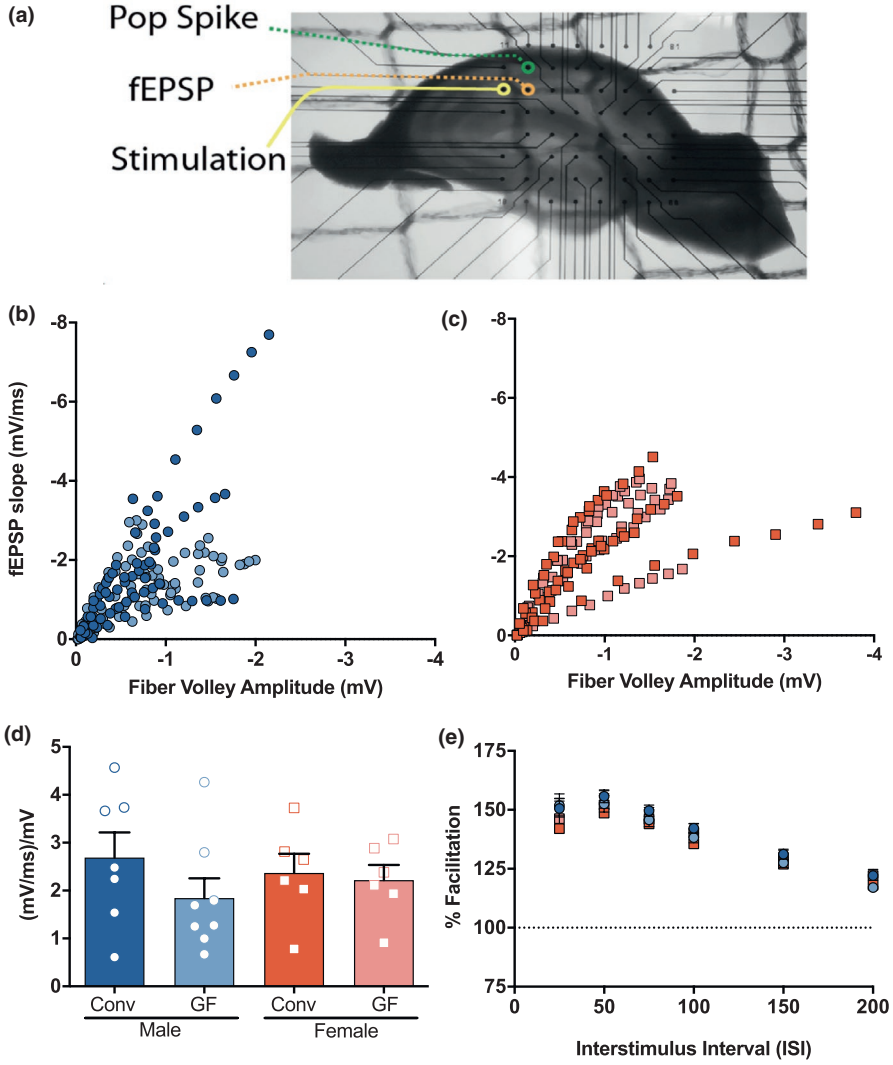

FIGURE 1 (a) Representative image of hippocampal slice on MEA, with target locations of stimulation and recording shown. The relationship between fibre volley amplitude and fEPSP slope of male (b) and female (c) slices. (d) Basal excitability summarised with the linear slope of each slice. (e) Paired-Pulse facilitation of fEPSPs. No statistically significant differences were found between germ-free and conventionally raised control slices. Error bars represent standard error of the mean. Male $N_{Conv/GF} = 7/8$, Female $N_{Conv/GF} = 6/6$

(indicating smaller fEPSP responses for the same fibre volley stimulus).

3.2 | Germ-free mice display normal pre-synaptic function

If two excitatory stimuli occur within a short space of time of each other, the second post-synaptic response, in conventional animals and under normal conditions, will be enhanced (Creager et al., 1980). This short-term facilitation is a result of pre-synaptic mechanisms leading to an increase in the amount of neurotransmitter release (Jackman & Regehr, 2017).

To establish whether GF hippocampal slices exhibited normal pre-synaptic function, we tested GF short-term plasticity using paired-pulse stimulus protocols. Slices from both GF and conventionally raised animals exhibited paired-pulse facilitation with inter-pulse intervals spanning 25–200 ms, with maximal facilitation of around 150% at an inter-pulse interval of 50 ms (Figure 1e), similar to that reported previously in the literature (Chong et al., 2011; Menigoz et al., 2016; Rohan et al., 2015). Mixed model analysis found no significant interaction between the sex and GF status ($F_{1,96.776} = 3.307$, $p = .072$), or main effects of either sex ($F_{1,96.776} = 1.392$, $p = .241$), or GF status ($F_{1,96.776} = 0.000$, $p = .995$).

3.3 | Germ-free males exhibit reduced Long-Term Potentiation after Theta-Burst Stimulation

After a minimum 30-min baseline recording period, LTP was induced using three trains of theta-burst stimulation (3xTBS). In conventionally raised animals, this led to a rapid potentiation of fEPSPs that lasted for 2 hr (males: mean = 153.0% *SEM* 8.5, females: mean = 138.6% *SEM* 5.4). Female GF slices matched this potentiation (mean = 140.9% *SEM* 5.4). Conversely, slices from male GF mice showed no immediate potentiation, with a slow upward trend for the duration of the recording, finishing at a mean of 125.7% *SEM* 7.9 (Figure 2).

Mixed-model analysis revealed a significant 3-way interaction between Time, Sex and GF status

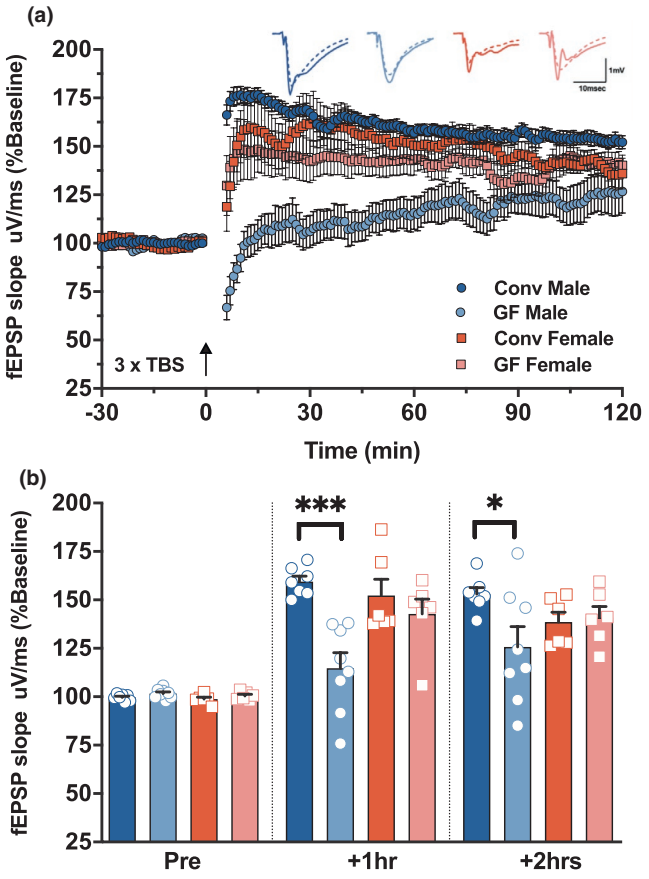

FIGURE 2 (a) Timeline of normalised CA1 fEPSP slopes for conventionally raised (dark shading) and germ-free (light shading) mice. Female (red) germ-free slices exhibited normal potentiation at 2 hr post LTP induction. Male (blue) germ-free slices exhibited a markedly decreased potentiation, that remained up to 2 hr post induction. Representative traces at both pre (dashed lines) and post (solid lines) LTP induction for each group. Scale bar is 1 mV × 10 ms. (b) Bar chart summarising pre and 2 hr post LTP induction fEPSP responses. Error bars represent standard error of the mean. Male $N_{Conv/GF}$ = 7/8, Female $N_{Conv/GF}$ = 6/6. *Mixed model $p = .035$. ***Mixed model $p < .005$

($F_{2,38.163} = 5.002$, $p = .012$). Subsequent analyses on each sex independently revealed no significant interaction between GF status and Time ($F_{2,20.669} = 0.476$, $p = .628$), nor main effect of GF status ($F_{1,17.733} = 0.162$, $p = .692$), leaving only the main effect of Time ($F_{2,20.669} = 86.924$, $p < .0005$) in female slices. Bonferroni corrected pairwise comparisons revealed that pre-TBS data were significantly different from both 1-hr ($p < .0005$) and 2-hr time points ($p < .0005$).

Conversely, for male slices, there was a significant interaction between GF status and time ($F_{2,18\cdot198} = 16.371$, $p < .0005$), suggesting GF status impacts LTP processes in male slices. Subsequent ANOVA found significant differences between GF and Conv. slices at both 1-hr ($p < .0005$) and 2-hr time points ($p = .035$).

3.4 | Theta-Burst stimulation induces normal potentiation of somatic responses in germ-free mice

With the advantages of multiple recording sites with the MEA system, we were able to concurrently record the population spike responses to TBS (Figure 3a). In both groups, TBS led to sustained potentiation of pop. spike amplitude for 2-hr post induction (Conv.: $Mean_{male}$.= 236.5% *SEM* 37.7, $Mean_{female}$ = 275.4% *SEM* 31.8, GF: $Mean_{male}$.=230.9% *SEM* 31.3, $Mean_{female}$ = 227.3% *SEM* 23.7).

Mixed model analysis revealed no significant 3-way or 2-way interactions, and only a main effect of Time ($F_{2,40.211} = 71.787$, $p < .0005$). Bonferroni corrected pairwise comparison showed significant differences between pre-TBS and both 1-hr ($p < .0005$) and 2-hr ($p < .0005$) post-TBS. Together, this shows that population spike potentiation in response to 3xTBS is comparable between GF and Conv. groups.

3.5 | GF slices exhibit altered E-S coupling

The stark contrast between the effects of TBS on the dendritic and somatic responses in GF slices suggested a difference in the E-S coupling relationship between conventionally raised and GF mice. After TBS stimulation, both conventional and GF slices exhibit an increase in the E-S coupling ratio (here representing an increase in pop. spike amplitude for a given fEPSP response) that is maintained for the duration of the recordings (Figure 3c). Notably, GF slices appear to have a greater E-S ratio both before and after LTP induction, with male slices exhibiting the greatest differences to conventional slices (Mean difference = 0.832 mV/(mV/ms)).

Mixed model analysis revealed no significant 3-way or 2-way interactions, but significant main effects of GF status ($F_{1,68.466} = 15.419$, $p < .0005$), and time ($F_{2,45.008} = 5.446$, $p = .008$).

In summary, GF slices exhibited a greater E-S ratio than Conv. slices, and this difference was not markedly altered by the effect of TBS.

4 | DISCUSSION

In the brain, activity-dependent modification of synapses has long been regarded as the brain's principal mechanism of assimilating transient experiences into perseverant memory engrams (Bliss & Lomo, 1973; Josselyn & Tonegawa, 2020), where an activity-dependent persistent strengthening of synaptic efficacy can be described as long-term potentiation

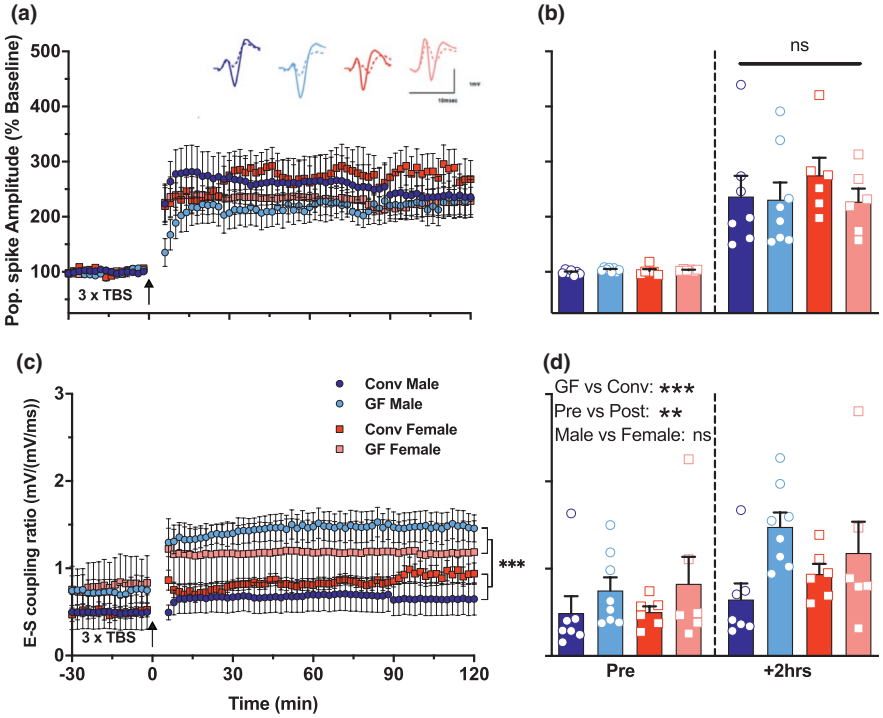

FIGURE 3 (a) Timeline of normalised CA1 pop. spike amplitudes for conventionally raised (dark shading) and germ-free (light shading) mice. Germ-free slices exhibited normal potentiation compared to conventionally raised slices. Representative traces at both pre (dashed lines) and post (solid lines) LTP induction for each group. Scale bar is 1 mV × 10 ms. (b) Bar chart summarising pre and 2 hr post LTP induction pop. spike responses. (c) Timeline of CA1 E-S coupling for conventionally raised (dark shading) and germ-free (light shading) mice. Both male and female germ-free slices exhibited markedly increased E-S coupling both pre and post LTP induction. (d) Bar chart summarising pre and 2 hr post LTP induction E-S coupling. Error bars represent standard error of the mean. Male $N_{Conv/GF} = 7/8$, Female $N_{Conv/GF} = 6/6$. ***Mixed model $p < .0005$. **Mixed model $p = .008$

(LTP) (Madison et al., 1991). Here, we show that the microbiome may play an important role in the expression of these processes.

In the unidirectional tri-synaptic excitatory pathway of the rodent hippocampus, any significant enhancement in synaptic activity lasting at least one hour post-tetanus is understood to be LTP (Nicoll, 2017). Therefore, LTP has been used as a reliable indicator of healthy brain function, and its impairment has been seen in rodent models of neurodegenerative disease. Deficits in LTP have been seen accompanying cognitive dysfunction (Li et al., 2015; Lin et al., 2014; Wieschollek & Manahan-Vaughan, 2013), and it is regarded as a reliable, functional readout for neurophysiological processes. Indeed, neuronal synaptic plasticity is particularly vulnerable to disruption by factors that alter cognition in psychiatric and neurological disease, including those that alter synaptogenesis (Bednarek & Caroni, 2011), axonal sprouting or pruning, and dendritic remodelling (Selemon, 2013; Ziv & Brenner, 2018), as well as neurogenesis (Ganguly & Poo, 2013), many of which have been shown to be influenced by gut-microbiota (Cryan et al., 2019). Furthermore, the hippocampus is highly responsive to glucocorticoids (Lupien et al., 2005), and it has been shown that hormone interaction with CA1 receptors in the hippocampus leads to a variety of different cellular responses, including changes in synaptic function, including neuronal injury (Chen et al., 2006).

GF animals have been utilized in examining the involvement of the microbiota in central synaptic plasticity, and here, we show that the absence of a microbiome significantly alters fundamental hippocampal neuronal plasticity processes. This fits with the evidence that GF animals exhibit altered molecular chemistry, morphology of the hippocampus as well as an altered behavioural phenotype associated with hippocampal activity (Gareau et al., 2011; Luczynski et al., 2016).

Specifically, the GF mice tested here appear to have normal ability to produce fEPSPs, and short-term potentiation (Figure 1), although male GF slices appear to have a small (nonsignificant) trend for lowered basal excitability (Figure 1d). However, testing post-synaptic LTP mechanisms (with 3xTBS) reveals a sex-dependent functional shift, with males showing greater deficits than females (Figure 2). Sex-specific impacts of the gut microbiome have been reported, however, there is no clear consensus with heterogeneous results possibly resulting from strain specificity of both microbiome and host (reviewed in Jaggar et al., 2020).

Whilst we have not investigated the molecular mechanisms underpinning this phenomenon, it is plausible that the altered genetic/transcriptional profile of immediate early genes

(Gareau et al., 2011) leads to a block of the dendritic potentiation signals. Additionally, a recent study has shown that microbial metabolites can translocate from the gut to the brain during early developmental periods (Swann et al., 2020). Interestingly, this included imidazole propionate, which activates mammalian target of rapamycin complex 1 (mTORC1), which is known to be involved in hippocampal LTP (Hoeffer & Klann, 2010). Furthermore, altered inflammatory signalling, widely implicated as a route of gut-brain interaction, has been shown to reduce hippocampal long-term potentiation via an interleukin-1 receptor dependent pathway (York et al., 2021).

When examining the spiking output from the CA1 area we find a normal potentiation of the pop. spike in response to 3xTBS in both male and female GF slices (Figure 3a). Moreover, when examining the input–output relationship between the dendritic fEPSP and somatic pop. spike (E-S coupling), we find that GF slices (both male and female) exhibit a significantly enhanced coupling (Figure 3b). This could suggest homeostatic mechanisms in the somatic integration of dendritic signals (E-S coupling) acting to rescue the functional deficits observed at the dendritic regions. Overall, this compensation may mask more severe microbiome driven influences, resulting in the milder behavioural phenotypes.

Whilst E-S coupling is a relatively understudied facet of neuronal plasticity, it is believed that it may be driven by a mixture of intrinsic factors as well as the excitation/inhibition balance, with one study suggesting around 40% of E-S coupling in hippocampus CA1 was driven by cell intrinsic factors (Daoudal et al., 2002). Interestingly, GF mice have previously been suggested to have a disrupted excitatory/inhibitory balance. This is supported by the finding that GF mice exhibit a reduced concentration of GABA in the hippocampus compared to specific-pathogen free mice (Kawase et al., 2017), and colonization of GF mice with Lactobacillus probiotics leads to increased GABAergic cells in the hippocampus (Mao et al., 2020). Disruption of the inhibitory/excitatory balance is thought to underpin many physiological disorders, including epilepsy. GF mice have not, to our knowledge, been reported to exhibit an epileptic phenotype, however, the microbiome has been implicated in disruption of the excitatory/inhibitory balance. Specifically, whilst a ketogenic diet was able to confer protective effects against a 6Hz electrical stimulation epilepsy paradigm, GF mice did not benefit from the same diet, but the protection was restored when supplementation of *Akkermansia* and *Parabacteroides* was given (Olson et al., 2018). This microbiome derived protection has been attributed to elevation of GABA inhibition (Dahlin & Prast-Nielsen, 2019). The morphological differences in hippocampal pyramidal neurons of GF mice (Luczynski et al., 2016) also suggests an immaturity of the glutamatergic synapses, which would most likely alter the inhibitory/excitatory balance of the network. Furthermore, microbiome/dietary based interventions have been proposed as an interesting approach to address epilepsy (De Caro et al., 2019).

GF mice often display altered measures of motor behaviour, as well as social interaction/cognition (reviewed in Spichak et al., 2018). Experience is well-known to modulate hippocampal plasticity processes. Whilst housing conditions between the Conv. and GF animals in this study were identical as far as possible, it is possible that the differences observed in our study are as a result of altered social experiences/interactions of the animals (driven by GF status) rather than as a direct result of any microbiome metabolite or signalling process. Further experiments will be needed to disentangle these possibilities.

Although translation of our findings to the human setting is not yet possible, there are still clear differences in the functional properties of the hippocampal circuit in vitro, supporting the idea that neurochemical and morphological alterations found in GF mice lead to altered functional activity. Further examination will be important to characterize changes in electrophysiological properties of circuits from animals with perturbed gut-microbiomes. Some principal questions that arise from this study are: Can hippocampal properties be altered with antibiotic knockdown of the microbiome? Are differences observed earlier in the lifespan? Are other forms of plasticity (i.e. long-term depression) influenced by the microbiota? Can these effects be reversed with pre/probiotic administration?

We conclude that the absence of a gut-microbiome leads to a selective alteration of dendritic LTP mechanisms, with males more severely affected, which may be compensated for by an E-S coupling driven hyperexcitability. This functional effect of hippocampal activity is likely to be important in the behavioural phenotype of GF animals and further implicates the microbiome in molecular memory processes.

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CONFLICTS OF INTEREST

J.F.C has been an invited speaker at meetings organized by Yakult, Alkermes, Ordesa, Servier, Lundbeck, Janssen, and AstraZeneca, and have received research funding from Mead Johnson, Cremo, Nutricia, Dupont, Pharmavite, and 4D Pharma. All other authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

H.T.D, K.J.OR, and J.F.C conceived and designed the experiment. H.T.D, M.K.C, and K.J.OR performed experiments,

and data analysis. K.J.OR prepared figures. H.T.D, K.J.OR, and J.F.C prepared the manuscript.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1111/ejn.15119.

DATA AVAILABILITY STATEMENT

Data used in this report are freely available upon reasonable request to the corresponding author.

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REFERENCES

Bednarek, E., & Caroni, P. (2011). beta-Adducin is required for stable assembly of new synapses and improved memory upon environmental enrichment. *Neuron*, *69*(6), 1132–1146. https://doi.org/10.1016/j.neuron.2011.02.034

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K. D., Verdu, E. F., & Collins, S. M. (2011). The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*, *141*(2), 599–609.e3. https://doi.org/10.1053/j.gastro.2011.04.052

Bercik, P., Verdu, E. F., Foster, J. A., Macri, J., Potter, M., Huang, X., Malinowski, P., Jackson, W., Blennerhassett, P., Neufeld, K. A., Lu, J., Khan, W. I., Corthesy–Theulaz, I., Cherbut, C., Bergonzelli, G. E., & Collins, S. M. (2010). Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. *Gastroenterology*, *139*(6), 2102–2112 e2101. https://doi.org/10.1053/j.gastro.2010.06.063

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *Journal of Physiology*, *232*(2), 331–356. https://doi.org/10.1113/jphysiol.1973.sp010273

Buffington, S. A., Di Prisco, G. V., Auchtung, T. A., Ajami, N. J., Petrosino, J. F., & Costa-Mattioli, M. (2016). Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. *Cell*, *165*(7), 1762–1775. https://doi.org/10.1016/j.cell.2016.06.001

Chen, J.-J., Zeng, B.-H., Li, W.-W., Zhou, C.-J., Fan, S.-H., Cheng, K. E., Zeng, L. I., Zheng, P., Fang, L., Wei, H., & Xie, P. (2017). Effects of gut microbiota on the microRNA and mRNA expression in the hippocampus of mice. *Behavioral Brain Research*, *322*(Pt A), 34–41. https://doi.org/10.1016/j.bbr.2017.01.021

Chen, Y., Fenoglio, K. A., Dube, C. M., Grigoriadis, D. E., & Baram, T. Z. (2006). Cellular and molecular mechanisms of hippocampal activation by acute stress are age-dependent. *Molecular Psychiatry*, *11*(11), 992–1002. https://doi.org/10.1038/sj.mp.4001863

Chong, S.-A., Benilova, I., Shaban, H., De Strooper, B., Devijver, H., Moechars, D., Eberle, W., Bartic, C., Van Leuven, F., & Callewaert, G. (2011). Synaptic dysfunction in hippocampus of transgenic mouse models of Alzheimer's disease: A multi-electrode array study. *Neurobiology of Diseases*, *44*(3), 284–291. https://doi.org/10.1016/j.nbd.2011.07.006

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, *18*(6), 666–673. https://doi.org/10.1038/mp.2012.77

Creager, R., Dunwiddie, T., & Lynch, G. (1980). Paired-pulse and frequency facilitation in the CA1 region of the in vitro rat hippocampus. *Journal of Physiology*, *299*, 409–424. https://doi.org/10.1113/jphysiol.1980.sp013133

Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., … Dinan, T. G. (2019). The Microbiota-Gut-Brain Axis. *Physiological Reviews*, *99*(4), 1877–2013. https://doi.org/10.1152/physrev.00018.2018

Dahlin, M., & Prast-Nielsen, S. (2019). The Gut microbiome and epilepsy. *Ebiomedicine*, *44*, 741–746. https://doi.org/10.1016/j.ebiom.2019.05.024

Daoudal, G., Hanada, Y., & Debanne, D. (2002). Bidirectional plasticity of excitatory postsynaptic potential (EPSP)-spike coupling in CA1 hippocampal pyramidal neurons. *Proc Natl. Acad Sci U S A*, *99*(22), 14512–14517. https://doi.org/10.1073/pnas.222546399

Davari, S., Talaei, S. A., Alaei, H., & Salami, M. (2013). Probiotics treatment improves diabetes-induced impairment of synaptic activity and cognitive function: Behavioral and electrophysiological proofs for microbiome-gut-brain axis. *Neuroscience*, *240*, 287–296. https://doi.org/10.1016/j.neuroscience.2013.02.055

De Caro, C., Iannone, L. F., Citraro, R., Striano, P., De Sarro, G., Constanti, A., Cryan, J. F., & Russo, E. (2019). Can we 'seize' the gut microbiota to treat epilepsy? *Neuroscience and Biobehavioral Reviews*, *107*, 750–764. https://doi.org/10.1016/j.neubiorev.2019.10.002

De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, A. J., Green, W., Denou, E., Silva, M. A., Santacruz, A., Sanz, Y., Surette, M. G., Verdu, E. F., Collins, S. M., & Bercik, P. (2015). Microbiota and host determinants of behavioural phenotype in maternally separated mice. *Nature Communications*, *6*(1), 7735. https://doi.org/10.1038/ncomms8735

Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2014). Microbiota is essential for social development in the mouse. *Molecular Psychiatry*, *19*(2), 146–148. https://doi.org/10.1038/mp.2013.65

Distrutti, E., O'Reilly, J. A., McDonald, C., Cipriani, S., Renga, B., Lynch, M. A., & Fiorucci, S. (2014). Modulation of intestinal microbiota by the probiotic VSL#3 resets brain gene expression and ameliorates the age-related deficit in LTP. *PLoS One*, *9*(9), e106503. https://doi.org/10.1371/journal.pone.0106503

Ganguly, K., & Poo, M. M. (2013). Activity-dependent neural plasticity from bench to bedside. *Neuron*, *80*(3), 729–741. https://doi.org/10.1016/j.neuron.2013.10.028

Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., MacQueen, G., & Sherman, P. M. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*, *60*(3), 307–317. https://doi.org/10.1136/gut.2009.202515

Heijtz, R. D., Wang, S., Anuar, F., Qian, Y., Bjorkholm, B., Samuelsson, A., Hibberd, M. L., Forssberg, H., & Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proc*

Natl Acad Sci USA, *108*(7), 3047–3052. https://doi.org/10.1073/pnas.1010529108

Hoban, A. E., Stilling, R. M., Moloney, G., Shanahan, F., Dinan, T. G., Clarke, G., & Cryan, J. F. (2018). The microbiome regulates amygdala-dependent fear recall. *Molecular Psychiatry*, *23*(5), 1134–1144. https://doi.org/10.1038/mp.2017.100

Hoeffer, C. A., & Klann, E. (2010). mTOR signaling: At the crossroads of plasticity, memory and disease. *Trends in Neurosciences*, *33*(2), 67–75. https://doi.org/10.1016/j.tins.2009.11.003

Jsackman, S. L., & Regehr, W. G. (2017). The mechanisms and functions of synaptic facilitation. *Neuron*, *94*(3), 447–464. https://doi.org/10.1016/j.neuron.2017.02.047

Jaggar, M., Rea, K., Spichak, S., Dinan, T. G., & Cryan, J. F. (2020). You've got male: Sex and the microbiota-gut-brain axis across the lifespan. *Frontiers in Neuroendocrinology*, *56*, 100815. https://doi.org/10.1016/j.yfrne.2019.100815

Josselyn, S. A., & Tonegawa, S. (2020). Memory engrams: Recalling the past and imagining the future. *Science*, *367*(6473), https://doi.org/10.1126/science.aaw4325

Kawase, T., Nagasawa, M., Ikeda, H., Yasuo, S., Koga, Y., & Furuse, M. (2017). Gut microbiota of mice putatively modifies amino acid metabolism in the host brain. *British Journal of Nutrition*, *117*(6), 775–783. https://doi.org/10.1017/S0007114517000678

Kim, C.-H., Takamiya, K., Petralia, R. S., Sattler, R., Yu, S., Zhou, W., Kalb, R., Wenthold, R., & Huganir, R. (2005). Persistent hippocampal CA1 LTP in mice lacking the C-terminal PDZ ligand of GluR1. *Nature Neuroscience*, *8*(8), 985–987. https://doi.org/10.1038/nn1432

Li, Y., Abdourahman, A., Tamm, J. A., Pehrson, A. L., Sanchez, C., & Gulinello, M. (2015). Reversal of age-associated cognitive deficits is accompanied by increased plasticity-related gene expression after chronic antidepressant administration in middle-aged mice. *Pharmacology, Biochemistry and Behavior*, *135*, 70–82. https://doi.org/10.1016/j.pbb.2015.05.013

Lin, N., Pan, X. D., Chen, A. Q., Zhu, Y. G., Wu, M., Zhang, J., & Chen, X. C. (2014). Tripchlorolide improves age-associated cognitive deficits by reversing hippocampal synaptic plasticity impairment and NMDA receptor dysfunction in SAMP8 mice. *Behavioral Brain Research*, *258*, 8–18. https://doi.org/10.1016/j.bbr.2013.10.010

Lu, J., Synowiec, S., Lu, L., Yu, Y., Bretherick, T., Takada, S., Yarnykh, V., Caplan, J., Caplan, M., Claud, E. C., & Drobyshevsky, A. (2018). Microbiota influence the development of the brain and behaviors in C57BL/6J mice. *PLoS One*, *13*(8), e0201829. https://doi.org/10.1371/journal.pone.0201829

Luczynski, P., Whelan, S. O., O'Sullivan, C., Clarke, G., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2016). Adult microbiota-deficient mice have distinct dendritic morphological changes: Differential effects in the amygdala and hippocampus. *European Journal of Neuroscience*, *44*(9), 2654–2666. https://doi.org/10.1111/ejn.13291

Luk, B., Veeraragavan, S., Engevik, M., Balderas, M., Major, A., Runge, J., Luna, R. A., & Versalovic, J. (2018). Postnatal colonization with human "infant-type" Bifidobacterium species alters behavior of adult gnotobiotic mice. *PLoS One*, *13*(5), e0196510. https://doi.org/10.1371/journal.pone.0196510

Lupien, S. J., Fiocco, A., Wan, N., Maheu, F., Lord, C., Schramek, T., & Tu, M. T. (2005). Stress hormones and human memory function across the lifespan. *Psychoneuroendocrinology*, *30*(3), 225–242. https://doi.org/10.1016/j.psyneuen.2004.08.003

Madison, D. V., Malenka, R. C., & Nicoll, R. A. (1991). Mechanisms underlying long-term potentiation of synaptic transmission. *Annual Review of Neuroscience*, *14*, 379–397. https://doi.org/10.1146/annurev.ne.14.030191.002115

Mao, J.-H., Kim, Y.-M., Zhou, Y.-X., Hu, D., Zhong, C., Chang, H., Brislawn, C. J., Fansler, S., Langley, S., Wang, Y., Peisl, B. Y. L., Celniker, S. E., Threadgill, D. W., Wilmes, P., Orr, G., Metz, T. O., Jansson, J. K., & Snijders, A. M. (2020). Genetic and metabolic links between the murine microbiome and memory. *Microbiome*, *8*(1), 53. https://doi.org/10.1186/s40168-020-00817-w

Menigoz, A., Ahmed, T., Sabanov, V., Philippaert, K., Pinto, S., Kerselaers, S., Segal, A., Freichel, M., Voets, T., Nilius, B., Vennekens, R., & Balschun, D. (2016). TRPM4-dependent postsynaptic depolarization is essential for the induction of NMDA receptor-dependent LTP in CA1 hippocampal neurons. *Pflugers Archiv. European Journal of Physiology*, *468*(4), 593–607. https://doi.org/10.1007/s00424-015-1764-7

Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology and Motility*, *23*(3), 255–264, e119. https://doi.org/10.1111/j.1365-2982.2010.01620.x

Nicoll, R. A. (2017). A brief history of long-term potentiation. *Neuron*, *93*(2), 281–290. https://doi.org/10.1016/j.neuron.2016.12.015

O'Dell, T. J., Kandel, E. R., & Grant, S. G. (1991). Long-term potentiation in the hippocampus is blocked by tyrosine kinase inhibitors. *Nature*, *353*(6344), 558–560. https://doi.org/10.1038/353558a0

Olson, C. A., Vuong, H. E., Yano, J. M., Liang, Q. Y., Nusbaum, D. J., & Hsiao, E. Y. (2018). The gut microbiota mediates the anti-seizure effects of the ketogenic diet. *Cell*, *173*(7), 1728–1741 e1713. https://doi.org/10.1016/j.cell.2018.04.027

O'Sullivan, E., Barrett, E., Grenham, S., Fitzgerald, P., Stanton, C., Ross, R. P., & Dinan, T. G. (2011). BDNF expression in the hippocampus of maternally separated rats: Does Bifidobacterium breve 6330 alter BDNF levels? *Benef Microbes*, *2*(3), 199–207. https://doi.org/10.3920/BM2011.0015

Pan, J.-X., Deng, F.-L., Zeng, B.-H., Zheng, P., Liang, W.-W., Yin, B.-M., Wu, J., Dong, M.-X., Luo, Y.-Y., Wang, H.-Y., Wei, H., & Xie, P. (2019). Absence of gut microbiota during early life affects anxiolytic Behaviors and monoamine neurotransmitters system in the hippocampal of mice. *Journal of the Neurological Sciences*, *400*, 160–168. https://doi.org/10.1016/j.jns.2019.03.027

Papouin, T., & Haydon, P. G. (2018). Obtaining acute brain slices. *Bio Protoc*, *8*(2), e2699. https://doi.org/10.21769/BioProtoc.2699

Rezaei Asl, Z., Sepehri, G., & Salami, M. (2019). Probiotic treatment improves the impaired spatial cognitive performance and restores synaptic plasticity in an animal model of Alzheimer's disease. *Behavioral Brain Research*, *376*, 112183. https://doi.org/10.1016/j.bbr.2019.112183

Rohan, J. G., Carhuatanta, K. A., McInturf, S. M., Miklasevich, M. K., & Jankord, R. (2015). Modulating hippocampal plasticity with in vivo brain stimulation. *Journal of Neuroscience*, *35*(37), 12824–12832. https://doi.org/10.1523/JNEUROSCI.2376-15.2015

Sarkar, A., Harty, S., Johnson, K.-.- A., Moeller, A. H., Carmody, R. N., Lehto, S. M., Erdman, S. E., Dunbar, R. I. M., & Burnet, P. W. J. (2020). The role of the microbiome in the neurobiology of social behaviour. *Biological Reviews of the Cambridge Philosophical Society*, *95*(5), 1131–1166. https://doi.org/10.1111/brv.12603

Selemon, L. D. (2013). A role for synaptic plasticity in the adolescent development of executive function. *Transl Psychiatry*, *3*, e238. https://doi.org/10.1038/tp.2013.7

Sgritta, M., Dooling, S. W., Buffington, S. A., Momin, E. N., Francis, M. B., Britton, R. A., & Costa-Mattioli, M. (2019). Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism spectrum disorder. *Neuron*, *101*(2), 246–259 e246. https://doi.org/10.1016/j.neuron.2018.11.018

Shaban, H., O'Connor, R., Ovsepian, S. V., Dinan, T. G., Cryan, J. F., & Schellekens, H. (2017). Electrophysiological approaches to unravel the neurobiological basis of appetite and satiety: Use of the multielectrode array as a screening strategy. *Drug Discovery Today*, *22*(1), 31–42. https://doi.org/10.1016/j.drudis.2016.09.003

Spichak, S., Guzzetta, K. E., O'Leary, O. F., Clarke, G., Dinan, T. G., & Cryan, J. F. (2018). Without a bug's life: Germ-free rodents to interrogate microbiota-gut-neuroimmune interactions. *Drug Discovery Today: Disease Models*, *28*, 79–93. https://doi.org/10.1016/j.ddmod.2019.08.002

Stilling, R. M., Moloney, G. M., Ryan, F. J., Hoban, A. E., Bastiaanssen, T. F. S., Shanahan, F., Clarke, G., Claesson, M. J., Dinan, T. G., & Cryan, J. F. (2018). Social interaction-induced activation of RNA splicing in the amygdala of microbiome-deficient mice. *Elife*, *7*, e33070. https://doi.org/10.7554/eLife.33070

Stilling, R. M., Ryan, F. J., Hoban, A. E., Shanahan, F., Clarke, G., Claesson, M. J., & Cryan, J. F. (2015). Microbes & neurodevelopment – Absence of microbiota during early life increases activity-related transcriptional pathways in the amygdala. *Brain, Behavior, and Immunity*, *50*, 209–220.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.-N., Kubo, C., & Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *Journal of Physiology*, *558*(Pt 1), 263–275. https://doi.org/10.1113/jphysiol.2004.063388

Swann, J. R., Spitzer, S. O., & Diaz Heijtz, R. (2020). Developmental signatures of microbiota-derived metabolites in the mouse brain. *Metabolites*, *10*(5), https://doi.org/10.3390/metabo10050172

Vagnerová, K., Vodička, M., Hermanová, P., Ergang, P., Šrůtková, D., Klusoňová, P., Balounová, K., Hudcovic, T., & Pácha, J. (2019). Interactions between gut microbiota and acute restraint stress in peripheral structures of the hypothalamic-pituitary-adrenal axis and the intestine of male mice. *Frontiers in Immunology*, *10*, 2655. https://doi.org/10.3389/fimmu.2019.02655

Wiescholleck, V., & Manahan-Vaughan, D. (2013). Persistent deficits in hippocampal synaptic plasticity accompany losses of hippocampus-dependent memory in a rodent model of psychosis. *Front Integr Neurosci*, *7*, 12. https://doi.org/10.3389/fnint.2013.00012

Woo, N. H., Teng, H. K., Siao, C.-J., Chiaruttini, C., Pang, P. T., Milner, T. A., Hempstead, B. L., & Lu, B. (2005). Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. *Nature Neuroscience*, *8*(8), 1069–1077. https://doi.org/10.1038/nn1510

York, E. M., Zhang, J., Choi, H. B., & MacVicar, B. A. (2021). Neuroinflammatory inhibition of synaptic long-term potentiation requires immunometabolic reprogramming of microglia. *Glia*, *69*(3), 567–578. https://doi.org/10.1002/glia.23913

Ziv, N. E., & Brenner, N. (2018). Synaptic tenacity or lack thereof: Spontaneous remodeling of synapses. *Trends in Neurosciences*, *41*(2), 89–99. https://doi.org/10.1016/j.tins.2017.12.003

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