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Interferon-γ potentiates $GABA_A$ receptor-mediated inhibitory currents in rat hippocampal CA1 pyramidal neurons

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ABSTRACT

The neural transmission and plasticity can be differentially modulated by various elements of the immune system. Interferon-γ (IFN-γ) is a "pro-inflammatory" cytokine mainly produced by T lymphocytes, activates its corresponding receptor and plays important roles under both homeostatic and inflammatory conditions. However, the impact of IFN-γ on the γ-aminobutyric acid (GABA)-mediated currents in the hippocampus, a major brain region involved in the cognitive function, has not been investigated. Here we detected abundant expression of both IFN-γ receptor subunit gene transcripts (*Ifngr1* and *Ifngr2*) in the rat hippocampus by quantitative PCR. In addition, we pre-incubated rat hippocampal slices with IFN-γ (100 ng/ml) and recorded GABA-activated spontaneous and miniature postsynaptic inhibitory currents (sIPSCs and mIPSCs) and tonic currents in hippocampal CA1 pyramidal neurons by the whole-cell patch-clamp method. The pre-incubation with IFN-γ increased the frequency but not the mean amplitude, rise time or decay time of both sIPSCs and mIPSCs in hippocampal CA1 pyramidal neurons, suggesting a presynaptic effect of IFN-γ. Moreover, the GABA-activated tonic currents were enhanced by IFN-γ. In conclusion, the potentiation of GABAergic currents in hippocampal neurons by IFN-γ may contribute to the disturbed neuronal excitability and cognitive dysfunction during neuroinflammation.

1. Introduction

Cytokines are a diverse family of signaling proteins produced mainly by immune cells to regulate immunological responses. In addition, neurons and glial cells such as microglia in the central nervous system (CNS) can also release cytokines. A variety of cytokines have been shown to modulate neuronal functions under both homeostatic and pathological conditions (Donzis and Tronson, 2014; Marin and Kipnis, 2013; Yirmiya and Goshen, 2011). One of such cytokines is interferon-γ (IFN-γ), well known for its antiviral activity, is produced predominantly by natural killer (NK) and T cells (Schoenborn and Wilson, 2007) and to a lesser extent by CNS cells including microglia (Monteiro et al., 2017). Notably, the levels of IFN-γ in the blood and brain are altered during aging and fasting as well as in neuroinflammatory, neuropsychiatric and neurodegenerative disorders (Arolt et al., 2000; Lee et al., 2006; Reale et al., 2009; Soderstrom et al., 1995; Wei et al., 2000). The released IFN-γ binds to the heterotetrameric IFN-γ receptor composed of two different subunits IFN-γR1 and IFN-γR2 that are expressed in various cell types including neurons (de Weerd and Nguyen, 2012). The expression level of the IFN-γ receptor in the brain varies among the sub-regions and changes after specific stimulations (Robertson et al., 2000; Utsuyama and Hirokawa, 2002). In addition to the important role of IFN-γ in inflammatory responses, accumulating evidence suggests that IFN-γ signaling also modulates neurological processes underlying cognitive behaviors including neurogenesis, neuronal excitability and plasticity (Filiano et al., 2016, Li et al., 2010, Monteiro et al., 2017).

The hippocampus, mostly known to be involved in learning and memory formation, expresses a broad range of cytokine receptors including the IFN-γ receptor (Arisi, 2014, Robertson et al., 2000). Several recent studies have suggested that the IFN-γ acts as a negative regulator of hippocampal functions. For example, the IFN-γ-deficient mice have shown impaired spatial memory, increased neurogenesis, dendritic arborizations, and probability of pre-synaptic neurotransmitter release in the hippocampal sub-regions (Litteljohn et al., 2014; Monteiro et al., 2016). Furthermore, intracerebroventricular injection of IFN-γ impairs the induction of long-term potentiation (LTP), an electrophysiological correlate of learning and memory, in hippocampal slices of rats (Maher

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et al., 2006). Interestingly, transgenic mice with limited overexpression of IFN-γ in the adult brain have displayed enhanced neurogenesis in the hippocampal dentate gyrus and improved cognitive performance (Baron et al., 2008). To date, little is known about the effects of IFN-γ on hippocampal excitability. In vitro experiments have revealed that the acute application of IFN-γ on cultured rat hippocampal slices increased the excitability of CA3 neurons (Muller et al., 1993) whereas the chronic IFN-γ treatment reduced the gamma oscillations in the CA3 region (Ta et al., 2019). In fact, the excitability of neuronal network is critically dependent on the balance between excitatory glutamatergic transmission and inhibitory GABAergic (γ-aminobutyric acid) transmission. The impact of IFN-γ on glutamatergic transmission has been demonstrated in cultured hippocampal neurons, where the frequency of AMPA receptor-mediated synaptic currents were significantly enhanced after 48-hour IFN-γ exposure but reduced after 4-week treatment (Vikman et al., 2001). However, the effect of IFN-γ on the GABAergic transmission has been sparsely investigated (Filiano et al., 2016).

There are two forms of GABA-activated inhibition namely phasic and tonic inhibitions, that are mediated by synaptic and extrasynaptic $GABA_A$ receptors ($GABA_A$Rs) respectively, and differentially modulate neuronal excitability (Farrant and Nusser, 2005; Pavlov et al., 2009). In the current study we first sought to examine the mRNA expression of the IFN-γ receptor subunits in the hippocampus of young rats and further explored the effect of IFN-γ on $GABA_A$R-mediated neuronal inhibition in rat hippocampal CA1 pyramidal neurons.

2. Materials and methods

2.1. Animals

Male Wistar rats, 16 to 22 days old, were used in all experiments. All animal care and experimental procedures were performed according to the guideline approved by the Uppsala Animal Ethical Board (Uppsala, Sweden).

2.2. Brain slice preparation

Animals were rapidly decapitated, brains were quickly removed and placed into ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): 124 NaCl, 3 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$, 2.5 Na_2HPO_4, and 10 glucose (pH 7.3–7.4 continuously bubbled with 95% O_2 and 5% CO_2, 300–310 mOsm). Sagittal brain sections containing the hippocampal region (400 μm thick) were prepared in the ice-cold aCSF using a vibratome (Leica VT1200, Leica, Germany). Slices were recovered in the same aCSF at 34 °C for half an hour and further incubated in aCSF or aCSF containing recombinant rat IFN-γ (100 ng/ml, PerproTech, UK) for 1–4 h at room temperature before being transferred to the recording chamber. The recombinant rat IFN-γ was reconstituted in sodium phosphate buffer (10 mM, pH 8.0) and further diluted in aCSF.

2.3. RNA isolation and reverse-transcription quantitative PCR (RT-qPCR)

The hippocampal region was isolated from sagittal brain sections of rats (8 sections per brain, 6 rats). Total RNA was isolated using GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, Germany), and quantified with Nanodrop (ThermoFisher Scientific, USA). Total RNA samples with absorbance ratios A260/A280 and A260/230 > 1.8 were further reverse transcribed to cDNA using SuperScript IV reverse transcriptase (ThermoFisher Scientific, USA). To exclude the potential genomic DNA contamination in the RNA samples, negative controls were also prepared by omitting reverse transcriptase in the reaction. Real-time quantitative PCR was performed using ABI PRISM 7900HT Sequence Detection System (ThermoFisher Scientific, USA) as previously described (Korol et al., 2018). The relative quantification assay using SYBR green dye was run in SDS2.4 program and followed by the melting curve analysis to confirm the specificity of the PCR product. Cycle threshold (Ct) values were determined by RQ Manager 1.2 program. The predesigned rat KiCqStart primer pairs were synthesized by Sigma-Aldrich, for IFN-γ receptor subunit 1, *Ifngr1* (from 5′ to 3′, forward F-CCATGATGACAGAGAAGAATC, reverse R-AGGGA GTACTTTGATTCAGG), IFN-γ receptor subunit 2, *Ifngr2* (F-CTACTGG GAAAAGACAGAAAC, R-GACAAGATACATTGCTCAGG) and reference genes *Ywhaz* (Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Zeta, F-TGACAAGAAAGGAATTGTGG, R-GGAGTTCAGGATCTCATAGTAG). The relative expression of IFN-γ receptor subunit genes was normalized to the reference gene *Ywhaz* that has been shown to be stably expressed in rat brain tissues (Bonefeld et al., 2008) and calculated with DataAssist V2.0 using the $2^{-\Delta Ct}$ method.

2.4. Whole-cell patch-clamp recording of GABA-activated currents and data analysis

Whole-cell voltage-clamp recordings were performed at room temperature as previously described (Jin et al., 2011b; Korol et al., 2015). The recording pipettes were pulled from borosilicate glass capillaries (Harvard Apparatus, UK) using the DMZ-Universal puller (Zeitz Instruments, Germany). The resistance of the pipette was 3–5 MΩ when filled with the pipette solution containing (in mM): 140 CsCl, 1 $CaCl_2$, 3 EGTA, 0.5 KCl, 1 $MgCl_2$, 2 ATP-Mg, 0.3 GTP-Na, 5 QX-314 bromide, and 10 TES (pH 7.25 adjusted with CsOH, and osmolarity 290–300 mOsm). The hippocampal slice was placed in the recording chamber and continuously perfused with aCSF containing kynurenic acid (3 mM, non-selective glutamate receptor antagonist, Sigma-Adrich, Germany) equilibrated with 95% O_2 and 5% CO_2 at room temperature. Whole-cell recordings of CA1 pyramidal neuron were performed under an upright microscope (Axon Examiner, Zeiss, Germany) as previously described (Jin et al., 2011a). $GABA_A$ receptor-mediated postsynaptic currents were recorded at −60 mV using a Multiclamp 700B amplifier (Molecular Devices, USA), 5–10 min after the establishment of stable whole-cell configuration. All recorded analog signals were filtered at 2 kHz, digitized at 10 kHz using a Digidata 1440A and pClamp 10 software (Molecular Device, USA). The recordings were rejected from the analysis if the access resistance changed > 20% during the experiment. The spontaneous or miniature inhibitory postsynaptic currents (sIPSCs or mIPSCs) were recorded in aCSF containing kynurenic acid (3 mM) or kynurenic acid (3 mM) together with tetrodotoxin (TTX, 1 μM, SigmaAdrich, Germany, sodium channel blocker to abolish action potential-dependent GABA release), respectively. $GABA_A$R antagonist bicuculline methbromide (100 μM, Sigma-Adrich, Germany) was

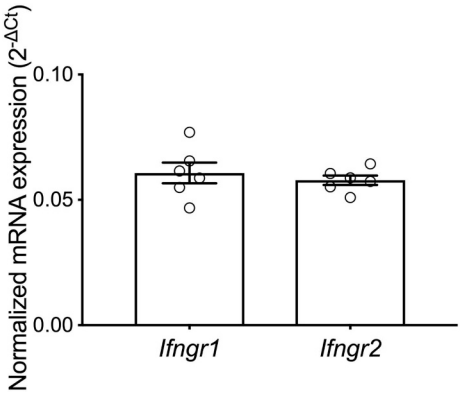

Fig. 1. Quantitative PCR analysis of IFN-γ receptor subunits *Ifngr1* and *Ifngr2* mRNA levels in the rat hippocampus.
The expression of *Ifngr1* and *Ifngr2* mRNA was normalized to the level of reference gene *Ywhaz* and calculated as $2^{-\Delta Ct}$ values. Data were presented as mean ± SEM (n = 6 per group of P16-22 Wistar rats).

applied to confirm the current mediation by $GABA_A$ receptors. TTX was dissolved in citrate buffer and further diluted in aCSF.

The analysis of sIPSCs and mIPSCs was performed on 3-minute current recording segments for each condition offline with MiniAnalysis 6.0.3 (Synaptosoft, USA). The frequency, inter-event intervals, amplitude, rise time (10–90%) and decay time (90–37%) of sIPSCs and mIPSCs were measured. To compute the average sIPSCs and mIPSCs, the synaptic events with single-peaks were aligned with the rise time. The amplitude of GABA-activated tonic current was measured as the difference in the baseline holding current before and after the application of bicuculline.

2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prism 8 software. Data were presented as mean ± standard error of the mean (SEM). The effect of IFN-γ on sIPSC/mIPSC frequency, median amplitude, rise time (10–90%) and decay time (90–37%) as well as tonic current amplitude was statistically analyzed using two tailed, unpaired Student *t*-test (normally distributed data) or Mann-Whitney test (non-normally distributed data). The D'Agostino-Pearson omnibus normality test was performed to determine the data distribution. The significance level was set at $p < .05$.

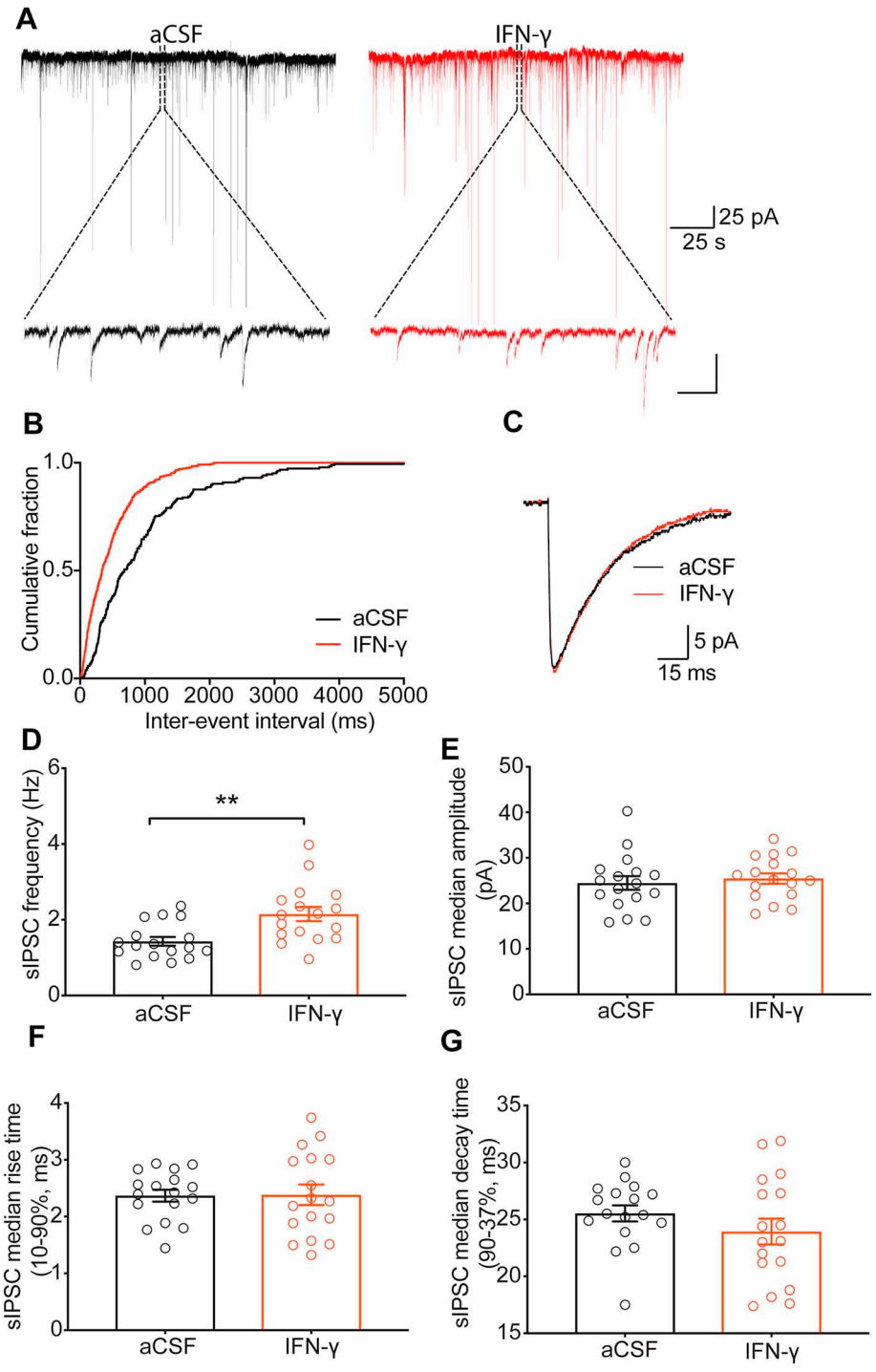

Fig. 2. IFN-γ increases the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) in rat hippocampal CA1 pyramidal neurons.
(A) Representative recordings (3 min) showing $GABA_AR$ -mediated sIPSC obtained from rat CA1 neurons in hippocampal slices incubated in artificial cerebrospinal fluid (aCSF) or aCSF with IFN-γ (100 ng/ml) for 3 h. The area between dashed lines was expanded to show the currents at a faster time scale in the lower panel. The neurons were held at −60 mV. **(B)** Cumulative probability plot showing inter-event intervals distribution of all sIPSC events detected in the upper panel of (A). **(C)** Rise time-scaled average sIPSC trace from individual sIPSC events in the upper panel of (A). (D) Frequency of sIPSC was significantly increased in rat CA1 neurons in hippocampal slices incubated in aCSF with IFN-γ (100 ng/ml) as compared to aCSF alone ($n = 17$ in each group; Unpaired student *t*-test, $^{**}p = .0023$). **(E–G)** There was no significant difference in sIPSC median amplitude (aCSF, 24.47 ± 1.51 pA, n = 17; IFN-γ, 25.46 ± 1.15 pA, n = 17; Unpaired student *t*-test, $p = .6025$), or median rise time (10–90%) (aCSF, 2.37 ± 0.11 ms, n = 17; IFN-γ, 2.39 ± 0.18 ms, n = 17; Unpaired student t-test, $p = .9421$), or median decay time (90–37%) (aCSF, 25.54 ± 0.71 ms, n = 17; IFN-γ, 23.94 ± 1.14 ms, n = 17; Mann-Whitney test, $p = .2279$) in aCSF with IFN-γ (100 ng/ml) as compared to aCSF alone. Data were presented as mean ± SEM.

3. Results

3.1. IFN-γ receptor subunit Ifngr1 and Ifngr2 mRNAs are expressed in the rat hippocampus

The IFN-γ receptor consists of two subunits, the α-chain (IFN-γR1) and the accessory β-chain (IFN-γR2), encoded by genes *Ifngr1* and *Ifngr2*, respectively (de Weerd and Nguyen, 2012). RT-qPCR was performed with IFN-γ receptor subunit specific primer pairs in RNA samples from rat hippocampal slices. Both *Ifngr1* and *Ifngr2* mRNAs were detected in the rat hippocampus ($n = 6$, Fig. 1). The expression of Ifngr1 and Ifngr2 mRNAs was normalized to the level of reference gene *Ywhaz* (Bonefeld et al., 2008).

3.2. Pre-incubation with IFN-γ increases GABA-activated sIPSC frequency and tonic currents in rat hippocampal CA1 pyramidal neurons

IFN-γ is a well-known cytokine involved in the neuroinflammatory response. To examine the effect of IFN-γ on $GABA_AR$-mediated neuronal currents, we pre-incubated rat brain slices with IFN-γ (100 ng/ml) for 1–4 h. The spontaneous postsynaptic currents (sIPSCs) were further recorded in hippocampal CA1 pyramidal neurons from slices perfused with aCSF containing kynurenic acid.

Representative current traces showed $GABA_AR$-mediated sIPSCs recorded from rat hippocampal CA1 pyramidal neurons in brain slices pre-incubated in aCSF or aCSF with IFN-γ (100 ng/ml) for 3 h (Fig. 2A). The cumulative probability analysis of sIPSCs in the same traces revealed that the distribution pattern of the inter-event interval in the IFN-γ pre-treated neuron was shifted to the left indicating increased sIPSCs frequency as compared to the aCSF control neuron (Fig. 2B). The average sIPSC from the above current traces were superimposed and showed similar amplitude and decay time (Fig. 2C). The frequency of sIPSCs was significantly increased in CA1 pyramidal neurons pre-incubated with IFN-γ (Fig. 2D, aCSF: 1.43 ± 0.12 vs. IFN-γ: 2.15 ± 0.19 Hz, $n = 17$, Unpaired student *t*-test, $p = .0023$), whereas the median amplitude, rise time (10–90%) and decay time (90–37%) of sIPSCs were not changed in IFN-γ-pre-treated CA1 neurons as compared to aCSF control neurons (Fig. 2E, F and G).

We next applied the $GABA_AR$ specific antagonist bicuculline (100 μM) to reveal the $GABA_AR$-mediated tonic currents at the end of each experiment (Fig. 3A, B). In the aCSF incubated hippocampal slices, no tonic current was detected in CA1 pyramidal neurons (Fig. 3A, C). In contrast, significant tonic current was present in CA1 pyramidal neurons from slices pre-incubated with 100 ng/ml IFN-γ (Fig. 3B, C, IFN-γ: 5.3 ± 1.18 pA, $n = 10$ vs aCSF: 0.5 ± 0.21 pA, $n = 11$; Unpaired student t-test, $p = .0012$). These data suggested that pre-incubation with IFN-γ potentiated GABA-activated phasic and tonic currents in the hippocampal CA1 pyramidal neurons.

3.3. Effect of IFN-γ on GABA-activated mIPSCs in rat hippocampal CA1 pyramidal neurons

To determine how IFN-γ affects action potential-independent release of GABA on rat hippocampal CA1 neurons, $GABA_AR$-mediated mIPSCs were recorded in CA1 pyramidal neurons from brain sliced pre-incubated with aCSF alone or with IFN-γ (100 ng/ml), in the presence of TTX (1 μM) (Fig. 4A). The cumulative probability analysis of mIPSCs showed that the distribution pattern of mIPSC inter-event interval in the IFNγ pre-treated neuron shifted to the left indicating the increase of mIPSC frequency, as compared to the neuron in the control condition (Fig. 4B). The superimposed average mIPSC from the above current traces revealed similar amplitude and decay time (Fig. 4C). The frequency of mIPSCs was significantly increased in CA1 neurons pre-incubated with IFN-γ (Fig. 4D, +TTX, $n = 11$: 1.66 ± 0.21 vs. IFN-γ + TTX, $n = 8$: 2.34 ± 0.25 Hz, Mann-Whitney test, $p = .0259$). In contrast, there was no significant change in the median amplitude, rise time (10–90%) and decay time (90–37%) of mIPSCs in IFN-γ-pre-treated CA1 neurons as compared to control neurons (Fig. 4E, F and G). These findings suggested that IFN-γ increased the probability of presynaptic release of GABA onto hippocampal CA1 neurons.

4. Discussion

In this study we demonstrate the abundant expression of mRNA transcripts of IFN-γ receptor subunits IFN-γR1 (*Ifngr1*) and IFN-γR2 (*Ifngr2*) in the rat hippocampus and the enhanced frequency of GABAergic synaptic currents and tonic current amplitude in CA1 pyramidal neurons of IFN-γ pre-treated hippocampal slices from young rats.

IFN-γ exerts pleiotropic cellular effects via activation of functional IFN-γ receptors composed of two ligand-binding IFN-γR1 subunits and two signal-transducing IFN-γR2 subunits (Schroder et al., 2004). Within

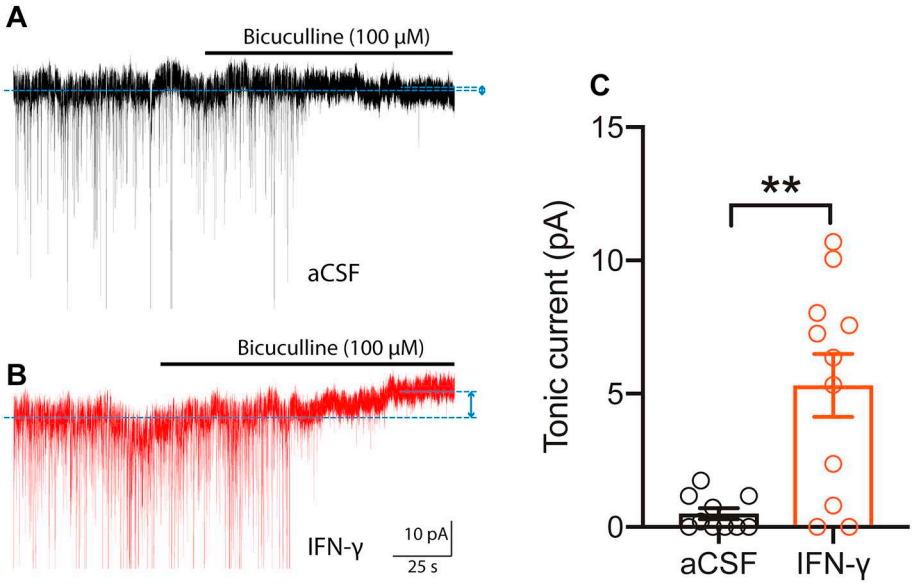

Fig. 3. Pre-incubation with IFN-γ potentiates GABA-mediated tonic currents in rat hippocampal CA1 pyramidal neurons.
(A and B) Representative current recordings showing the increased GABA-activated tonic currents in CA1 neurons of rat hippocampal slices incubated in artificial cerebrospinal fluid (aCSF) with IFN-γ (100 ng/ml) for 3 h **(B)** as compared to aCSF alone **(A)**. The amplitude of tonic current (arrow) was measured as the difference in the baseline holding current level (dashed lines) before and after the application of $GABA_AR$ antagonist bicuculline (100 μM). The neurons were held at −60 mV. **(C)** Summary scatter plots displaying significantly higher GABA-activated tonic currents in CA1 neurons from IFN-γ-incubated slices compared to aCSF controls (IFN-γ: $n = 10$ vs aCSF: $n = 11$; Unpaired student *t*-test, $p = .0012$). Data were presented as mean ± SEM.

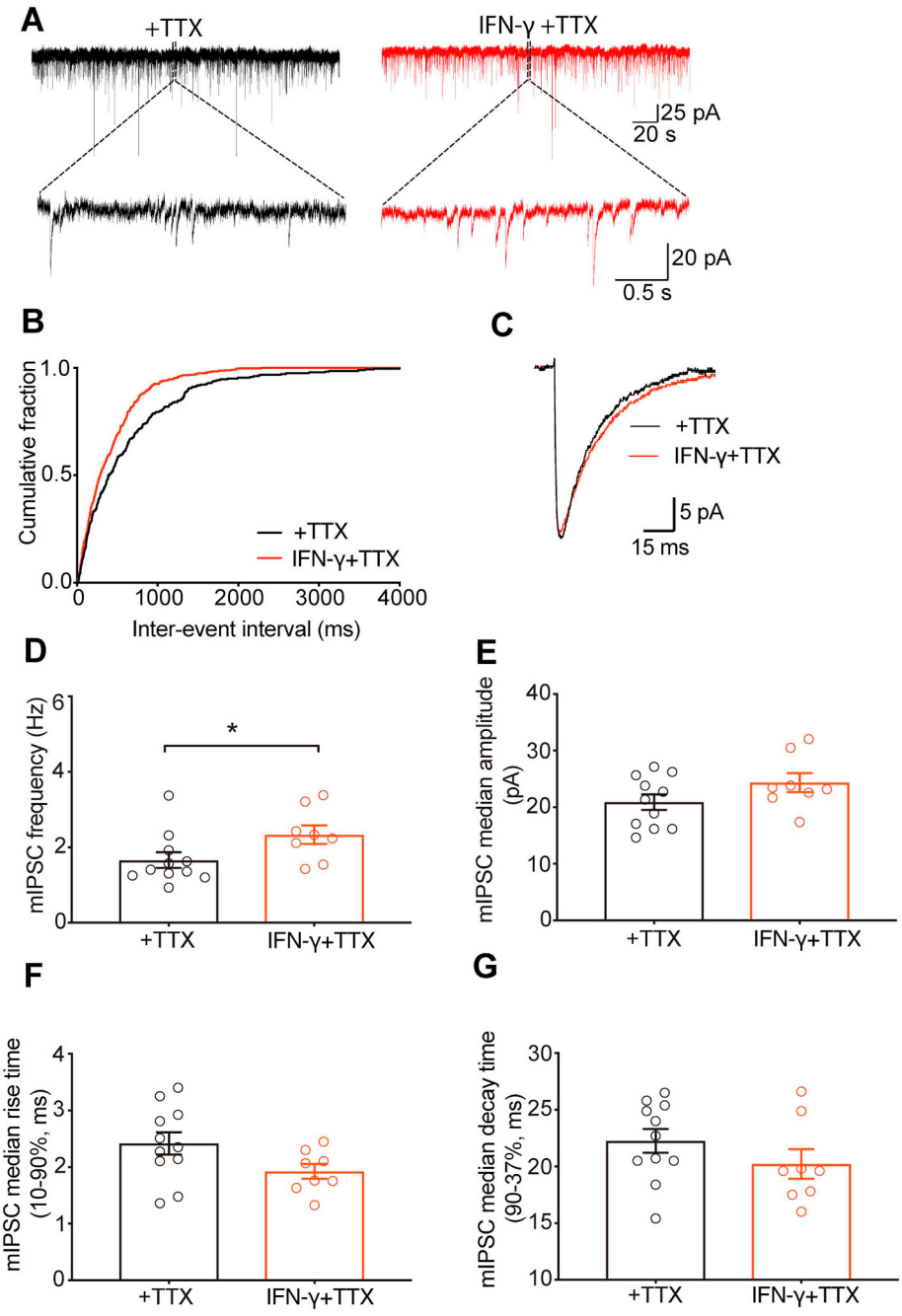

Fig. 4. IFN-γ increases the frequency of miniature inhibitory postsynaptic currents (mIPSCs) in rat hippocampal CA1 pyramidal neurons.
(A) Representative recordings (3 min) showing $GABA_AR$ -mediated mIPSC obtained from rat CA1 neurons in hippocampal slices pre-incubated in aCSF alone or with IFN-γ (100 ng/ml) for 3 h, in the presence of TTX (1 μM). The area between dashed lines was expanded to show the currents at a faster time scale in the lower panel. The neurons were held at −60 mV. **(B)** Cumulative probability plot showing inter-event intervals distribution of all mIPSC events detected in the upper panel of (A). **(C)** Rise time-scaled average mIPSC trace from individual mIPSC events in the upper panel of (A). **(D)** Frequency of mIPSC was significantly increased in rat CA1 neurons in hippocampal slices pre-incubated in aCSF with IFN-γ (100 ng/ml) as compared to aCSF alone (+TTX, n = 11; IFN-γ + TTX, $n = 8$; Mann-Whitney test, $^*p = .0259$). **(E–G)** There was no significant difference in mIPSC median amplitude (+TTX, 20.9 ± 1.36 pA, n = 11; IFN-γ + TTX, 24.34 ± 1.68 pA, n = 8; unpaired student t-test, $p = .1281$), or median rise time (10–90%) (+TTX, 2.42 ± 0.2 ms, n = 11; IFN-γ + TTX, 1.93 ± 0.13 ms, n = 8; unpaired student t-test, $p = .073$), or median decay time (90–37%) (+TTX, 22.25 ± 1.05 ms, n = 11; IFN-γ + TTX, 20.23 ± 1.3 ms, n = 8; unpaired student *t*-test, $p = .2369$) in aCSF with IFN-γ (100 ng/ml) as compared to aCSF alone. Data were presented as mean ± SEM.

the brain, IFN-γ receptors are broadly expressed in many cell types including microglia, astrocytes, oligodendrocytes, endothelial cells, neural precursor cells and neurons (Hashioka et al., 2010; Hausler et al., 2002; Li et al., 2010; Mizuno et al., 2008; Ni et al., 2014). Notably, the levels of IFN-γ receptor expression differ among cell types, brain regions and species and can alter with external stimuli and in vivo/in vitro conditions (Hashioka et al., 2010; Lee et al., 2006; Li et al., 2010; Robertson et al., 2000). The detection of both *Ifngr1* and *Ifngr2* gene transcripts in hippocampal tissue from rats between postnatal days 16 and 22, is consistent with previous observations that IFN-γR1 is present in the hippocampus (Mizuno et al., 2008; Neumann et al., 1997; Robertson et al., 2000; Utsuyama and Hirokawa, 2002). However, there are some discrepancies among different studies regarding the spatial distribution of IFN-γ receptor in the hippocampus. For instance, Neumann et al. (1997) have shown that in the rat hippocampal neuronal culture all electrically active neurons confirmed by patch-clamp recordings express the *Ifngr1* mRNA transcript. In addition, the IFN-γR1 immunoreactivity was detected in both neurons and glial cells (Neumann et al., 1997). In contrast, another immunohistochemical study reported that the immunostaining of IFN-γR1 was observed in the molecular layer of the dentate gyrus but not in CA1 or CA2 fields in the adult rat hippocampus (Robertson et al., 2000). We have also examined a recently released RNA-seq database Hipposeq (https://hipposeq.janelia.org) (Cembrowski et al., 2016) and found that both *Ifngr1* and *Ifngr2* gene transcripts are constitutively expressed in glutamatergic neurons of CA1–3 and dentate gyrus as well as parvalbumin (PV) or somatostatin (SST) expressing interneurons of mouse hippocampus. Furthermore, the presence of IFN-γ receptor in hippocampal microglia and astrocytes has been demonstrated, although the levels of expression differ depending on species and in vivo/in vitro conditions (Hashioka et al., 2010; Papageorgiou et al., 2016; Ta et al., 2019). Thus, the constitutive expression of IFN-γ receptors in several hippocampal cell

types indicates that the IFN-γ receptor signaling can regulate hippocampal functions by modulating the activity of a number of cell-types.

It is noteworthy that IFN-γ is normally present at low concentrations in the brain (Rady et al., 1995) and regulates normal brain physiology such as neurogenesis and synaptic plasticity (Filiano et al., 2016; Li et al., 2010; Monteiro et al., 2016). IFN-γ levels can increase under many pathological conditions particularly neuroinflammation (Ottum et al., 2015). In this study, we have pre-incubated the rat hippocampal slices with IFN-γ for 1–4 h at a concentration of 100 ng/ml, which has been shown to evoke maximal effects in microglia (Hausler et al., 2002; Ta et al., 2019). Notably, the actual IFN-γ concentration within the slices may be lower due to lack of blood flow, longer diffusion distance and limited extracellular space in hippocampal slices (McBain et al., 1990; Ta et al., 2019). The studies of the effects of IFN-γ on the neurotransmission have been mainly focused on the glutamatergic system (Mizuno et al., 2008; Sonekatsu et al., 2016; Vikman et al., 2001). Our current study demonstrates that the pre-treatment of IFN-γ enhanced GABAergic phasic and tonic currents, as evidenced by the increase in the frequency of both sIPSCs and mIPSCs as well as tonic current amplitude in CA1 neurons from IFN-γ pre-treated hippocampal slices. These results are somehow in line with a recent study showing acute application of IFN-γ potentiates GABA-mediated tonic currents in mouse prefrontal cortex layer 2/3 pyramidal neurons although the effect of IFN-γ on GABA-activated synaptic currents is not examined (Filiano et al., 2016). Additionally, deletion of IFN-γ receptor in inhibitory interneurons eliminates IFN-γ-enhanced tonic currents in layer 2/3 pyramidal neurons, indicating a pre-synaptic site of action (Filiano et al., 2016). In contrast, two previous studies have shown that IFN-γ reduces GABAergic activity in neurons (Muller et al., 1993; Vikman et al., 2007). Muller et al. (1993) has shown that acute application of IFN-γ decreased the amplitude of evoked inhibitory postsynaptic potential (IPSP) in cultured hippocampal CA3 neurons, suggesting a presynaptic mechanism. Intrathecal administration of IFN-γ in rats disrupts the GABAergic inhibition in the spinal dorsal horn neurons, as shown by that $GABA_AR$ antagonist bicuculline fails to decrease paired-pulse depression in the IFN-γ treated animals (Vikman et al., 2007). Several factors may contribute to this discrepancy, including (1) the concentration of IFN-γ and the duration of application; (2) experimental models (acute brain slice preparation vs. brain slice culture); (3) the brain sub-regions and neuronal subtypes investigated (Filiano et al., 2016; Muller et al., 1993; Vikman et al., 2007). Importantly, in this study the increase in the frequency but not the mean amplitude, rise time (10–90%) or decay time (90–37%) of both sIPSCs and mIPSCs in CA1 neurons lends the support for a presynaptic mechanism. Indeed, the increased sIPSC and mIPSC frequencies could be explained by an enhancement in interneuron excitability and activity-independent quantal release of GABA, respectively. Additionally, the pre-incubation of hippocampal slices with IFN-γ could first activate microglia and astrocytes and secondly affect the GABAergic neurotransmission by mechanisms such as the release of other cytokines and modulation of GABA reuptake (Losi et al., 2014; Ta et al., 2019).

The level of GABA-activated tonic inhibition depends on the neuronal cell types, the composition of extrasynaptic $GABA_ARs$ and the extracellular GABA concentration (Birnir and Korpi, 2007; Semyanov et al., 2003). In the aCSF-incubated hippocampal slices, we detected no or minor tonic current in CA1 pyramidal neurons when no exogenous GABA or GABA transporter blocker was added, which is in accordance with previous reports (Jin et al., 2011b; Semyanov et al., 2003). In contrast, the tonic currents in CA1 pyramidal neurons are potentiated in slices pre-treated with IFN-γ, which can be explained by the increase of extracellular GABA concentration due to GABA spillover from synapses or reverse transport of GABA by GABA transporters, or modulation of extrasynaptic $GABA_ARs$ in CA1 pyramidal neurons. The binding of IFN-γ to its heterotetrameric receptor results in the receptor dimerization and the predominant activation of the Janus associated kinase/signal transducer and activator of transcription-1 (JAK/STAT) signaling pathway (de Weerd and Nguyen, 2012). Interestingly, it has been shown that the modulation of JAK/STAT pathway affects the expression of specific $GABA_AR$ subunits in hippocampal neurons (Lund IV et al., 2008; Raible et al., 2015). However, the actual mechanisms underlying IFN-γ-mediated potentiation of GABAergic transmission in hippocampal CA1 neurons should be addressed in future studies.

The presence of IFN-γ receptors in both neurons and glia suggests that IFN-γ signaling can directly or indirectly influence the GABAergic neurotransmission. The altered GABAergic inhibition inevitably affects neurocircuits and contributes to cognitive dysfunctions in various neurological disorders (Contestabile et al., 2017; Xu and Wong, 2018). During the inflammatory processes triggered by aging, infection, injury or neurological disorders, specific immune cells (including T cells) that normally patrol in the compartments surrounding the brain parenchyma can infiltrate into the brain parenchyma and produce cytokines including IFN-γ (Filiano et al., 2017; Gemechu and Bentivoglio, 2012). Therefore, our results suggest that the augmentation of GABAergic inhibition in hippocampal CA1 pyramidal neurons by IFN-γ may play a role in mediating cognitive dysfunctions under pathological conditions.

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Declaration of Competing Interest

None.

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