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Interleukin-15 alters hippocampal synaptic transmission and impairs episodic memory formation in mice

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Abstract

Cytokines are potent immunomodulators exerting pleiotropic effects in the central nervous system (CNS). They influence neuronal functions and circuit activities with effects on memory processes and behaviors. Here, we unravel a neuromodulatory activity of interleukin-15 (IL-15) in mouse brain. Acute exposure of hippocampal slices to IL-15 enhances gamma-aminobutyric acid (GABA) release and reduces glutamatergic currents, while chronic treatment with IL-15 increases the frequency of hippocampal miniature inhibitory synaptic transmission and impairs memory formation in the novel object recognition (NOR) test. Moreover, we describe that serotonin is involved in mediating the hippocampal effects of IL-15, because a selective 5-HT_3A receptor antagonist prevents the effects on inhibitory neurotransmission and ameliorates mice performance in the NOR test. These findings provide new insights into the modulatory activities of cytokines in the CNS, with implications on behavior.

Key words

IL-15, synaptic transmission, behavior, microglia, cytokine-CNS interplay

1. Introduction

Cytokines are one superfamily of proteins traditionally seen as belonging to the immune system, where they regulate the innate and adaptive immune functions in physiological conditions and upon pathological insults (Vezzani and Viviani, 2015; Van der Meide et al., 1996; Belardelli, 1995). In addition to this classical view, several pieces of evidence demonstrated that cytokines are also released by different cells in the brain, including neurons, astrocytes, endothelial cells and microglia, shaping CNS functions and exerting pivotal roles in the neuroimmune crosstalk (Bezzi et al., 2001; Beattie et al., 2002; Prinz and Priller, 2017; Alves de Lima et al., 2020). The interactions between cytokines and the CNS have been mostly studied in the context of neuroinflammation (Viviani et al., 2003; Czerniawski, 2015; Choi et al., 2016; Filiano et al., 2016; Habbas et al., 2015; Shin Yim et al., 2017; Di Filippo et al., 2021). However, emerging studies reveal additional roles in shaping neuronal plasticity and behavior under non-pathological conditions (Brombacher et al., 2017; Herz et al., 2021; Matejuk et al., 2021; Wang et al., 2021; Li et al., 2023).

The cytokine interleukin-15 (IL-15) is considered a bridge between the adaptive and the innate immune system (Pan et al., 2013; Jabri et al., 2015). IL-15 belongs to the interleukin-2 (IL-2) family cytokines, and interacts with a heterotrimeric receptor that consists of a specific high-affinity IL-15-binding alpha subunit (IL-15Rα) associated with a β chain (shared with IL-2) and a common γ chain (γ_c, shared with IL-2, IL-4, IL-7, IL-9, and IL-21) signaling complex, leading to the recruitment of Janus kinase (JAK) JAK1 by the β chain and activation of JAK3 constitutively associated with the γ chain (Perera et al., 2012; Kennedy et al., 1996). Activated JAK1 and JAK3 phosphorylate signal transducer and activator of transcription (STAT) proteins STAT3 and STAT5 respectively, to mediate IL-15 effects in T lymphocytes. The differential expression of these cytokines and the alpha chains of their receptors within various tissues and cell types suggests that IL-2 and IL-15 perform distinct functions. IL-15 can be trans-presented by IL-15Rα to adjacent cells that express IL-2/IL-15Rβγ complex, with no need of the heterotrimeric receptor complex (Schluns et al., 2005). IL-15 is also able to bind to the intermediate affinity IL-2/IL-15Rβγ_c signaling complex without the requirement for the IL-15Rα high affinity binding protein, and signal through the recruitment of other non-receptor tyrosine kinases such as Lck, Fyn, Lyn, Syk and cross talk with signaling proteins of the PI3K and MAPK pathways (Giri et al., 1994; Dubois et al., 2002; Nandi et al., 2021). IL-15 has pleiotropic effects and promotes the survival, proliferation, and cytotoxicity of NK cells and CD8+ T cells (Jarjour et al., 2022; Lodolce et al., 2002; Geginat and Granucci, 2023; Madi et al., 2023; Ma et al., 2022). In the CNS, IL-15 and its receptors are expressed by both glial cells and neurons, and show developmental changes and regional differentiation (Hanisch et al., 1997; Schluns et al., 2005).

In several pathological conditions, IL-15 orchestrates the immune response and neuronal and glial functions. High levels of IL-15 are reported in the serum and cerebrospinal fluid of multiple sclerosis patients and in the CNS of experimental autoimmune encephalomyelitis (EAE) mouse models, where IL-15 contributes to the amplification of T-cell inflammatory properties (Laurent et al., 2020; Rentzos et al., 2006). In brain tumors, IL-15 has an antitumoral effect, with particular focus on NK cells as crucial components of innate immunity (Ma et al., 2022). In a syngeneic glioma mouse model, microglial-derived IL-15 increases the infiltration of NK cells in the tumor mass and reduces the tumor volume, enhancing the mice survival (Garofalo et al., 2015, 2017; Mormino et al., 2021). Moreover, IL-15 is a mediator of the crosstalk between astrocytes and microglia, and astrocyte-derived IL-15 exacerbates brain injury following intracerebral hemorrhage (Shi et al., 2020), aggravating postischemic brain damage via the propagation of CD8+ T and NK cell-mediated immunity (Li et al., 2017).

Interestingly, in previous studies, it has been shown that IL-15 or IL-15Rα deletion affects behaviour and neurotransmitters levels, suggesting a major role for this signaling in physiological brain functions (Pan et al., 2013; He et al., 2010; Nguyen et al., 2017). In particular, IL-15Rα KO mice show decreased retention of spatial memory and contextual fear, and a reduction in the hippocampal GABA concentration. Interestingly, the alterations in GABA levels are associated with an increase in the number of interneurons in the stratum oriens, increased expression of GABA-synthesizing enzyme glutamic acid decarboxylase (GAD-65) in the hippocampus, and increased GABA uptake in synaptosomal preparations, suggesting effects of IL-15Rα deletion on GABA turnover (He et al., 2010).

Here, we investigated the effects of IL-15 treatment on the synaptic activities of hippocampal CA1 pyramidal neurons at steady state. We found that acute IL-15 treatment of hippocampal brain slices enhances the probability of GABA release while miniature glutamatergic current amplitudes are reduced. The effect on inhibitory transmission is reproduced in vivo in IL-15-treated mice. Interestingly, the IL-15-induced synaptic alterations are associated with impairment in episodic memory formation, as assessed by the novel object recognition (NOR) task. Notably, the antagonist of 5-HT_3A receptors prevents IL-15-dependent inhibition and restores performance in the NOR test, indicating the involvement of the serotonin neuromodulatory pathway in mediating IL-15 effects. Overall, these data indicate that perturbation of IL-15- signaling increases GABAergic neurotransmission with impairment of memory, unraveling a new role for IL-15 in regulating CNS functions.

2. Methods

2.1 Animals

Procedures using laboratory animals were in accordance with the Italian and European guidelines and were approved by the Italian Ministry of Health (protocol n. D117C.N.ASY) in accordance with the guidelines on the ethical use of animals from the European Communities Council Directive of September 20, 2010 (2010/63/UE). Wild-type mice (C57BL/6J background) were either bred in-house or were purchased from the Jackson Laboratory (JAX 000664). Mice were maintained in the animal facility for at least one week prior to the start of any experiment. All procedures were performed at 6-8 weeks of age. Only male mice were used in all experimental procedures.

All efforts were made to minimize suffering and the number of animals used. Mice were housed in standard breeding cages at constant temperature (22 ± 1◦C) and relative humidity (50%), with a 12:12 h light:dark cycle (light on 07.00–19.00 h). Food and water were available *ad libitum*.

2.2 Hippocampal slices preparation

Transverse acute hippocampal slices were obtained from 6-8 weeks old mice. Animals were decapitated under halothane anesthesia. Whole brains were rapidly removed from the skull and immersed in an ice-cold artificial cerebrospinal fluid (ACSF) solution containing (in mM): 87 NaCl, 75 Sucrose, 2 KCl, 7 MgCl2, 0.5 CaCl2, 25 NaHCO3, 1.2 NaH2PO4 and 10 glucose, pH 7.3, 300–305 mOsm. The ACSF was continuously oxygenated with 95% O2 and 5% CO2

to maintain the physiological pH. Transverse 300 µm thick slices were cut at 4°C using a Vibratome (ThermoScientific HM 650 V) and placed in a chamber filled with oxygenated ACSF containing (in mM): 125 NaCl, 2 KCl, 1.2 MgCl2, 2 CaCl2, 25 NaHCO3, 1.2 NaH2PO4 and 10 glucose, pH 7.3, 300–305 mOms.

2.3 Single-cell Real Time PCR analysis

For single-cell RT-PCR experiments, acute hippocampal slices were prepared as described in the previous section, except for the use of autoclaved bidistilled water, autoclaved glassware and glass pipette for patching. Precautions were taken throughout the study to ensure a 'ribonuclease-free environment' according to previous reports (Durand et al., 2006; Devienne et al., 2018).

For cell harvest, the slices were placed in a recording chamber and perfused with standard ACSF. Cells were visualized using an upright microscope (Leica DM-LFS) equipped with a water immersion 40× objective (Leica) and a digital DCC camera (C8484, Hamamatsu). Patch clamp recordings were performed by using a Multiclamp 700B amplifier (Molecular Devices, USA). Signals were acquired (sampling 10 kHz, low-pass filtered at 2 kHz) with DigiData-1440A using pCLAMP-v10 software (Molecular Devices, USA). Patch clamp recordings were made using an intracellular solution containing (in mM):135 KCl, 5 NaCl, 5 BAPTA, 2 MgCl2, 10 HEPES, 2 Mg-ATP and 0.3 NaGTP (pH 7.35 adjusted with KOH, osmolarity 290-295 mOsm).

To collect cellular contents, the cell cytoplasm was harvested through the patch pipette after electrophysiological characterization of passive membrane properties of the cells such as resting membrane potential, cell capacitance and voltage responses to incremental current injections. The cytoplasm was extracted from the cell by applying suction to the back of the recording pipette. Immediately following harvesting, the contents of an individual cell were expelled from the patch electrode by breaking its tip into a PCR thin-layer tube containing 8.5 µl of reverse transcription mixture. The cDNA was synthesized using the iScript Advanced cDNA synthesis kit for RT-PCR (Bio-Rad #1725038) according to the manufacturer's instructions, under the following conditions: incubation at 46°C for 20 min, then the enzyme was inactivated at 95°C for 1 min. For the amplification of the cDNA products, a nested PCR amplification was performed, using SsoAdvanced PreAmp Supermix (Bio-Rad #172-5160) with specific primers. The first preamplification step consisted of 12 cycles of denaturation at 95 °C for 3 min and annealing/extension at 58 °C for 4 min.

The amplification product was diluted 1:5 and the PCR protocol consisted of 40 cycles of denaturation at 95 °C for 30 s and annealing/extension at 60 °C for 30 s. The Ct values from each gene were normalized to the Ct value of *Gapdh* in the same RNA samples. Relative quantification was performed using the $2^{\Delta DDCt}$ method and expressed as fold change in arbitrary values. For negative controls, amplifications were performed using water as PCR template or omitting the reverse transcriptase step in the first strand synthesis reaction. For single-cell PCR, reactions were also performed on the content of patch pipettes dipped into the solution bathing the cells, to rule out contaminations from extracellular material. Of four samples tested, none was found to be positive.

2.4 Patch clamp recordings of CA1 pyramidal neurons from ex vivo hippocampal brain slices

After cutting, brain slices were allowed to recover at least for 1 h before recording at room temperature, then transferred to a recording chamber within 1–6 h after slice preparation. All recordings were performed at room temperature on slices submerged in ACSF and perfused with the same solution in the recording chamber at a rate of approximately 2 ml/min by using a gravity-driven perfusion system.

Spontaneous (sIPSCs, sEPSCs), miniature (mIPSCs, mEPSCs) and evoked inhibitory and excitatory postsynaptic currents (eIPSCs, eEPSCs) were recorded from CA1 pyramidal neurons using the patch clamp technique in whole-cell configuration, which allowed recording currents through multiple channels simultaneously, over the membrane of the entire cell. Patch clamp recordings were performed by using a Multiclamp 700B amplifier (Molecular Devices, USA). Signals were acquired (sampling 10 kHz, low-pass filtered 2 kHz) with DigiData-1440A using pCLAMP-v10 software (Molecular Devices, USA); the analysis was performed off-line using Clampfit 10 (Molecular Devices) and MiniAnalysis (Mini Analysis, Synaptosoft Fort Lee, NJ, USA). Series resistance (Rs) was not compensated during voltage clamp experiments to avoid increased electrical noise in the trace. Rs was constantly monitored over time and recordings in which it changed more than 20% were discarded.

Glass electrodes (3–5 MΩ) were pulled with a vertical puller (PC-10, Narishige). Pipette were filled with 148 mM Cs Methanesulfonate, 10 mM Hepes, 0.5 mM EGTA, and 2 mM Mg-ATP, Na3-GTP 0.3 mM, MgCl2 2 mM (295–300 mOsm, pH 7.2).

GABAergic membrane currents were recorded with the neuron clamped at 0 mV. At this voltage, Cl− mediated inhibitory events are outward currents (estimated ECl = − 80 mV) whereas excitatory currents are inward but of small amplitude as they would occur close to their reversal potential. Although it was possible to isolate sIPSCs pharmacologically, by using 20 μM DNQX plus 10 μM AP-5 to block both the AMPA and NMDA receptor components of sEPSCs, this antagonist mixture sometimes attenuated or occasionally completely blocked sIPSCs. This presumably reflected impediment of excitatory synaptic drive to the inhibitory interneurons that were responsible for sIPSC generation. In view of this variable effect of DNQX/AP-5 on sIPSCs, we elected to use a holding potential of 0 mV rather than pharmacological methods to separate sIPSCs from sEPSCs. The validity of this approach is supported by the observation that 100μM picrotoxin (PTX) completely eliminated all spontaneous outward current activity recorded at 0 mV (data not shown). Miniature EPSCs/IPSCs were recorded during an initial 10 min baseline period, followed by application of tetrodotoxin, TTX (0.5μM, Tocris Bioscience, Bristol, United Kingdom) for 10 min. After stabilization of TTX effect, exogenous IL-15 (10nM, PeproTech EC Ltd., London, UK) was applied for 10 min. By using the same conditions, excitatory post-synaptic currents (EPSCs) were recorded clamping the cell at − 70 mV. In a subset of experiments, the glutamatergic nature of the mEPSC recordings was confirmed at the end of the experiment by total blockade of mEPSCs by DNQX (20 μM; data not shown).

For evoked post-synaptic currents, paired-pulse protocol and input/output curve, QX314-Br (2mM) was daily added to block voltage-gated Na^+ channels. A concentric bipolar stimulating electrode (SNE-100 × 50 mm long Elektronik-Harvard Apparatus GmbH, Crisel Instruments, Rome, Italy) was positioned in the stratum radiatum to evoke eIPSCs (holding the cell at 0 mV) or eEPSCs (Vh: -70 mV) from CA1 pyramidal neurons. For paired pulse experiments pairs of stimuli (interstimulus intervals, ISI, 25, 50, 100 and 700ms) were applied every 20 sec.

Stimulus intensity was about 50% of maximal amplitude, delivered through a A320R Isostim Stimulator/Isolator (WPI). The paired pulse ratio (PPR) of eIPSCs was calculated as the ratio between the amplitude evoked by the second stimulus (A2) over the first (A1; A2/A1) and the amplitude of each IPSC was measured relative to a 2 ms long baseline period starting 3 ms before stimulation. To measure the amplitude of the second peak the baseline was adjusted to zero. The stimulus intensity was adjusted accordingly to the experiment. For input/output curves, inhibitory fibers were stimulated by applying a single pulse at increasing intensities (0.1-10 mA). Each pulse of a given intensity was repeated 3 to 6 times to obtain an average response.

For current clamp recordings, micropipettes (4–5 MΩ) were usually filled with a solution containing the following composition (in mM): KCl 135, NaCl 5 BAPTA 5, MgCl2 2, HEPES 10, Mg-ATP 2 and NaGTP 0.3 (pH 7.35 adjusted with KOH, osmolarity 290-295 mOsm). Resting Membrane Potential (RMP) was measured as the voltage with no injected current. Membrane capacitance was estimated as the total charge (i.e., the current integral, Qstep) mobilized in each cell by a 10 mV hyperpolarizing step (Vstep): Qstep/Vstep. The somatic Rinput was measured from the steady-state current evoked by a 10 mV hyperpolarizing step injected from the holding potential of -70 mV. Relations between firing frequency and injected current were examined by measuring the number of action potentials elicited by somatic current injection steps incremented by 10 pA from RMP.

2.5 Analysis of miniature post-synaptic currents

Miniature excitatory post-synaptic currents (mEPSCs) were isolated by adding Tetrodotoxin (0.5 mM, TOCRIS) to ACSF in the perfusion line for at least 10 min before starting acquisition. Signals were acquired (sampling 10 kHz, low-pass filtered 2 kHz) with DigiData-1440A using pCLAMP-v10 software (Molecular Devices, USA). Analysis of mEPSCs and mIPSCs was performed offline using MiniAnalysis software (Mini Analysis, Synaptosoft Fort Lee, NJ, USA) with the threshold for detection set at 3 folds the RMS noise. Only data from the last 2 min of each recording epoch were analyzed to ensure that drugs had fully equilibrated.

Within a single cell, the values for mPSC inter-event-intervals (IEI), amplitudes, 10-90 rise times and t decay showed a non-Gaussian distribution. Therefore, the respective parameters were calculated as a median value per cell. However, as the median values for all cells within one group followed a Gaussian distribution, the population data are presented as mean ± SEM.

2.6 Mice treatment

Eight-week-old male C57BL/6J mice were randomly grouped for the treatments. For IL-15 (PeproTech EC Ltd., London, UK) infusion in the hippocampus, mice were anesthetized with a mixture of tiletamine and zolazepam (1:1, 40 mg/kg) and xylazine (10 mg/kg, Mediser, ITA), and a guide cannula was placed AP +2.3mm, ML +1.75mm, DV –1.75mm depth from the bregma, according to the Allen mouse brain atlas, and it was fixed with quick-setting cement. After 7 days, mice were infused via cannula with vehicle (PBS) or IL-15 (25 ng/ml in 3 µL PBS), every 2 days. IL-15 doses were selected to mimic those achieved in the *ex vivo* experiments. After 8 days behavioral test was performed. To study the involvement of serotonin receptor, the 5-HT_3R inhibitor (Granisetron hydrochloride – Tocris, 0.25µg/0.5µl)

was added to IL-15 solution during the last infusion in the mouse hippocampus, 1h before the behavioral test.

2.7 Novel object recognition (NOR) test

Experimental groups were blinded and randomly assigned before the start of behavioral experiments and remained blinded until all data were collected. Sample sizes were chosen on the basis of a power analysis using estimates from previously published experiments. Before the experiments, mice were transported to the behavior room and left for at least 30 min to habituate. This test analyzes the non-spatial working memory function. NOR test was conducted in an observation chamber (40 cm × 40 cm × 30 cm) with discriminated objects (A, B and C) identically sized in a dimly (30–40 lx) illuminated soundproof room. Stimulus objects were made of ceramic or plastic and varied in color, size, and shape. The role (familiar or novel) and the relative position of the objects was counterbalanced and randomly permuted for each mouse. All objects have been previously tested to avoid biased preference. The NOR test consists of two sessions: the training session and the test session (see **Fig.5 A**). A preceding 10 min of acclimatization to the experimental set-up was carried out to reduce the impact of anxiety and stress on the outcomes. The two identical objects (object A and B) were symmetrically fixed to the floor of the box. The training session was carried out by placing each mouse in the middle of the two objects, and each mouse was allowed to explore the objects for 10 min. After the training, the animals were immediately returned to their home cages, and the observation chamber and objects were cleaned with 70% ethanol to avoid innate odorant cues from previous animal during the training phase. An animal was considered to be exploring the object when its head was facing the object i.e., the distance between the head and object is approximately 1 cm or less, or when it was touching or sniffing the object. Test sessions were carried out 1h and 24 h after the habituation session. In the test session, object B used during the training session was replaced with a new object (C, novel to the mice). Mice were placed back in the box and allowed to freely explore objects A and C for 5 min, and the all behavioral parameters were calculated by software ANY MAZE 7.0.

The discrimination index (D.I.) was calculated as the ratio of the difference between the exploration time of the unfamiliar (UF) minus the familiar (F) object and the total exploration time: DI = (UF − F)/Total exploration time × 100 (Garofalo et al., Nat Comm. 2023).

2.8 PLX5622 treatment

Microglial depletion was achieved by administering the Plexxikon CSF1R inhibitor PLX5622 (1200 PPM added to AIN-76A chow, Research Diets) to C57BL/6N mice at least 7 days before experiments and its administration continued until the end of the experiments. The reduction of the microglial population, was confirmed by immunofluorescence staining against ionized calcium-binding adapter molecule 1 (IBA1, specific microglial/macrophages marker. After anesthesia, mice were intracardially perfused with phosphate buffered saline solution (PBS, Sigma) and 4% paraformaldehyde (PFA, Santa Cruz Biotechnology). Brains were then isolated and fixed in 4% formaldehyde and snap frozen. Cryostat sections (20 μm) were washed in PBS, blocked (3% goat serum in 0.3% Triton X-100) for 1 h at RT, and incubated overnight at 4°C with a specific antibody for IBA1 (1:500). After several washes, sections were stained with the fluorophore-conjugated antibody and Hoechst for nuclei visualization and analyzed using a

fluorescence microscope. Data are expressed as the percentage of reduction of the area occupied by fluorescent cells in PLX5622-treated mice versus control (by converting pixel to mm) (as reported in Garofalo et al., 2017, Corsi et al., 2022). We report that in the hippocampus the microglial depletion was 93.89 ± 2.76% complete.

2.9 Statistical analysis

All data are presented as mean ± SEM. Statistical analyses comprising the calculation of degrees of freedom were done using Sigma Plot 11.0 and Origin 8 (OriginLab Corporation, Northampton, Massachusetts, USA) software was used for the statistical analysis of electrophysiological data. Statistical significance was determined by paired t-test for electrophysiological experiments with acute application of IL-15. Unpaired t-test was used to compare electrophysiological parameters and behavioral outcome between VEH, IL-15 and Granisetron-IL-15 treated groups as indicated. Levels of significance were set as *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. In the legend, the number of cells and the number of animals are expressed together in the form n.cells/n.animals. Criteria for animal exclusion/inclusion were pre-established; animals considered for the analysis were selected for age. At weaning, pups from different colonies were mixed and mice were randomly treated. The investigators performing the different analyses always received the samples from a third laboratory member, who was not involved in that specific experiment, to ensure blinding to the group allocation.

3. Results

3.1 IL-15 acutely regulates synaptic transmission in the hippocampus

To investigate the effect of IL-15 on synaptic transmission, we tested the effect of an acute application (10 nM) on the miniature inhibitory post-synaptic currents (mIPSCs) recorded from CA1 pyramidal neurons (PC) under action potential block with tetrodotoxin (TTX, 0.5 mM). After 10 min of IL-15 perfusion, the mean frequency of mIPSCs is enhanced ($p = 0.031$) and the IEI is reduced ($p = 0.037$) while the mean amplitude of events is not modified ($p = 0.33$) (**Fig.1 A-C, Table 1**), indicating that IL-15 has a pre-synaptic effect on the inhibitory synapses onto PC cells. No changes in the rise time and decay time of the events are observed upon IL-15 ($p = 0.39$ and $p = 0.57$ respectively) while the decay time is increased during the wash out period, probably linked to a general increase of series resistance at the end of the experiments due to long lasting period of recordings ($p = 0.036$, **Table 1**).

Changes in the paired pulse ratio (PPR) are strongly correlated with modifications in the neurotransmitter release probability (Dobrunz and Stevens, 1997). To verify whether IL-15 modulates the probability (Pr) of GABA release, we measured the PPR of the inhibitory post-synaptic currents evoked in PC by extracellular stimulation with a bipolar electrode positioned in the stratum radiatum. We found that the PPR of the responses evoked by two consecutive stimuli (ISI: 50 ms) is reduced after IL-15 treatment and rescues to control values after wash ($p = 0.02$, **Fig.1 D-F, Table 1**) confirming that IL-15 acts by transiently enhancing the probability of GABA release.

We then investigated the modulation of acute IL-15 treatment (10 min, 10 nM) on the excitatory transmission in PC. Recordings of miniature excitatory post-synaptic currents (mEPSCs) reveal that the amplitude of events is reduced ($p = 0.022$, **Fig.2 A-B, Table 1**) while neither the mean frequency ($p = 0.62$) nor the IEI of mEPSCs are modified ($p = 0.44$, **Fig.2 C, Table 1**) by IL-15 treatment, suggesting post-synaptic alteration(s) at the excitatory synapses. Interestingly, while we found no changes in the rise time ($p = 0.66$, **Table 1**), the decay time of mEPSCs was significantly reduced in IL-15 treated slices compared to wash out condition, indicating modifications of AMPA receptor properties upon IL-15 treatment ($p = 0.008$, **Table 1**).

We then recorded the evoked eEPSC from PC by stimulating the Schaffer collaterals and we found that during paired pulse stimulation, the amplitude of both responses is decreased ($p = 0.05$ and $p = 0.03$ respectively), leaving the PPR unchanged ($p = 0.32$, **Table 1, Fig.2 D-F**). These results indicate that IL-15 acts acutely on excitatory synapses onto PC, decreasing the excitation through post-synaptic mechanisms.

Altogether these results indicate that IL-15 has a general inhibitory effect on hippocampal neurotransmission in mice hippocampus, increasing GABAergic neurotransmission and depressing glutamatergic currents.

3.2 IL-15 modulates inhibition in CA1 hippocampal area and impairs episodic memory in mice

To evaluate the contribution of IL-15 in the modulation of synaptic transmission in the hippocampus in vivo, we treated mice with vehicle or IL-15 in hippocampus via an intracerebral cannula. After 8 days of treatment, mice were sacrificed, and hippocampal brain

slices were harvested. Capacitance, input resistance, resting membrane potential along with firing properties are not different between the VEH- and IL-15-treated groups, indicating that the biophysical properties and the intrinsic excitability of PC cells are not affected by IL-15 treatment (**Fig.S2**). Interestingly, and in accordance with the data shown in **Fig.1**, the recordings of mIPSCs from PC showed an enhancement of the frequency and a reduction of the IEI in the IL-15 group ($p = 0.04$ and 0.05 respectively, **Fig.3 A-C, Table 2**), compared to the VEH group, while the amplitude of mIPSCs are similar ($p = 0.34$, **Fig.3 D, Table 2**). Analysis of kinetics shows no change in the rise time and decay times of events in the IL-15 group compared to VEH ($p = 0.27$ and $p = 0.24$ respectively, **Table 2**). These observations indicate that similarly to acute treatment, in vivo delivery of IL-15 in the hippocampus enhances the inhibitory transmission through presynaptic mechanisms.

We also looked at excitatory transmission by recording mEPSCs from PC cells in slices harvested from the VEH and IL-15 groups (**Fig.3 E**). Events analysis revealed that the mean frequency ($p = 0.49$), IEIs ($p = 0.99$) and amplitudes ($p = 0.16$) of mEPSCs are similar in both VEH and IL-15 groups while we found a faster rise time and decay time in the IL-15 group. ($p = 0.005$ and $p = 0.01$ respectively, **Fig.3 F-G, Table 2**). These results suggest modifications of AMPA receptors properties induced by chronic IL-15 treatment, in line with what is found during acute application of IL-15 (**Fig.2**). Consequent to the alterations in the inhibitory currents, measurements of mIPSC (at 0 mV) and mEPSC (-70 mV) from individual PC cells

confirm a reduced excitation/inhibition, E/I, ratio calculated from the frequencies ($p = 0.04$, **Fig.3 H**) in IL-15-treated mice while no difference in E/I amplitude is observed ($p = 0.27$, **Fig.3 H**).

To investigate whether the increase in mIPSCs frequency induced by in vivo treatment with IL-15 is due to increased inhibitory connectivity, we analysed the input/output curve (I/O curve) by measuring the amplitude of eIPSCs elicited by stimuli of graded intensities. The inhibitory post-synaptic currents increased similarly in the VEH and IL-15 groups, at increasing stimulation intensity (**Fig.4 A-B**), suggesting that the strength of inhibitory input onto CA1 pyramidal neurons is not affected by chronic IL-15. Evoked inhibitory currents were then recorded during pairing of extracellular stimuli at different interstimulus intervals. In line with what was observed with acute IL-15 application, the paired pulse ratio is reduced in the IL-15 group at 200 ms ISI, indicating that IL-15 treatment enhances pre-synaptic GABA release by increasing release probability ($p = 0.04$, **Fig.4 C-D**).

Inhibition of pyramidal neurons by GABAergic interneurons plays a critical role in the regulation of information flow through the hippocampus. Recently, it has been found that specific hippocampal interneurons in CA1 control episodic-like memory consolidation in a GABA-dependent manner (Oliveira da Cruz et al., 2020) (Rong et al., 2023). Moreover, it has been reported that selective deletion of IL-15 or IL-15Rα leads to behavioral and cognitive impairments related to hippocampus (He et al, 2010; Nguyen et al., 2017)). To further explore the potential role of IL-15 in memory processes related to hippocampal formation, we evaluated the episodic memory with the novel-object recognition (NOR) test. We observed that IL-15-treated mice show memory impairment both in the short (1h) and in the long term (24h) memory task, as indicated by a reduction of the discrimination index ($p = 0.024$ and $p = 0.023$, **Fig.5**).

3.3 Microglial depletion does not preclude IL-15-mediated effects on GABAergic neurotransmission but prevents IL-15 effects on NOR memory consolidation

The expression of IL-15Rα, the specific subunit of the IL-15 receptor, has been found in human fetal brain extracts from different regions, including the hippocampus, while at cellular level it has been found in microglia, in astrocytes and in neural cell lines (Hanisch et al., 1997; Satoh et al., 1998; Kurowska et al., 2002; Lee et al., 1996). However, no evidence of IL-15Rα expression by different neuronal subtypes has been reported. Here, we performed single cell qRT-PCR analysis of CA1 pyramidal cells (PC), interneurons (INT), microglia (MIC) and astrocytes (AST), extracting the cytoplasm via a patch pipette. Electrophysiological characterization of patched cells before collecting the cellular content allows the identification of the different cell types (**Fig.S1**). By single cell RT-PCR analysis we found that all the cells tested, with the exception of INT, express the IL-15Rα subunit (**Fig.6 A**), with high levels in microglia.

To investigate whether microglia have a role in the neuromodulatory activity of IL-15 on GABAergic transmission, we studied the effect of IL-15 in mice treated with PLX5622, a CSFR1 inhibitor added to the diet, that eliminates more than 90 % of microglia in the brain

(Corsi et al., 2022). We found that upon microglia depletion, the intracerebral injection of IL-15 produces no changes in the mean amplitude of mIPSCs ($p = 0.97$) while the treatment increases the frequency and reduces the IEIs of mIPSCs ($p = 0.044$ and $p = 0.039$, **Fig.6 B-C**) compared to depleted mice injected with vehicle. The effects of IL-15 treatment in microglia-depleted mice on inhibitory currents are similar to those observed in control mice (see **Fig.3**), suggesting that other mechanisms could be involved in the neuromodulatory activity of IL-15 on GABAergic neurotransmission.

Interestingly, in the NOR test, PLX5622 treated mice injected with IL-15 show a reduction in the discrimination index at 1h but not at 24h compared to VEH depleted mice ($p = 0.012$ and $p = 0.190$, respectively, **Fig.6 D**). This difference suggests that microglia might control distinct phases of NOR memory consolidation mediated by IL-15.

3.5 Serotonin receptors mediates IL-15 effects on GABA release and NOR memory

In the hippocampus, the activity of inhibitory interneurons is modulated by a number of inputs from the subcortical monoaminergic nuclei, including the serotonergic midbrain raphe nuclei (Lambert and Wilson, 1993; Fink and Ghothert, 2007, Varga et al., 2009), and the activation of 5-HT_3 receptors modulates the release of several neurotransmitters, including GABA (Katsurabayashi et al., 2003; Turner et al., 2004). Furthermore, it has been shown that IL-15Rα knock out mice show an alteration in the serotonin system and increased depressive-like behavior (Miettinen and Freund, 1992a, 1992b; Freund et al., 1990; Papp et al., 1999). For these reasons, we investigated the possible involvement of 5-HT_3A receptors in the IL-15-induced potentiation of GABA release, analyzing the mIPSCs in hippocampal slices pretreated with a selective inhibitor for the serotonin 5-HT_3A receptor, granisetron (30 nM). We observed that while acute treatment of slices with IL-15 increases mIPSC frequency and IEIs ($p = 0.03$ and $p = 0.028$ respectively, **Fig. 7 A-B left panels**), pretreatment of the hippocampal slices with granisetron prevents these effects ($p = 0.87$ and $p = 0.94$ respectively, **Fig. 7 A-B right panels**). Furthermore, the mIPSCs amplitude is unaffected in all the conditions (**Fig.7 C**, CTR: TTX vs IL-15 $p = 0.20$; +GRANISETRON: TTX vs IL-15 $p = 0.67$). These results indicate that the activation of 5-HT_3A receptors is required for the presynaptic effect of IL-15 on inhibitory circuitry.

Serotonin dysregulation is known to contribute to cognitive symptoms and NOR impairment associated with several brain disorders and different subtypes of 5-HT receptors play a role in object recognition memory (Meneses, 2013). 5-HT3R antagonists have been reported to improve NOR deficits in kindled rat, schizophrenic and depressive mouse models (Sayahi et al., 2022; Bozkurt and Unal, 2023; Pehrson et al., 2018) while in non-pathological conditions a 5-HT3 receptor antagonist, tropisetron, facilitates cognition in female rats by improving the recognition of familiar information (Sawyer et al, 2012). To demonstrate a potential role of the 5-HT_3A receptor in the effects induced by IL-15 on episodic memory, we intracranially injected granisetron (0.25μg/0.5μl), simultaneously with IL-15. This local treatment inhibited the effects induced by IL-15 on the NOR test (**Fig.7 D**), indicating a role for 5-HT_3AR in the regulation of non-spatial memory mediated by IL-15.

4. Discussion

The role of the cytokine network in the regulation of the homeostatic functions of the CNS is still far from being completely understood, but several evidence support the notion that cytokines contribute to neuronal circuit function and mice behavior. Here, we demonstrated that the administration of IL-15 in the hippocampus induces changes in neuronal transmission, with alterations in cognitive performance in mice. In particular, we found that IL-15 increases the frequency of miniature GABAergic currents and reduces the PPR of evoked inhibitory responses, both *ex vivo* and *in vivo*. These effects, together with the observation that the I/O relationship of inhibitory transmission is not modified by IL-15, indicate that IL-15 increases GABAergic transmission by enhancing the probability of GABA release rather than altering the number of inhibitory connections/interneurons.

Here we also showed that the strength of both spontaneous and evoked glutamatergic transmission is reduced upon acute administration of IL15 on slices, suggesting post-synaptic changes at excitatory synapses. Interestingly, upon chronic delivery of IL-15 into the brain, the mean amplitude of mEPSCs is not affected but the decay time of mEPSCs is reduced, an effect also observed during acute IL-15 treatment, suggesting alterations of the AMPARs properties (Jonas, 2000). Our single cell PCR analysis on hippocampal cell populations showing IL-15Rα expression by PC indicates that a direct action of IL-15 onto pyramidal cells could underlie these effects. However, we cannot exclude additional mechanisms involving the release of mediators by glia cells (Dubois et al., 2002).

We observed that IL-15 differently affects the amplitude of glutamatergic current upon acute or chronic treatment: we speculate that the long-lasting alteration in basal neurotransmission could activate compensatory mechanisms, i.e. homo-or hetero-synaptic plasticity, to counteract synaptic alterations mediated by IL-15 (Field et al., 2020). In accordance with the specific increase of mIPSCs frequency, we reported that IL-15-treated mice display a reduction in the E/I frequency ratio on PC, thus suggesting an impairment in hippocampal circuit function. Indeed, the balance of excitatory to inhibitory (E/I) synaptic inputs is thought to control information processing and behavioral output of the central nervous system (Vogels et al., 2011) while an unbalanced E/I ratio may underlie impairment in social behavior, sensory perception and memory (Mendez et al.,2018). Interestingly, we found impairment in the NOR task upon IL-15 treatment, indicating that the increase in inhibition and the altered E/I balance disturb hippocampal activity and may underlie the episodic memory deficits. Our data are also in line with studies reporting that an increased GABAergic transmission and augmented GABA tone appears to exert inhibitory effects on learning and memory in the hippocampus (Koh et al., 2023; Rong et al., 2023). Moreover, a recent report reveals that a specific subgroup of interneurons controls memory involved in NOR tasks through a GABA mediated mechanism (Oliveira da Cruz et al., 2020).

Considering our data from single cell RT-PCR showing high levels of IL15Rα on microglia, we investigated whether microglial cells are involved in the neuromodulatory activity of IL-15. Mice partially depleted of microglia show an increase in GABAergic spontaneous activity upon chronic treatment with IL-15, and an impairment in short term memory in the NOR test, similarly to IL-15 injected control mice, suggesting that microglia are not directly involved in the IL-15 mediated effect on inhibition. Nevertheless, the effect of IL-15 on long term episodic memory is prevented by microglia depletion, suggesting that microglia might control distinct phases of NOR memory consolidation mediated by IL-15. Indeed, recent advances in proteomics and genomic tools have revealed that microglia display heterogeneous transcriptomic and functional profiles with differences linked to the brain region and the circuit they are associated with (Ochocka and Kaminska, 2021). Accordingly, we previously described

that depletion of microglia by PLX5622 administration affects hippocampal synaptic plasticity in a different manner at ventral and dorsal hippocampal pole (De Felice et al., 2022), two regions that are differently connected to cortical and subcortical areas and showing functional segregation with specific behavioral implications (Hargraves et al., 2005). Related to the NOR memory, a recent paper showed that a subpopulation of microglia expressing Arginase-1 control the late phase of NOR memory but not the short-term phase through regulation of cholinergic innervation in the hippocampus (Stratoulias et al., 2023), thus suggesting that other neuromodulators could be involved in the effects observed on episodic memory.

Taking into account the complex cell interactions mediated by cytokines, we can speculate that different cell types could be involved in the effects of the IL-15/Il-15 Rα system on neuronal functions. We found no expression of IL-15Rα subunit by interneurons, indicating that the effects of IL-15 on inhibition and memory could imply either a "trans-presentation" mode, by which the IL-15/IL-15Rα complex expressed on the surface of a producing cell triggers signaling through IL-15Rβγ_c on a neighbouring cell (Dubois et al., 2002) or heterogeneity of IL-15 expression by different INT subpopulations. Alternatively, we can speculate that other mediators able to modulate the release of GABA by interneurons onto CA1 pyramidal cells could be involved.

To investigate the last point, we considered that different inputs originating from distal brain regions are known to control the activity of interneurons and modulate the release of GABA by a wide variety of neurotransmitters and neuromodulators (Lambert and Wilson, 1993; Maggi et al., 2001; Fink and Ghothert, 2007). Among them, serotonin increases firing activity of interneurons (Staubli and Xu, 1995; McMahon and Kauer JA, 1997, Varga et al., 2009) and exerts a facilitatory action on the release of GABA by acting through presynaptic 5-HT_3 receptors (Katsurabayashi et al., 2003; Turner et al., 2004; Ciranna, 2006; Dorostkar and Boehm, 2007). Antagonism of 5-HT3A with ondasetron reduced the between-burst hyperpolarization in CA1, enhancing both the frequency of the naturally occurring theta rhythm and the induction of long-term potentiation, a molecular correlate of memory, thus facilitating learning (Staubli and Xu, 1995). Indeed, several studies showed modulation of cognitive performance and memory tasks upon administration of selective agonist or antagonists of the 5-HT receptors (Buhot, 1997, 2000) while 5-HT_3R antagonists, such as ondansetron, have been shown to improve cognition in different models of memory impairment (Staubli and Xu, 1995; Reznic and Staubli, 1997; Fontana et al., 1995; Boast et al., 1999; Mishra and Goel, 2016; Dale et al., 2016). Interestingly, IL-15Rα KO mice have alterations of the serotoninergic system and behavioral abnormalities (Wu et al., 2010, 2011). We described that inhibition of the 5-HT_3 receptor prevents the increase in mIPSCs frequency upon IL-15 treatment, indicating that 5-HT signaling is involved in the IL-15 mediated effects on inhibitory neurotransmission. We here did not investigate directly the expression of IL15 Rα subunit on serotoninergic afferents neither the molecular mechanisms linking IL-15/IL-15 Rα signaling to serotoninergic pathway. However, modulation of serotoninergic system by IL-15 could be achieved by altering serotonin metabolism, as reported for other inflammatory cytokines such as TNFα and IL-1, known to increase the expression and function of the reuptake pumps (transporters) for serotonin through activation of mitogen activated protein kinase (MAPK) such as p38 MAPK, signaling molecules also activated by the IL-15 pathway. (Pelletier et al., 2002, Zhu et al., 2006). Previous studies reported altered expression of some 5-HTRs in IL-15Rα KO and reduced serotonin uptake in both IL-15 treated and IL-15Rα KO synaptosomal preparation (Wu et al., 2010, 2011). Alternatively, IL-15 can influence the release of serotonin by increasing the production of NO, known to modulate the release of serotonin in hippocampus

(Lorrain and Hull, 1993, Kaehler et al., 1999). In future experiments it would be interesting to investigate the mechanisms by which IL-15 affects serotoninergic system in our model.

We speculate that the synaptic and behavioral alterations observed upon IL-15 treatment could be related to several pathological conditions characterized by chronic inflammation. In the brain, IL-15 signaling may be involved in the inflammatory reactions in CNS disorders and it is upregulated in multiple sclerosis, Alzheimer's and Parkinson's disease and it exacerbates ischemic brain injury (Rentzos et al., 2006a, 2006b; Li M. et al., 2016; Fehniger and Caligiuri, 2001). Notably, the release of pro-inflammatory cytokines during pathological conditions significantly impacts on the activity of the nervous system, promoting sickness behavior and other behavioral abnormalities (Salvador et al., 2021) and patients affected by these disorders suffer from memory impairments (Zhang et al.; 2012; Jahn, 2013; Davis et al., 2016; Valentine et al., 2023). Our data demonstrated a correlation between the cytokine IL-15 and memory impairments, highlighting a new potential mechanism that participates in brain dysfunctions. Recently, many evidence demonstrated that immune cells tune neuronal transmission and neurotransmitter levels with effects on mice behavior (Alves de Lima et al., 2020; Garofalo et al., 2023). Since IL-15 is fundamental to sustain and activate immune cell functions, this cytokine could represent a molecular bridge among the adaptive and innate immune cells and the brain, and part of its effects in vivo could be due to the activation and recruitment of immune cells in the brain, with mechanisms worth to be further investigated.

5. Conclusions

Our data disclosed new modulatory mechanisms of IL-15 in the brain under physiological conditions, and identified the serotonin 5-HT_3A receptor as one of the mediators involved in the modulation of inhibitory neurotransmission and episodic memories by IL-15.

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Declaration of Competing Interest

None of the authors declares a conflict of interest.

Data availability

Data will be made available on request.

Figure Legends

Figure 1. IL-15 increases spontaneous and evoked GABAergic transmission in acute hippocampal slices. A) Scheme and B) representative recordings of mIPSCs performed from CA1 pyramidal neurons (PC) in acute hippocampal slices with TTX and after 10 min application of IL-15 (10nM), at 0 mV holding potential. C) Histograms representing the mean ± SEM of the frequency, IEIs and amplitude before, after 10 min of IL-15 treatment in the bath and after 10 min wash out period. Single dots represent the median value for each cell recording (n = 9/6). D) Representative timeline graph of a paired stimulation protocol experiment (ISI: 50 ms). Evoked IPSC amplitudes of the first peak (P1), the second peak (P2) and the paired pulse ratio (PPR) are shown. E) Representative traces of the experiment shown in D. F) Histograms of the mean values for PPR with dots indicating single cell recordings (n = 5/3). Paired t-test, * ≤ 0.05.

Figure 2. IL-15 transiently decreases the amplitude of glutamatergic transmission in acute hippocampal slices. A) Scheme and representative recordings of mEPSCs performed from CA1 pyramidal neurons at – 70 mV in acute hippocampal slices with TTX and after 10 min application of IL-15 (10nM). Histograms representing the mean ± SEM of the mEPSCs amplitude (B), frequency and IEIs (C) before, after 10 min of IL-15 treatment in the bath and after 10 min wash out period. Single dots represent the median value for each cell recording (n = 6/3). D) Representative timeline graph of a paired stimulation protocol experiment (ISI: 50 ms). Evoked EPSC amplitudes of first peak (P1), second peak (P2) and PPR are shown. E) Graph showing the mean values of the PPR (left) and the mean values of the first (P1) and the second peak (P2) amplitude (right) for each recording (single dot) and the average (histogram) before and after IL-15 treatment (n = 6/3). Paired t-test, * ≤ 0.05. F) Representative eEPSC traces of the experiment shown in D. Horizontal scale bar: 50 ms, vertical 200 pA.

Figure 3. Effects of intracerebral injection of IL-15 in mice on spontaneous hippocampal synaptic transmission. A) Schematic representation of IL-15 injections in mice. B) Scheme and representative recordings of mIPSCs from CA1 PC at 0 mV from VEH and IL-15 treated mice. Horizontal scale bar: 250 ms, vertical scale bar: 25 pA C) Histograms showing the mean frequency and IEIs of mIPSCs with single dots representing the median value for each cell (VEH n = 23/5, IL-15 n = 30/7). D) Histograms of mIPSCs amplitude in VEH and IL-15 groups. E) Representative traces of mEPSCs recorded from CA1 PC at -70 mV. F) Histograms of the mean frequency and IEIs for mEPSCs (VEH n = 11/5, IL-15 n = 14/6). G) Mean values and single cell median values of mEPSCs amplitudes. H) Excitation/inhibition ratio calculated as the ratio between mEPSC/mIPSC recorded from the same cell, for the frequency and amplitude respectively in vehicle (0.41 ± 0.11, 0.67 ± 0.04, n = 9/4) and IL-15 treated mice (0.15 ± 0.03, 0.61 ± 0.04, n = 13/7). * ≤ 0.05. Unpaired t-test.

Figure 4. In vivo delivery of IL-15 impacts on paired pulse ratio but not on inhibitory strength. A) Traces of IPSCs evoked elicited by stimuli of graded intensities recorded from VEH and IL-15 mice. B) Input/output curves of eIPSCs averaged from cells recorded in VEH (n = 7/4) and IL-15 treated mice (n = 9/3). C) Representative traces of paired eIPSCs in control and IL-15 mice. D) Paired pulse ratio measured at 50, 100, 200 and 700 ms interstimulus

intervals (ISIs) in vehicle (50 ms: 0.79 ± 0.08, 100 ms: 0.91 ± 0.06, 200 ms: 0.92 ± 0.04, 700 ms: 0.85 ± 0.03) and IL-15 injected mice (50 ms: 0.71 ± 0.05, 100 ms: 0.81 ± 0.06, 200 ms: 0.80 ± 0.04, 700 ms: 0.88 ± 0.04) * ≤ 0.05, unpaired t-test.

Figure 5. IL-15 impairs episodic memory formation. A) Scheme of NOR test depicting the training and the test sessions. B) Discrimination index analyzed after 1h (short term memory - STM) and 24h (long term memory - LTM) in mice treated with VEH or IL-15 (n = 10 each group, *p=0.024 STM, *p=0.023 LTM two-tailed Student's t-test).

Figure 6. **Role of microglia in IL-15 mediated effects on synaptic transmission and episodic memory.** A) Histogram showing the results of single cell qPCR for IL-15Rα mRNA from pyramidal cells (PC), interneurons (INT), microglia (MIC) and astrocyte (AST). B) Scheme of PLX5622 mice treatment. C) Histograms showing the mean amplitude, the frequency and IEIs of mIPSCs in microglia depleted mice treated intracerebrally with vehicle (n = 12/2) or IL-15 (n = 16/3). D) Discrimination index analyzed after 1h (STM, left graph) and 24h (LTM, right graph) in mice fed with control chow (left bars) and PLX 5622 chow (right bars) treated with VEH (n = 5) or IL-15 (n = 7).

Figure 7. 5-HT_3 receptors activation mediates the effects of IL-15 on inhibitory transmission and episodic memory. A) Single data plot of mIPSCs frequency in control, after 10 min of IL-15 acutely applied in slice, and after 10 min of wash out, without (n = 5/3, left) and with pretreatment of the slice with the 5-HT_3A R antagonist, Granisetron (n = 6/3, right). B) Histograms showing the mean of IEIs values in TTX, after 10 minute of IL-15 application and after 10 min wash out without (left) and with (right) Granisetron pretreatment. Values are normalized to TTX in each condition. C) Normalized values to TTX condition without and with Granisetron pretreatment for mean mIPSCs amplitudes. D) Discrimination index analyzed after 1h (short term memory - STM) and 24h (long term memory - LTM) in mice treated with VEH (n = 5), IL-15 (n = 7) or Granisetron + IL-15 (n = 6).

Figure 8. Graphical summary showing the observed effects of IL-15 on synaptic transmission in CA1 hippocampal region and behaviour.

Exogenous application of IL-15 on acute slices and in mice produces presynaptic effects at the inhibitory synapses. We observed (red signs) an increase in the frequency of mIPSCs and a reduction of PPR eIPSC, both indicating enhanced GABA release upon IL-15. Granisetron (30 nM), an antagonist of 5-HT3ARs reported to be expressed on interneurons (INT) where regulates firing and presynaptic release of GABA, precludes the effect of IL-15 on inhibitory currents, indicating that 5-HT (released from serotoninergic afferents originating from dorsal raphe nucleus (DRN)) is involved in the IL-15 mediated effects on GABA release. Depletion of microglia (MIC) with PLX5622 do not prevent the effects of IL-15 on inhibitory currents. IL-15 treatment causes post-synaptic changes at the excitatory synapses between Schaffer

collaterals (Sch) and CA1 pyramidal neurons (PC), reducing the amplitude of mEPSC and eEPSC. The alterations in inhibitory activity are linked to impairment in the episodic memory, observed as reduction in both short (STM) and long-term memory (LTM) in the novel object recognition task. STM and LTM impairment are rescued in IL-15 treated mice by Granisetron while PLX5622 treatment leads to a recovery only of LTM.

Fig.S1. Example traces recorded for electrophysiological characterization of patched cells in single cell qPCR to identify A), pyramidal cells (PC) B), interneurons (INT) C) microglia (MIC) and D) astrocyte (AST). Cell capacitance and firing pattern of pyramidal cell (A) and interneurons (B) are shown. For PC and INT, voltage responses are elicited by somatic current injection steps incremented by 10 pA from RMP. For MIC C) and AST (D), currents are evoked by hyperpolarizing and depolarizing voltage steps incremented by 10 mV.

Fig.S2. Graphs showing the mean values of cell capacitance (A) and input resistance (B) measured from pyramidal cells recorded in slices harvested from vehicle (VEH: 93.09 ± 6.73 pF, 313.81 ± 29.87 MΩ, n = 22/5) and IL-15 treated mice (110.87 ± 9.04 pF, 302.69 ± 30.58 MΩ, n = 29/7). Dots indicate the value for each cell recordings. (C) Membrane potential values from vehicle (-65.64 ± 1.86 mV, n = 8/3) and IL-15 treated mice (-65.88 ± 1.45, n = 7/2). D) Firing pattern and E) relationship between somatic current injection and number of action potential recorded in current clamp mode from PC in VEH (n = 8/3) and IL-15 treated mice (n =7/2). F) Threshold for action potential in VEH (-42.00 ± 2.67 mV, n = 8/3) and IL-15 mice (-42.67 ± 0.95 mV, n = 7/2).

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		TTX	IL-15	WASH
mIPSC	**Frequency (Hz)**	3.59 ± 0.58 Hz	**4.05 ± 0.56 Hz** *	3.94 ± 0.68
	IEI (ms)	256.77 ± 58.59	**224.64 ± 51.64** *	244,96 ± 57.99
	Amplitude	12.08 ± 0.82	11.73 ± 0.97	11.31 ± 0.71
	Rise time (ms)	1.77 ± 0.09	1.95 ± 0.09	1.83 ± 0.15
	Decay time (ms)	10.64 ± 0.85	10.96 ± 0.97	**13.98 ± 1.45** *
mEPSC	**Frequency (Hz)**	0.50 ± 0.04	0.52 ± 0.06	0.44 ± 0.03
	IEI (ms)	2009.52 ± 229.94	2126.05 ± 236.31	2431.63 ± 171.43
	Amplitude	8.73 ± 0.38	**7.79 ± 0.26** * #	8.94 ± 0,39
	Rise time (ms)	1.96 ± 0.14	1.88 ± 0.11	2.01 ± 0.14
	Decay time (ms)	4.49 ± 0.44	**3.94 ± 0.35** #	4.92 ± 0.39
		ACSF	**IL-15**	**WASH**
eEPSC	**1st peak (pA)**	387.49 ± 87.39	**312.59 ± 68.68** *	354.34 ± 84.67
	2nd peak (pA)	547.902 ± 117.801	**467.343 ± 94.104** *	494.289 ± 123.046
	PPR	1.47 ± 0.09	1.59 ± 0.12	1.67 ± 0.11
IPSC	**1st peak (pA)**	326.64 ± 60.51	350.48 ± 60.80	308.37 ± 59.304
	2nd peak (pA)	182.82 ± 30.97	169.54 ± 30.58	184.43 ± 43.19

	PPR	0.69 ± 0.12	**0.56 ± 0.11** * #	0.79 ± 0.15

Table 1.

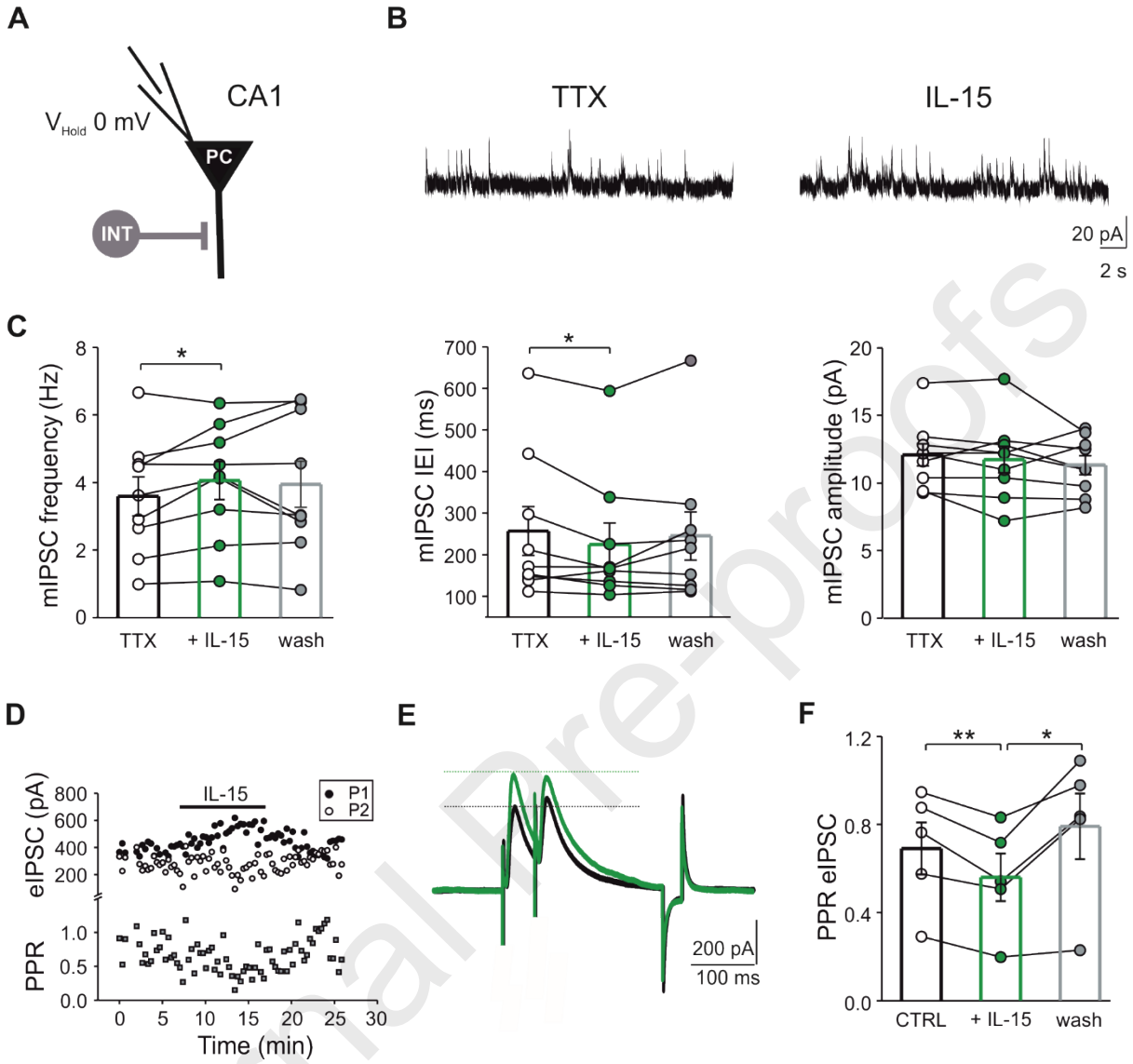
Legend Table 1. Electrophysiological parameters of excitatory and inhibitory synaptic transmission recorded from CA1 pyramidal neurons before, after 10 min of IL-15 (10nM) application and following 10 min of IL-15 wash out, in acute hippocampal slices. IEI: inter-event-intervals; PPR: paired pulse ratio. Values are indicated as mean ± sem. * comparison versus TTX or ACSF $p \leq 0.05$, # comparison versus WASH $p \leq 0.05$.

Table 2

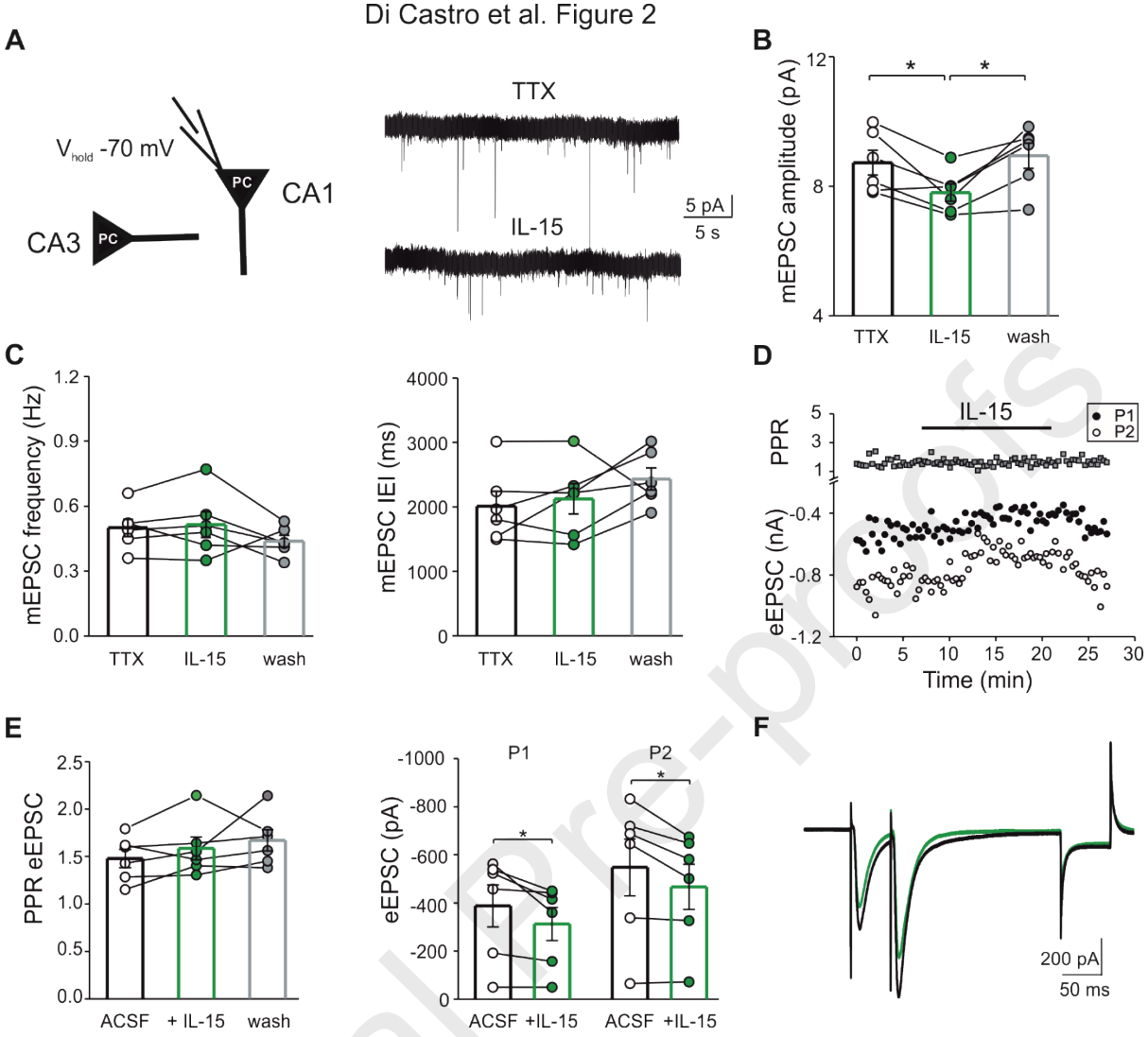
		Vehicle	***IL15***
mIPSC	**Frequency (Hz)**	3.44 ± 0.36	**5.12 ± 0.59 ***
	IEI	242.21 ± 29.77	**174.88 ± 19.22 ***
	Amplitude (pA)	12.02 ± 0.48	12.67 ± 0.47
	Rise time (ms)	1.79 ± 0.13	1.94 ± 0.08
	Decay time (ms)	9.19 ± 0.42	8.63 ± 0.25
mEPSC	**Frequency (Hz)**	0.69 ± 0.13	0.59 ± 0.08
	IEI	1327.87 ± 339.66	1323.89 ± 177.19
	Amplitude (pA)	7.91 ± 0.35	7.12 ± 0.41
	Rise time (ms)	2.49 ± 0.14	**2.03 ± 0.07 ***
	Decay time (ms)	4.69 ± 0.29	**3.55 ± 0.28 ***

Legend Table 2. Electrophysiological parameters of miniature excitatory and inhibitory post synaptic currents recorded from CA1 pyramidal neurons in acute hippocampal slices from vehicle and intracerebrally IL-15 treated mice. Values are indicated as mean ± sem. * $p \leq 0.05$.

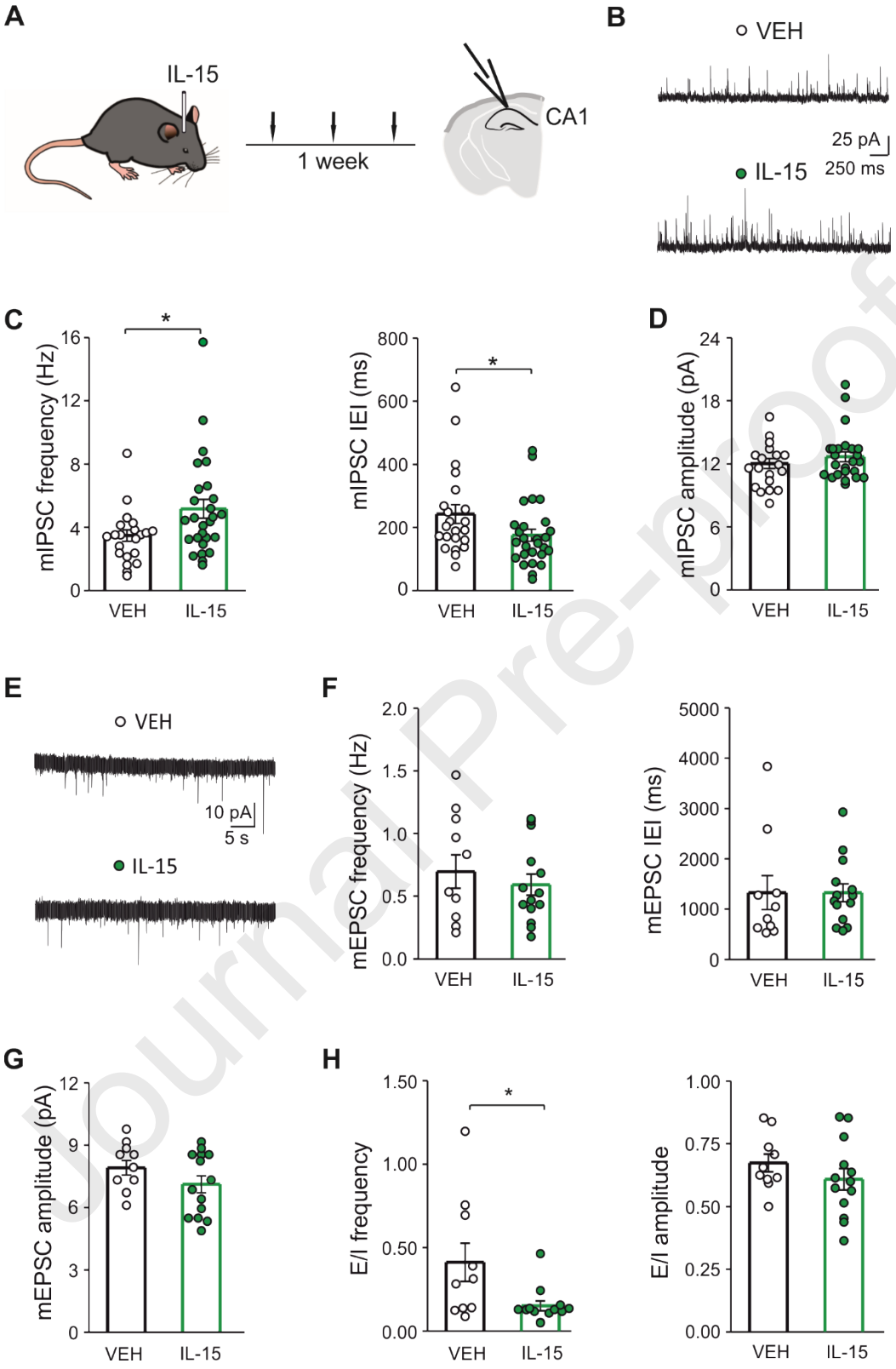
Di Castro et al. Figure 1



Di Castro et al. Figure 2



Di Castro et al. Figure 3



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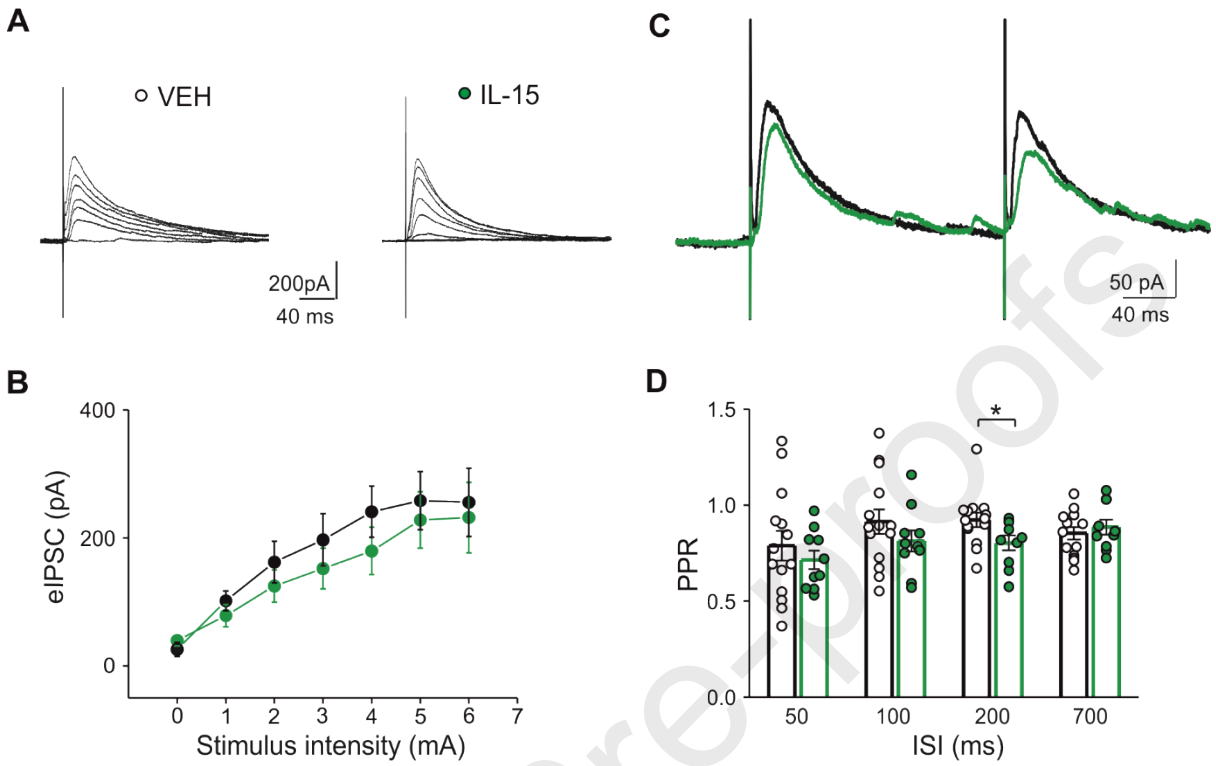
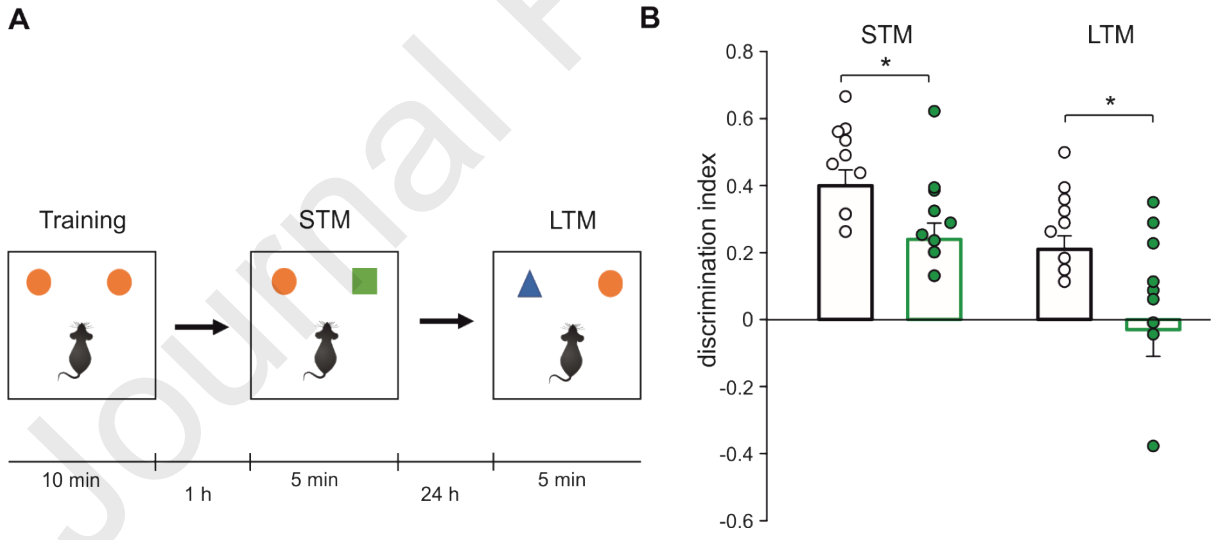
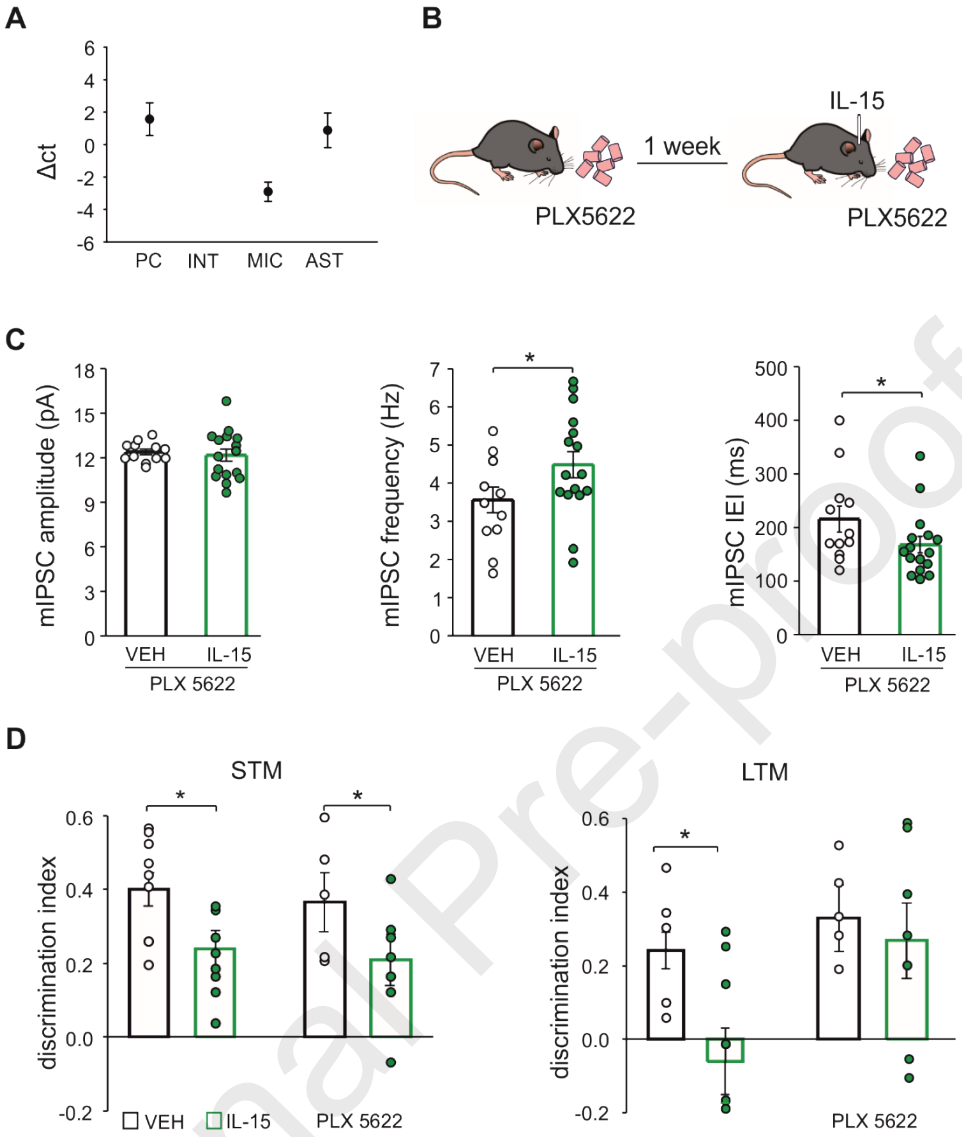
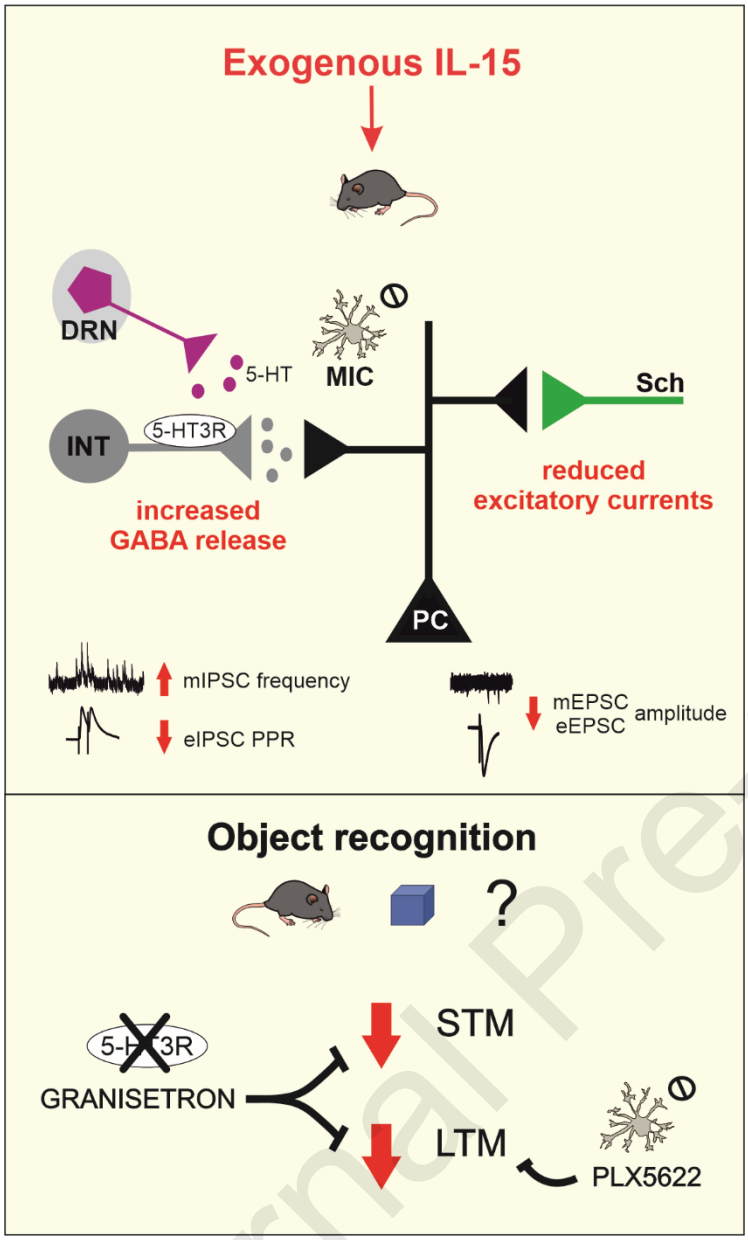


Di Castro et al. Figure 5



Di Castro et al. Figure 6





Interleukin 15 alters hippocampal synaptic transmission and impairs episodic memory formation in mice

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Highlights

- IL-15 modulates synaptic GABAergic synaptic transmission in hippocampal CA1 area.
- IL-15 impairs short and long term episodic memory formation in the Novel Object Recognition test.
- 5-HT3 receptor mediates the IL-15 effects on inhibitory transmission and episodic memory.
- Microglial cells participate in the IL-15 effects on the formation of the short term memory.