Role of type I interferon signaling and microglia in the abnormal long term potentiation and object place recognition deficits of male mice with a mutation of the Tuberous Sclerosis 2 gene

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Abstract

Tuberous Sclerosis Complex (TSC) is a genetic disorder associated with high rates of intellectual disability and autism. Although previous studies focused on the role of neuronal deficits in the memory phenotypes of rodent models of TSC, the results presented here demonstrate a role for microglia in these deficits. Mice with a heterozygous null mutation of the *Tsc2* gene (*Tsc2*^{+/-}), show deficits in hippocampal dependent tasks, as well as abnormal long-term potentiation (LTP) in the hippocampal CA1 region. Here, we show that microglia and type I interferon signaling (IFN1) have a key role in the object place recognition (OPR; a hippocampal dependent task) deficits and abnormal LTP of *Tsc2*^{+/-} male mice. Unexpectedly, we demonstrate that male, but not female, *Tsc2*^{+/-} mice showed OPR deficits. Importantly, these deficits can be rescued by depletion of microglia, as well as by a genetic manipulation of a signaling pathway known to modulate microglia function (interferon-alpha/beta receptor alpha chain null mutation).

In addition to rescuing the OPR deficits, depletion of microglia also reversed the abnormal LTP of the $Tsc2^{+/-}$ mice. Altogether, our results suggest that altered IFN1 signaling in microglia cause the abnormal LTP and OPR deficits of male $Tsc2^{+/-}$ mice.

Introduction

Tuberous Sclerosis (TSC) is a genetic condition caused by mutations in either *Tsc1* (1) or *Tsc2* (2) genes. Autism Spectrum Disorder (ASD) (3-6), and cognitive deficits (7-9) are common in TSC. Prior studies have suggested that humans (8, 10), as well as *Tsc1* (7, 11) and *Tsc2* (9, 12) rodent models of TSC, show hippocampal dependent spatial memory deficits, as assessed in the Morris water maze (7, 9, 12) and radial arm maze (9). In addition, other forms of hippocampal dependent memory, including contextual fear memory (7, 9, 11, 12), are impaired in TSC mouse models. Previous studies suggested that abnormal hippocampal long-term potentiation (LTP) may underlie these memory deficits (9, 13), thus suggesting that neuronal deficits have a principal role in the memory phenotypes of rodent models of TSC (14, 15).

The Object Place Recognition (OPR) task is designed to assess spatial memory and discrimination. This test is based on rodent's tendency to spend more time investigating a familiar object that has been moved to a new location (16, 17). Previous studies showed that impairing hippocampal function with lesions (16, 18), pharmacological antagonists (19), and genetic manipulations (20) resulted in impairments in OPR, demonstrating a role for the hippocampus in this task (16, 18-21). Here, we show that male, but not female, $Tsc2^{+/-}$ mice have profound OPR deficits, that can be reversed by depletion of microglia, as well as by an interferon-alpha/beta receptor alpha chain null mutation. In addition to rescuing the OPR deficits, depletion of microglia also rescued the abnormal LTP of the $Tsc2^{+/-}$ mice, a result that demonstrates a previously unrecognized role of this cell type in the spatial and LTP abnormalities of animal models of TSC.

Results

Male, but not female $Tsc2^{+/-}$ mice show deficits in OPR.

Male and female $Tsc2^{+/-}$ mice and their wild type littermates (WT mice) were tested as adults (4-6 months old) in the OPR task (Fig.1A). Only male $Tsc2^{+/-}$, but not female $Tsc2^{+/-}$ or WT mice, showed OPR deficits (no preference for the object in the novel location vs the object in the same location as during training; Fig.1B). Interestingly, all groups tested (fig.S1A) showed no deficits and equivalent performance in the object recognition memory task (NOR) (22) (fig.S1B), indicating that the OPR deficits of male $Tsc2^{+/-}$ mice are not due to deficits in objects recognition. Recently, our laboratory has shown that early post-natal immune activation in $Tsc2^{+/-}$ mice leads to social phenotypes (23). To determine whether immune activation can reveal OPR deficits in female $Tsc2^{+/-}$ mice, male and female $Tsc2^{+/-}$ mice were injected with either polyinosinic:polycytidylic acid (Poly I:C; 20mg/kg), or Saline (control) i.p. at post-natal day 3 (P3), P7 and P14 (fig.S2A). We then tested the mice as adults (4-6 months old) in the OPR task. Only male $Tsc2^{+/-}$ mice injected with Poly I:C early post-natally ($Tsc2^{+/-}$ Ep) or saline, but not female $Tsc2^{+/-}$ mice, showed OPR deficits (fig.S2B). These data demonstrated that early post-natal immune activation did not reveal OPR deficits in female $Tsc2^{+/-}$ mice.

A single adult treatment with a mTOR inhibitor (rapamycin) can temporarily rescue the OPR deficits of male $Tsc2^{+/-}$ mice.

The $Tsc2$ gene is known to regulate mTOR signaling (24-27). Accordingly, reductions of $Tsc2$ cause upregulation of mTOR signaling in rodents and humans (28, 29). Previous results showed that an mTOR inhibitor (rapamycin) was capable of reversing multiple phenotypes observed in $Tsc2^{+/-}$ mice (30-32). To test whether the inhibition of mTOR signaling in adult male $Tsc2^{+/-}$ mice can reverse their OPR deficits, we treated those mice with rapamycin (5 mg/kg) or vehicle (DMSO) as adults (Fig.2A) for 5 days. Before treatment with rapamycin, the $Tsc2^{+/-}$ mice showed deficits in OPR (Fig.2B), while $Tsc2^{+/-}$ mice treated with rapamycin show normal OPR (preference for the object in the novel location) (Fig.2B). However, 2 months after the Rapamycin treatment, $Tsc2^{+/-}$ mice show again OPR deficits (Fig.2B). These results demonstrate the critical role of mTOR signaling in the OPR deficits of adult $Tsc2^{+/-}$ male mice, and show that a 5-day treatment of rapamycin can only temporarily rescue the OPR deficits of $Tsc2^{+/-}$ male mice.

Adult depletion of microglia results in the permanent rescue of the OPR deficits of male $Tsc2^{+/-}$ mice.

Previous results from our laboratory (33) showed that microglia play a critical role in the social memory deficits of $Tsc2^{+/-}$ mice with early post-natal immune activation. Therefore, we tested whether depletion of microglia could also reverse the OPR deficits of $Tsc2^{+/-}$ male mice. Inhibition of the Colony Stimulating Factor 1 Receptor (CSF1R) leads to the depletion of ~99% of all microglia brain-wide (34) (Fig.3B). Thus, we treated male $Tsc2^{+/-}$ mice with a CSF1R inhibitor (PLX5622 chow) or vehicle control chow (Fig.3A) for 21 days, and then tested them for OPR. Treatment with PLX5622 rescued the OPR deficits of male $Tsc2^{+/-}$ mice (Fig.3C). Remarkably, we also found that this treatment results in a permanent rescue of OPR in the male $Tsc2^{+/-}$ mice since these the mice showed normal OPR when they were tested 2 months after microglia depletion (Fig.3D). Histological studies demonstrated that at this time microglia had already repopulated the brain of these mice (fig 3B). These results demonstrate a role for microglia in the OPR deficits of male $Tsc2^{+/-}$ mice.

As we mentioned above, our laboratory has previously shown that early post-natal immune activation reveals social memory deficits in $Tsc2^{+/-}$ mice ($Tsc2^{+/-}$ Ep) (33). Thus, we determined whether the depletion of microglia is still capable of rescuing the OPR deficits of $Tsc2^{+/-}$ Ep adult mice (4-6 months old). Remarkably, treatment with PLX5622 also rescued the OPR deficits of male $Tsc2^{+/-}$ Ep mice (fig.S3B), and this rescue persisted for at least 2 months (fig.S3C).

The $Tsc2^{+/-}$ mutation restricted to microglia during development, but not in adults, results in OPR deficits

To test whether the $Tsc2^{+/-}$ mutation restricted to the microglia (35) is sufficient to cause OPR deficits, we used a mouse model with a floxed $Tsc2$ allele specifically deleted in microglia by a Cre recombinase expressed from a microglia specific promoter ($Cx3cr1$; $Cx3cr1^{Cre}$ - $Tsc2^{Fllox}$) (35). We tested those mice as adults (4-6 months old) for OPR (Fig.4A). Similar to germ line $Tsc2^{+/-}$ male mice, male $Cx3cr1^{Cre}$ - $Tsc2^{Fllox}$, but not control mice, showed OPR deficits (Fig.4B).

The results presented above (Fig.3D and fig.S3C), showed that depletion of microglia permanently recue the OPR deficits of male $Tsc2^{+/-}$ mice. So, once the microglia is depleted in adult mice, the $Tsc2^{+/-}$ mutant microglia that repopulate the adult brain of these mice (after PLX treatment in adults) is not able to trigger OPR deficits. This suggests that the $Tsc2$ mutation, specifically during development is critical for the OPR deficits of male $Tsc2^{+/-}$ mice. To test this hypothesis, we used mice with a tamoxifen-inducible $Tsc2^{+/-}$ mutation restricted to microglia (the

Cx3cr1^{CreER}-Tsc2^{Flox} mouse model) (36), and that could be specifically induced in adults. Thus, we activated the Tsc2 mutation in the microglia by administering 4-Hydroxytamoxifen in adult male Cx3cr1^{CreER}-Tsc2^{Flox} mice (Fig.4C). Then, we tested those mice for OPR. Remarkably, the adult induction of the Tsc2^{+/-} mutation in microglia failed to induce OPR deficits in male Cx3cr1^{CreER}-Tsc2^{Flox} mice (Fig.4D), contrary to what happened when the Tsc2^{+/-} mutation was induced in microglia during development, a result that suggests that the Tsc2^{+/-} mutation affects microglia during development.

Microglia depletion reverses the abnormal LTP of male Tsc2^{+/-} mice.

Previous studies suggested that abnormal hippocampal CA1 LTP may underlie the spatial memory deficits of Tsc2^{+/-} mice (9, 13). The results presented above demonstrated that microglia play a critical role in the OPR deficits of male Tsc2^{+/-} mice (Fig.3C and 3D; fig.S3B and S3C). To test if microglia is also responsible of the abnormal LTP of Tsc2^{+/-} mice, we treated adult male Tsc2^{+/-} Ep mice with PLX5622 or control chow and four months later, we tested their LTP (Fig.5A). We tested Tsc2^{+/-} Ep mice, and not Tsc2^{+/-} mice, because of the hippocampal memory deficits of the Tsc2^{+/-} Ep mice (they also include social memory deficits) are more severe than those of the Tsc2^{+/-} mice (23). Remarkably, the results show that the depletion of microglia rescued the abnormal LTP of male Tsc2^{+/-} Ep mice (Fig.5B-5D). These results demonstrate that microglia have a critical role in the abnormal LTP of Tsc2^{+/-} mice, and suggest that this may contribute to their spatial learning and memory deficits.

Normal short-term memory for OPR in Tsc2^{+/-} male mice

Prior results (9), as well as the LTP experiments described above (Fig.5B-5D) revealed that Tsc2^{+/-} mice (with or without early-post-natal immune activation) have normal LTP tested 60 minutes post-induction, although LTP tested 3 hours post-induction, was abnormal. In the experiments described above (Fig.1), OPR memory was tested 24-hours after training. Additionally, TSC2 affects mTOR signaling (28, 29), and this signaling pathway has been implicated in memory consolidation (37). Thus, we next tested whether adult Tsc2^{+/-} male mice have deficits in OPR at a time both prior to memory consolidation (60 min after OPR training), and when their LTP is normal (Fig.5B-5D). Consistent with the idea that abnormal LTP may contribute to the OPR memory deficits of the Tsc2^{+/-} male mice, we found that these mutant mice show normal OPR

when tested 60 min after training (Fig.6B). When tested 24 hours after training, the Tsc2^{+/-} male mice once again revealed OPR deficits (Fig.6B). These results support the hypothesis that the abnormal LTP of the Tsc2^{+/-} mice contributes to their OPR memory deficits.

The interferon-alpha/beta receptor alpha chain KO (IFNAR1^{-/-}) prevents the OPR deficits in male Tsc2^{+/-} mice.

In our previous work, we determined that type I-IFN plays a critical role in the social memory deficits of male Tsc2^{+/-} mice (33). Here, we determined whether type I-IFN signaling also has a role on the OPR deficits of male Tsc2^{+/-} mice (Fig.7A). Remarkably, although male Tsc2^{+/-} mice showed robust OPR deficits (Fig.7B; as we observed in Fig.1B), male Tsc2^{+/-} mice with a null mutation for the interferon-alpha/beta receptor alpha chain gene (Tsc2^{+/-}/IFNAR1^{-/-}) showed normal OPR (Fig.7B). These results demonstrate the role of type I IFN signaling in the OPR deficits of male Tsc2^{+/-} mice.

Discussion

Although previous studies focused on the role of neuronal deficits in the memory phenotypes of rodent models of TSC (14, 15), the results presented here demonstrate a role for microglia in these memory deficits. In addition to rescuing their OPR deficits, we show that depletion of microglia also reversed the abnormal LTP of the Tsc2^{+/-} mice.

TSC-associated neuropsychiatric disorders, include autism, intellectual disability, and a range of other psychiatric and behavioral symptoms (3-9, 38). Additionally, epilepsy affects up to 80% of TSC patients, with severe seizures that can be treatment resistant, and that could contribute to their behavioral abnormalities (39). While neuronal mechanisms have played a key role in explanations of autism and intellectual disability phenotypes of TSC patients (14, 15), glia have been increasingly recognized to play important roles in TSC (40). For example, there is growing evidence for the role of astrocytes, microglia, and oligodendrocytes in the pathophysiology of epilepsy in TSC (40). Our previous results (33), including those presented here, also suggest that glial cells, particularly microglia, have a role in the cognitive phenotypes of TSC, and that targeting glial mechanisms could be a viable therapeutic approach for addressing their cognitive deficits.

Pathology studies of tubers dissected from TSC epileptic patients revealed evidence of microglial activation (41, 42). However, seizures are common in TSC patients, and themselves may cause microglia activation (43), thus making it difficult to determine whether microglia activation is a consequence of other pathological causes in TSC, or a contributing cause for TSC phenotypes. For example, previous studies showed that mice with an astrocyte specific *Tsc1* mutation showed evidence of microglia activation, as well as increased microglia size and number (44). The results presented here make a strong case that abnormalities in microglia may not simply be a consequence of changes in other cell types involved in the pathophysiology of TSC, such as astrocytes and neurons, since we showed that the *Tsc2*^{+/-} mutation restricted to microglia recreated the hippocampal memory deficits associated with animal models of TSC (7, 23, 45-47). Additionally, we also showed that the hippocampal CA1 LTP abnormalities and OPR deficits of the *Tsc2*^{+/-} mice could be reversed by a manipulation that depleted most microglia from the brain of these mice. Importantly, we demonstrated that a null mutation (*IFNAR1*^{-/-}) of a key signaling pathway in microglia (48) also prevented the hippocampal-dependent memory deficits of these mice. Importantly, previous RNA sequencing studies in ASD patients (49) pointed to a lasting upregulation of microglia and interferon response pathways, suggesting that our findings with an animal model of TSC may be more generally significant.

All together the results presented here demonstrate that microglia and type I interferon signaling have a key role in the electrophysiological and memory phenotypes of an animal model of TSC, a result consistent with the hypothesis that abnormal type I-IFN signaling in microglia is responsible for abnormal LTP and for hippocampal memory deficits in *Tsc2*^{+/-} mice. Importantly, these phenotypes can be reversed by depletion of microglia, a result that suggests that therapeutical strategies targeting this cell type may be a viable strategy to address cognitive impairments in TSC.

Materials and Methods

The Chancellor's Animal Research Committee at the University of California, Los Angeles, approved the research protocols used here.

Experimental Design and subject details.

1) $Tsc2^{+/-}$ mice. We first crossed male $Tsc2^{+/-}$ mice (28) with female wild type (WT) mice. $Tsc2^{+/-}$ male breeders were on a C57BL/6NcrJ genetic background (Charles River Laboratories, Cat.#: 027). We used C57BL/6J females (JAX, Cat.#: 000664) to generate experimental mice. Pregnant females were single housed and left undisturbed except for weekly cage changes. Pregnancy was determined by checking for abdominal distension. For all of the mice studied here, pregnant females were checked every day to determine the exact day when the pups were born (designated P0). Tail biopsies for genotyping were taken around P40.

2) $IFNAR1^{-/-}$ // $Tsc2^{+/-}$ mice. To test the importance of type I interferon α and β (IFN α , β) for the phenotype we observed in male $Tsc2^{+/-}$ mice, we generated mice that included both the $Tsc2^{+/-}$ and $IFNAR1^{-/-}$ null mutations by crossing $Tsc2^{+/-}$ male breeders with $IFNAR1^{-/-}$ females (bred on a C57BL/6J background) followed by intercrosses. Tail biopsies for genotyping were taken around P40.

3) $Cx3cr1^{Cre}$ - $Tsc2^{Flox}$ mice. To confirm whether microglia play a critical role in the hippocampal-dependent memory deficits that male $Tsc2^{+/-}$ mice show, we crossed $Cx3cr1^{Cre}$ mice (JAX stock #025524) with $Tsc2^{Flox}$ mice (JAX stock #027458) to generate mice that carry the $Tsc2$ mutation only in microglia. Tail biopsies for genotyping were taken around P40.

4) $Cx3cr1^{CreER}$ - $Tsc2^{Flox}$ mice. We used this mouse model to test whether the heterozygous deletion of the $Tsc2$ gene specifically in adult microglia can trigger spatial memory deficits in adult male mice. We also used those mice to determine if this $Tsc2$ gene deletion in adult microglia can create a window of vulnerability where a post-natal immune activation could trigger spatial memory deficits in female $Tsc2^{+/-}$ mice. We crossed $Cx3Cr1^{CreER}$ mice expressing tamoxifen-inducible Cre recombinase (JAX stock # 021160) with $Tsc2^{Flox}$ mice (JAX stock #027458) to

generate mice that carry the $Tsc2^{+/-}$ mutation only in microglia of adults. The experimental mice were injected intraperitoneally with 4-Hydroxytamoxifen (4-OHT) (Sigma Aldrich; Cat.#:H6278-50mg; 75mg/kg) once per day during 4 consecutive days. Tail biopsies for genotyping were taken around P40.

Method details.

1) Object Place Recognition test (OPR).

Prior to the test, mice were handled for eight minutes daily for 6 consecutive days. Then, in the next two days, they were habituated in the OPR open field (41.5 x 41.5 x 40.5 cm) 2 days for 12 minutes. During the training session, mice were placed back in the OPR open-field, this time with 2 identical objects, and they were allowed to explore freely for 7 minutes. After either a 1 hour or a 24h interval, mice were tested for OPR with the previously presented objects, one of them in a new location. The object on the new location during test was counterbalanced between trials. The open-field was cleaned after each session. Sessions were video recorded and scored offline by either one or two observers, as described above. The variation between observers was normally less than 2 seconds in the 7 minutes exploration sessions. Exploration was counted only when the test mouse touched with its nose one of the objects. All experiments and scoring were carried out blind to genotype and treatment condition. The objects included in this study were small bottles or containers of different shapes.

2) Novel Object Recognition test (NOR).

The Novel Object Recognition test (NOR) was carried out as described previously (22), twenty-four hours after OPR. During the training session, mice were placed in the NOR open-field (same as the OPR open field), with 2 identical objects, and they were allowed to explore freely for 7 minutes. After a 24h interval, mice were tested for object memory with the previously presented object and a new object. Location of the new object during the test was counterbalanced between trials. Mice were trained or tested once per day. The open-field was cleaned after each session. Sessions were video recorded and scored offline as described above. The variation between observers was normally less than 2 seconds in the 7 minutes exploration sessions. Exploration was counted only when the test mouse touched with its nose the objects. All experiments and scoring

were carried out blind to genotype and treatment condition. The objects included in this study were small bottles or containers of different shapes.

3) Poly I:C administration.

Poly I:C potassium salt (Sigma; Cat.#: P9582-50MG; Poly I:C is supplied at 10% of the total weight of the salt; dosage was based on the weight of poly I:C itself) was dissolved in vehicle solution (0.9% sterile saline) before use. Tsc2^{+/-} mice were injected intraperitoneally with Poly I:C (20mg/kg) or vehicle at P3, P7 and P14.

4) Rapamycin treatment

Rapamycin (5 mg/kg; LC Laboratories, Cat.#: R-5000) was freshly dissolved in vehicle solution (100% DMSO; Sigma-Aldrich, Cat.#: D5879-500ML) before use. Adults (4-6 months) Tsc2^{+/-} mice were treated with a single intraperitoneal injection of rapamycin (5 mg/kg) or vehicle (DMSO) daily for 5 days prior to the OPR test. Mice were tested for OPR 18h after the last injection of rapamycin or DMSO as well as 2 months later.

5) PLX5622 treatment

PLX5622 and control rodent diet was provided by Plexxikon Inc. and formulated in AIN-76A standard chow by Research Diets. Adult (4-6 months old) Tsc2^{+/-} mice were fed with PLX5622 (1200mg/kg; Cat.#: D11100404i) or control chow (Cat.#: D10001i) for 21 days (50). At day 21 of PLX treatment, mice were tested for OPR test. Mice were tested again in the OPR test, 2 months after the last day of PLX treatment.

6) 4-Hydroxytamoxifen (4-OHT) treatment

A solution of 4-Hydroxytamoxifen was prepared using pure Ethanol (Sigma Aldrich; Cat.#:E7023-1L) and corn oil (Sigma Aldrich; Cat.#: C8267-500ML). First, 4-OHT (125mg/ml; Sigma Aldrich, Cat.#: H6278-50mg) was added to ethanol (100%; pure) and incubated at 55 °C with vortexing for 15 min. Then, corn oil was added to a final concentration of 4-OHT of 12.5 mg/ml. Adult experimental mice were injected intraperitoneally with either 75mg/kg 4-OHT or vehicle once a day for 4 consecutive days.

7) Immunohistochemistry

Immunohistochemistry was performed as described earlier (51). Briefly, mice were perfused transcardially with a fixative containing 4% paraformaldehyde, and cryoprotected with 30% sucrose. Coronal and sagittal brain sections 60 μm thick were incubated overnight at 4°C with monoclonal rabbit anti-Iba1 (1:1000 dilutions; Wako Chemicals, Cat.#: 019-19741). The sections were then incubated for 90 minutes with Alexa Fluor 488 goat anti-rabbit IgG (1:500 dilutions; Invitrogen, Cat.#: A11011). After that, the sections were incubated in DAPI (1:1000 dilutions) for 15 minutes and then in PBS for 14 minutes. The immunofluorescence labeling was detected with a confocal microscope.

8) Hippocampal slice physiology

Coronal hippocampal slices (350 μm in thickness) were freshly made with a Leica VT-1000 vibratome in ice-cold cutting solution containing: 7mM MgSO_4 , 1mM CaCl_2 , 2.5 mM KCl, 26mM NaHCO_3 , 1mM NaH_2PO_4 , 30mM Glucose, 1.3mM sodium L-ascorbate, 1 mM kynurenic acid, 3mM sodium pyruvate, and 119mM choline chloride. Slices were recovered in a solution, consisting of 85 mM NaCl, 2.5 mM KCl, 4 mM MgCl_2 , 0.5 mM CaCl_2 , 1.25 mM NaH_2PO_4 , 24 mM NaHCO_3 , 25 mM glucose and 50 mM sucrose, for at least 1 h at room temperature. Slices were then transferred to the submerged recording chamber and continuously perfused with artificial cerebrospinal fluid (ACSF, 30 °C) at a rate of ~3 ml/min. ACSF contains (in mM) 120 NaCl, 3.5 KCl, 1.3 MgCl_2 , 2.5 CaCl_2 , 1.25 NaH_2PO_4 , 26 NaHCO_3 and 10 D-glucose. All solutions (PH 7.2~7.4, Osmo 290~310) were oxygenated with 95% O_2 / 5% CO_2 mixture gas.

Field excitatory postsynaptic potentials (fEPSPs) in the Schaffer collateral-CA1 (SC-CA1) in the dorsal hippocampus were evoked with a FHC bipolar platinum microelectrode (Ehninger et al., 2008). The input-output (I/O) curve was constructed by varying stimuli intensity (10~100 μA), while measuring the corresponding pre-synaptic volley and the initial fEPSPs slope. Long-term potentiation (LTP) of SC-CA1 synapses was induced by a 100Hz (1s) tetanus. All stimulation pulses were 100 μs in duration and at 1/3~1/2 of the intensity that induces a maximal fEPSP response. Data were obtained using MultiClamp 700B amplifier equipped with a digidata 1440A interface and pCLAMP 10.0 software (Axon Instruments). Digitalized signals were sampled at 10 kHz and filtered at 2 kHz. Initial fEPSPs slopes after tetanic stimulation were normalized to the

average baseline fEPSP slopes. For statistical analyses, two-way repeated measure ANOVAs and a Sidak's multiple comparisons test were used on the average of the first 5 min and the last 20 min of recording after LTP induction.

Statistical Analysis

Mouse behavioral data are presented as mean $\pm$ SEM as well as individual data. For behavioral experiments, statistics presented in the figures were based on Student's t-tests. The data was also analyzed using two-way ANOVA plus Holm-Sidak post-hoc analyses with identical results. $P < 0.05$ was considered significant. $P > 0.05$, $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$ were denoted as n.s. (non-significant), *, **, ***, ****, respectively. GraphPad Prism 7 software was used to perform statistical analyses and for generating graphical representations of the data.

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Data and materials availability: PLX5622 was obtained under a material transfer agreement with Plexxikon. All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

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Figures

Fig. 1. Male but not female Tsc2^{+/-} mice show OPR deficits.

(A) OPR outline. (B) Graph shows the % of time that mice spent actively exploring the objects in the novel and familiar locations. WT/male (n=14; $P < 0.0001$, $t = 7.81$), WT/female (n=15; $P < 0.0001$, $t = 6.35$) and Tsc2^{+/-}/female (n=8; $P < 0.0001$, $t = 7.59$), show normal OPR (they explore significantly more the object in the novel location than the object in the familiar location) but Tsc2^{+/-}/male (n=16; $P = 0.25$, $t = 1.16$) mice show OPR deficits (show no preference for the object in the novel location). Data represent means $\pm$ SEM as well as individual data.

Fig. 2. Effects of rapamycin on the OPR deficits of male Tsc2^{+/-} mice.

(A) Outline of the treatment with rapamycin (Rap) or DMSO and behavior approach. (B) Tsc2^{+/-} male mice before rapamycin (n=13; P=0.11, t=1.64) treatment show OPR deficits. Tsc2^{+/-} mice during rapamycin treatment (n=12; P<0.0001, t=8.90) show normal OPR. In contrast, Tsc2^{+/-} mice 2-months after rapamycin (n=9; P=0.58, t=0.55) show again OPR deficits. Data represent means ± SEM as well as individual data points.

Fig. 3. Evidence for a critical role of microglia in the OPR memory deficits of male *Tsc2*^{+/-} mice.

(A) Outline of the treatment with PLX5622 (PLX; depletes microglia) or Control chow and behavior approach. (B) IBA1 immuno staining of *Tsc2*^{+/-} Control, PLX-mice and mice 2 months after PLX. The treatment with PLX5622 led to the elimination of microglia in the whole brain (Hippocampus shown as example) compared to the control group (Hippocampus shown as

example). Two months after PLX the microglia had repopulated the brain (Hippocampus shown as example). **(C)** $Tsc2^{+/-}$ /PLX mice ($n=3$; $P<0.01$, $t=7.59$), but not $Tsc2^{+/-}$ /Control ($n=10$; $P=0.73$, $t=0.33$) mice, show normal OPR memory. **(D)** $Tsc2^{+/-}$ mice two months after PLX ($n=5$; $P<0.001$, $t=5.92$), but not $Tsc2^{+/-}$ mice 2 months after Control ($n=3$; $P=0.76$, $t=0.31$), show normal OPR memory. Data represent mean $\pm$ SEM as well as individual data. Bar: 200 μ m.

A

B

C

D

Fig. 4. The Tsc2 mutation restricted to microglia during development (but not in adults mice) leads to OPR memory deficits.

(A) Outline of Behavior approach for Cx3cr1^{Cre} / Tsc2^{Flox}. (B) Male WT (n=7; P<0.05, t=2.69) show normal OPR. In contrast, Cx3cr1^{Cre} / Tsc2^{Flox} (Tsc2^{+/-}) (n=11; P=0.76, t=0.3) show deficits in OPR. (C) Time line for injections of 4-Hydroxyramoxifen or vehicle and behavior approach. (D) Male Cx3cr1^{CreER}/Tsc2^{Flox}/vehicle mice (n=5; P<0.01, t=4.8) and male Cx3cr1^{CreER}/Tsc2^{Flox}/4-OHT mice (n=4; P<0.01, t=3.85), show normal OPR.

Data represent means $\pm$ SEM as well as values for individual mice. As indicated, in (B), Tsc2^{+/-} represents Cx3cr1^{Cre}/Tsc2^{Flox} and in (D), Tsc2^{+/-} represents Cx3cr1^{CreER}/Tsc2^{Flox}.

Fig. 5. Effects of microglia depletion in CA1 LTP of male *Tsc2*^{+/-} Ep mice.

(A) Outline of Poly I:C injections, treatment with PLX5622 and analysis approach. (B) Initial fEPSP slopes recorded in the CA1 region of hippocampal slices are shown before (baseline, 15 min) and following LTP induction (with a 1-s, 100-Hz tetanus delivered at time 0) for the tetanized pathway and for a separate, untetanized pathway (control). Data are plotted in 1-min blocks and fEPSP slopes are normalized to the average baseline response. Sample traces show responses (5 responses were averaged) during baseline (grey) and during the last 5 min of recordings (color). Scale bars, 1 mV and 10 ms. (C) Averaged fEPSPs during the last 20 min of LTP recording. Regular two-way ANOVA, pathway $\times$ genotype interaction: $F_{(2,54)} = 10.51$, $p < 0.001$; Sidak's multiple comparisons for tetanized vs. control pathway fEPSPs, **** $p < 0.0001$ for *Tsc2*^{+/-}; VEH group, not significant (ns) for other two groups; Tukey's multiple comparisons for tetanized pathway fEPSPs, ** $p < 0.01$ for *Tsc2*^{+/-}; PLX group vs. *Tsc2*^{+/-}; VEH group. (D) Averaged post-tetanus fEPSPs (5 min, PTP) recorded immediately after a 100-Hz tetanus. Regular two-way ANOVA, pathway factor $F_{(1,54)} = 105.1$, $P < 0.001$, genotype factor $F_{(2,54)} = 1.488$, $p > 0.05$; Sidak's multiple comparisons for tetanized vs. control pathway, **** $p < 0.0001$ for all three groups. *Tsc2*^{+/-}; VEH slices, n=10 from 6 mice. *Tsc2*^{+/-}; PLX slices, n=11 from 7 mice. WT; PLX slices, n = 9 slices from 5 mice. All data are shown as means $\pm$ SEM.

Fig. 6. Male Tsc2^{+/-} mice show normal OPR memory 60 min after training.

(A) Outline of behavior approach. (B) Graph shows the % of time the mice spent actively exploring the objects in the novel and familiar locations. Tsc2^{+/-}/60 min (n=10; P<0.0001, t=6.97) show normal OPR (they explore significantly more the object in the novel location than the object in the familiar location) but Tsc2^{+/-}/24h (n=10; P=0.73, t=0.33) mice show OPR deficits (show no preference for the object in the novel location). Data represent means $\pm$ SEM as well as individual data.

A

B

Fig. 7. Effects of the null homozygous mutation of type I IFN receptor (IFNAR1^{-/-}) in Tsc2^{+/-} OPR deficits.

(A) Behavior approach. (B) Tsc2^{+/-} (n=9; P=2.24, t=1.19) show OPR deficits. WT (n=6; P<0.001, t=5.71), IFNAR1^{-/-} (n=6; P<0.0001, t=6.34) and Tsc2^{+/-}/IFNAR1^{-/-} (n=8; P<0.0001, t=6.78) show normal OPR. Data represent means ± SEM as well as values for individual mice.