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Astrocytic IGF-IRs induce adenosine-mediated inhibitory down regulation and improve sensory discrimination.

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ABSTRACT

Insulin-like growth factor-I (IGF-I) signalling plays a key role in learning and memory processes. While the effects of IGF-I on neurons have been studied extensively, the involvement of astrocytes in IGF-I signalling and the consequences on synaptic plasticity and animal behavior remain unknown. We have found that IGF-I induces long-term potentiation (LTP, here called LTP_{IGFI}) of the postsynaptic potentials that is caused by a long-term depression (LTD) of inhibitory synaptic transmission in mice. We have demonstrated that this long-lasting decrease in the inhibitory transmission is evoked by astrocytic activation through its IGF-IRs. We show that LTP_{IGFI} not only increases the output of pyramidal neurons, but also favours the N-methyl-D-aspartate (NMDAR) dependent LTP, resulting in the crucial information processing at the Barrel cortex since specific deletion of IGF-IR in cortical astrocytes ($IGF\text{-}IR^{-/-}$) impairs the whisker discrimination task. Our work reveals a novel mechanism and functional consequences of IGF-I signalling on cortical inhibitory synaptic plasticity and animal behavior, revealing that astrocytes are key elements in these processes.

SIGNIFICANCE STATEMENT

Insulin-like growth factor-I (IGF-I) signaling plays key regulatory roles in multiple processes of brain physiology, such as learning and memory. Yet, the underlying mechanisms remain largely undefined. Here we demonstrate that astrocytes respond to IGF-I signaling, elevating their intracellular Ca^{2+} and stimulating the release of ATP/adenosine, which triggers the LTD of cortical inhibitory synapses, thus regulating the behavioral task performance related to cortical sensory information processing. Thus, the present work represents a major conceptual advance in our knowledge of the cellular basis of IGF-I signaling in brain function, by including for the first time astrocytes as key mediators of IGF-I actions on synaptic plasticity, cortical sensory information discrimination and animal behavior.

INTRODUCTION

Insulin-like growth factor-I (IGF-I) is a peptide involved in learning and memory (Ross, 2005;Aleman and Torres-Alemán, 2009). Indeed, a deficiency in circulating IGF-I results (Nishijima et al., 2010) in an impairment of the processing speed and deficiencies in both spatial and working memories (Deijen et al., 1996; Lijffijt et al., 2003; Sytze Van Dam, 2005; Kołtowska-Häggström et al., 2006;Adamsky et al., 2018), whereas replacing IGF-I reverses many of these cognitive deficits (Trejo et al., 2007). IGF-I regulates neuronal firing (Nuñez et al., 2003; Gazit et al., 2016) and modulates excitatory synaptic transmission in many areas of the brain (Nilsson et al., 1988; Araujo et al., 1989; Castro-Alamancos and Torres-Aleman, 1993; Seto et al., 2002; Maya-Vetencourt et al., 2012). IGF-I produces a long-lasting depression of glutamate-mediated GABA release by Purkinje cells in the cerebellum (Castro-Alamancos and Torres-Aleman, 1993), or a long-term potentiation of GABA release in the olfactory bulb (Liu et al., 2017). However, whether IGF-I is involved in the long-lasting plasticity of inhibitory synaptic transmission in the neocortex remains unexplored.

The level of IGF-I modulates the plasticity of the somatosensory cortex in rats exposed to sensory motor restriction (Mysoet et al., 2015). The Barrel cortex is the area of the somatosensory cortex involved in processing tactile information coming from the vibrissae, which is an extremely important factor in survival (Carvell and Simons, 1990). Interestingly, the uptake of IGF-I by the brain correlates with frequency-dependent changes in cerebral blood flow in the Barrel cortex during whisker stimulation (Nishijima et al., 2010), indicating that IGF-I-induced plasticity at the Barrel cortex is crucial in information processing in this cortical area. Moreover, somatosensory cortex astrocytes respond to sensory stimuli and regulate the sensory-evoked neuronal network (Lines et al., 2020). Indeed, astrocytes are of key importance

in the induction of the long-term modulation of excitatory synaptic transmission induced by the spike timing dependent protocol (Andrade-Talavera et al., 2016). They respond with Ca^{2+} elevations to neurotransmitters released by neurons and induce changes in neuronal excitability and synaptic transmission by releasing gliotransmitters (Araque et al., 2001; Nedergaard et al., 2003; Volterra and Meldolesi, 2005; Haydon and Carmignoto, 2006; Perea et al., 2009; Parpura and Zorec, 2010; Singh et al., 2014). In addition to glutamate, astrocytes also release ATP that in turn depresses excitatory synaptic transmission in the hippocampus (Serrano, 2006; Andersson et al., 2007; Chen et al., 2013). Moreover, in the neocortex, the exocytosis of ATP from astrocytes leads to a short term down regulation of inhibitory synaptic currents by inhibiting postsynaptic and extrasynaptic $GABA_A$ receptors in layer II/III pyramidal neurons (Lalo et al., 2014). Further, astrocytes can release IGF-I (Chisholm and Sohrabji, 2016), and several studies have shown the presence of IGF-I and IGF-IRs in neurons, astrocytes and microglia (Ocrant et al., 1988; Zhou et al., 1999; Garwood et al., 2015; Rodriguez-Perez et al., 2016). However, the effect of astrocytic IGF-IR activation on the long-term synaptic plasticity in the Barrel cortex is yet to be elucidated.

Here, we have investigated whether astrocytic IGF-IR activation is participating in long-term synaptic transmission changes. We found that IGF-I induces a LTP of the post-synaptic potentials (PSPs) (LTP_{IGFI}) that matches a presynaptic LTD of IPSCs that depend on an increase in cytosolic calcium in the astrocytes, and the activation of A_{2A} adenosine receptors. This LTD is absent in mice in which IGF-IR has been deleted specifically in astrocytes ($IGF\text{-}IR^{-/-}$ mice). We show that the LTP_{IGFI} favours the NMDAR dependent LTP of the PSPs, in contrast to $IGF\text{-}IR^{-/-}$ mice that show impairment in their performance of a whisker discrimination task. We show that the activation of astrocytes by IGF-I is essential for inducing Hebbian plasticity. Therefore,

we present a novel mechanism of long-term synaptic depression of inhibition at the Barrel Cortex induced by the activation of astrocytic IGF-IRs and ATP/Adenosine (ATP/Ado) release from astrocytes that have an important impact on the processing of somatosensory information occurring during the whisker discrimination task.

MATERIALS AND METHODS

Materials: 2-(2-Furanyl)-7-(2-phenylethyl)-7*H*-pyrazolo[4,3-*e*][1,2,4] triazolo [1,5-*c*] pyrimidin-5-amine (SCH58261 (SCH), 100 nM) and 8-Cyclopentyltheophylline (CPT, 5 µM) and BAPTA-AM (10 µM) were purchased from Tocris. NVP AEW541 (NVP, 400 nM) was purchased from Cayman. Recombinant Human IGF-I (IGF-I, 10 nM) was from Peprotech. BAPTA-K4 (40 mM) was from Santa Cruz Byotechnology. Fluo-4-AM (2 µM) was purchased from Invitrogen and the ATP Assay Kit was from Abcam. All the remaining drugs were purchased from Sigma-Aldrich (CNQX 20µM and D-AP5 50M).

Key resources table

Bacterial and Virus Strains

AAV8-GFAP-mCherry (3.13 x 10^{13} GC/ml) from UMN vector core.

AAV8-GFAP-mCherry-CRE (3.8 x 10^{12} GC/ml) from UMN vector core.

AAV5-pZac2.1-gfaABC1D-cyto-GCAMP6f (7×10^{12} vg/mL) from Penn Vector Core.

Experimental Models: Organisms/Strains

IGF-IR flox/flox	The Jackson Laboratory	JAX #012251
CreERT2.GFAP	The Jackson Laboratory	JAX #012849

Ethics statement and animals. All animal procedures were approved by the Ethical Committee of the Universidad Autónoma of Madrid, Cajal Institute and University of Minnesota Animal Care and Use Committee (IACUC) and are in accordance with Spanish (R.D. 1201/2005) and European Community Directives

(86/609/EEC and 2003/65/EC) and the National Institutes of Health guidelines for the care and use of laboratory animals in the USA. Male C57BL/6J or transgenic mice were housed under a 12-h/12-h light/dark cycle with up to five animals per cage and were used for slice electrophysiology. Transgenic mice with a deletion of IGF-IR in astrocytes (GFAP-IGF-IR$^{-/-}$ mice) were obtained by injecting IGF-IR$^{f/f}$ mice (B6, 129 background; Jackson Labs) with AAV8-GFAP-mCherry (UMN vector core) or AAV8-GFAP-mCherry-CRE viral vectors (UNC vector core). In the Behavioural studies, adult male and female control (C57BL/6JolaHsd; 6-12 months old; 28-35g), and transgenic mice were used. Transgenic mice with tamoxifen-regulated deletion of IGF-IR in astrocytes (IGF-IR-/- mice) were obtained by crossing IGF-IRf/f mice (B6, 129 background (IGF-IRflox/flox mice; Jackson Labs; stock number: 012251) with CreERT2.GFAP mice (C57B&/6xSJL/J mix background; Jackson Labs, stock number: 012849, see (Ganat et al., 2006) for further details.

Slice preparation. Barrel cortex slices were prepared as described previously (Díez-García, 2017). Briefly, the mice were sacrificed, and their brains submerged in cold (4 °C) cutting solution containing (in mM): 189.0 sucrose, 10.0 glucose, 26.0 $NaHCO_3$, 3.0 KCl, 5.0 Mg_2SO_4, 0.1 $CaCl_2$, 1.25 $NaH_2PO_4.2H_2O$. Coronal slices (350 µm thick) were cut using a Vibratome (Leica VT 1200S), and incubated (> 1h, 25–27 °C) in artificial cerebrospinal fluid (ACSF) containing (in mM): 124.00 NaCl, 2.69 KCl, 1.25 KH_2PO_4, 2.00 Mg_2SO_4, 26.00 $NaHCO_3$, 2.00 $CaCl_2$, and 10.00 glucose). The pH was stabilized at 7.3 with a 95 % O_2, 5 % CO_2 mixture. The slices were transferred to a 2 ml chamber fixed to an upright microscope stage (BX51WI; Olympus, Tokyo, Japan) equipped with infrared differential interference contrast video (DIC) microscopy and superfused at room temperature with 95 % O_2, 5 % CO_2-bubbled ACSF (2 ml/min).

Electrophysiological recordings in slices. Patch-clamp recordings from layer II/III pyramidal neurons of the Barrel cortex were performed in whole-cell voltage-clamp and current-clamp configurations as described previously (Díez-García, 2017). Briefly, patch pipettes (4–8 MΩ) filled with an internal solution that contained (in mM): 135 $KMeSO_4$, 10 KCl, 10 HEPES-K, 5 NaCl, 2.5 ATP-Mg^{+2}, and 0.3 GTP-Na^{+}, pH 7.3. In some experiments, a BAPTA-based intracellular solution was used (in mM): 40 BAPTA-K_4, 2 ATP-Na^{2+}, 10 mM HEPES, 1 $MgCl_2$ and 8 NaCl, pH 7.3. The holding potential was adjusted in a range from -70 to -80 mV, and series resistance was compensated to ≈ 80 %. Layer II/III pyramidal neurons located over the Barrels (layer IV) were accepted only when the seal resistance was >1 GΩ and the series resistance (10–20 MΩ) did not change (>20 %) during the experiment. Inhibitory synaptic currents (IPSCs) were isolated in the presence of AMPAR and NMDAR antagonists (20 µM CNQX and 50 µM D-AP5, respectively). IPSCs were evoked with a bipolar stimulation electrode pulled from a theta borosilicate glass capillary (tip diameter, 5–8µm), filled with ACSF, and connected to a Grass S88 stimulator and a stimulus isolation unit (Quincy, USA digital stimulator) through chloride-silver electrodes. The stimulating electrodes were placed at layer IV of the Barrel cortex near the tip (~100-150 µm) of the recording pipette. Paired pulses (200-µs duration and 50 ms-interval) were continuously delivered at 0.33 Hz. After recording 5 minutes of stable baseline of the IPSCs, IGF-I was added to the bath for 35 minutes. To check for the induction of long lasting synaptic plasticity the IGF-I was washed for at least 10 minutes and the amplitude of the IPSCs after IGF-I washout was continuously checked. The pre- or postsynaptic origin of the observed regulation of synaptic currents was tested by estimating changes in the paired pulse ratio (PPR) of the IPSCs (Creager et al., 1980; Clark et al., 1994; Kuhnt and Voronin, 1994). To estimate the modification of the synaptic current variance, we

calculated the noise-free coefficient of variation (CV_{NF}) of the synaptic responses before (control conditions) and during IGF-I (Maglio et al., 2018).

Electrophysiological recording in vivo. We studied three-month-old male C57BL6/J mice weighing 25-30 g. Mice were group housed in clear plastic cages under a 12 h light/dark cycle and given *ad libitum* access to food and water. Surgery and recording were performed as previously described (Díez-García, 2017). Briefly, the mice were placed in a stereotaxic device under urethane (1.4 g/kg) in which surgical procedures and recordings were made. Local field potentials (LFPs) were obtained through tungsten microelectrodes (0.5-1 MΩ) located into the cortex. Signals were filtered (0.3-300 Hz for ECoG) and amplified via an AC P15 preamplifier (Grass, West Warwick, USA). After a stable baseline recording, IGF-I or saline (Six application of 84nL, one every 5 min, at a speed of 84 nl per minute) were administered intracraneal with a Hamilton Gasstight coupled to a MicroSyringePump Controller (WPI) stereostaxically placed at layer II/III of the Barrel cortex.

Stereotaxic surgery and virus delivery. Mice between 8-10 weeks' old were anesthetized with ketamine (100 mg/kg)/xylazine (10 mg/kg) mix. To selectively delete the IGF-IRs in cortical astrocytes, $IGF\text{-}IR^{f/f}$ mice were injected in the Barrel cortex with 500 nl of the viral vector AAV8-GFAP-mCherry-CRE (or AAV8-GFAP-mCherry as a control). To monitor the astrocyte calcium levels, the mice were injected with 500 nl AAV5-pZac2.1-gfaABC1D-cyto-GCAMP6f (Penn Vector Core). Injections were made at layer II/III of Barrel cortex with a Hamilton syringe attached to a 29-gauge needle at a rate of 0.1 µl/min. The coordinates used to reach the area were: anterior–posterior: -1.0 mm; medial–lateral: +/−3.50 mm; dorsal–ventral: −0.15 mm. Three weeks after the injection, successful delivery was confirmed by the location of the virus based on

mCherry expression. Immunohistochemistry analysis confirmed the selective expression of the virus in cortical astrocytes.

Ca^{2+} imaging. In GFAP-IGF-$IR^{-/-}$ mice and their controls, Ca^{2+} levels in the astrocyte soma and processes were obtained by two-photon microscopy (Leica DM6000 CFS upright multiphoton microscope with TCS SP5 MP laser) using the GCaMP6f viral vector targeted at astrocytes. In some experiments, the somatic Ca^{2+} levels were obtained using epifluorescence microscopy and the Ca^{2+} indicator Fluor-4-AM (5 μM in 0.01 % pluronic, incubated for 30-45 min at room temperature). Loaded cells were illuminated every 100 ms at 490 nm with a monochromator (Polychrome IV; TILL Photonics), and successive images were obtained at 1 Hz with a cooled monochrome CCD camera (Hammamatsu, Japan) attached to the Olympus microscope equipped with a filter cube (Chroma Technology). The camera control and synchronization for the epifluorescence were made by ImagingWorkbench software (INDEC-BioSystems) and for two-photon imaging by the Leica LAS software.

Astrocyte cultures and ATP release. Pure astroglial cultures were prepared as described (Fernandez et al., 2007). Postnatal (day 0-2) brains from C57BL/6J (WT) and constitutively GFAP IGF-$IR^{-/-}$ mice (Hernandez-Garzón et al., 2016) were removed and immersed in ice-cold Earle's balanced salt solution (ThermoFisher, Waltham, MA USA). The cortices were dissected and mechanically dissociated. The resulting cell suspension was centrifuged and plated in DMEM/F-12 (ThermoFisher) with 10 % fetal bovine serum (FBS, ThermoFisher) and 100 mg/ml of antibiotic-antimycotic solution (Sigma, St. Louis, MO, USA). Cell cultures were maintained in an incubator at 37 °C and 95 % humidity with 5 % CO2. Pure astrocyte monolayer cultures were replated at 2.5×10^5 cells/well in DMEM/F-12 with 10 % FBS medium. After two days the

medium was replaced by DMEM/F-12 for 3 hours followed by a one-hour treatment with 10 nM IGF-I (PreproTech, Rocky Hill, NJ, USA) or a one-hour treatment with inhibitors: 400 nM NVP-AEW541 (IGF-I receptor antagonist, Cayman, Ann Arbor, MI, USA), 10 µM BAPTA-AM (intercellular Ca^{2+} chelator, Sigma) followed by a one-hour stimulation with 10 nM IGF-I.

The amount of ATP released into the medium was measured using an ATP Assay Kit (Fluorometric, ab83355; Abcam, Cambridge, UK) according to the manufacturer's instructions. Firstly, the cells were washed with cold phosphate-buffered saline and resuspended in 100 µl of ATP assay buffer. Then, the cells were quickly homogenized by pipetting up and down and centrifuged for 2 min at 4 °C (18 000 g) to remove any insoluble material. The supernatant was collected and transferred to a clean tube and kept on ice. An ATP assay buffer (400 µL) and 100µL of ice cold 4M perchloric acid were added to the homogenate to deproteinate the samples. The homogenates were briefly vortexed and incubated on ice for 5 min, centrifuged at 18 000 g for 2 min at 4 °C, and the supernatant transferred to a fresh tube. The supernatant volume was measured and an equal volume of ice cold 2 M KOH was added. Finally, the homogenate was centrifuged at 18 000 g for 15 min at 4 °C and the supernatant collected. ATP reaction mix (50 µL) and 50 µL of sample were added to each well and incubated at 24 °C for 30 min, protected from light. The samples were measured on a FLUOstar microplate reader (BMG LabTech, Ortenberg, Germany) at 535/587 nm. Each sample was run in duplicate. The concentrations of ATP released from the astrocyte primary cultures were obtained from standard curves and normalized to the total amount of protein.

Behavioural experiments. Adult male and female control (C57BL/6JolaHsd; 6-12 months old; 28-35g), and transgenic mice with tamoxifen-regulated deletion of IGF-

IR in astrocytes (IGF-IR-/- mice, see above) were used. The IGF-IR activity was knocked down after the administration of tamoxifen, as described elsewhere (Hirrlinger et al., 2006). Tamoxifen was injected for 5 consecutive days intraperitoneally (75 mg/kg, Sigma) from the age of 1 month, and the mice were used at 2-7 months old. The controls used were their siblings treated only with the vehicle for tamoxifen injections (corn oil). Using the tdTomato/eGFP reporter mouse to detect Cre-mediated deletion in response to tamoxifen administration in CreERT2.GFAP mice, we previously documented that it was restricted to the astrocytes (García-Cáceres et al., 2016). Multiplex PCR for mouse genotyping included a common forward primer (P3, 5'−CTG TTT ACC ATG GCT GAG ATC TC−3') and two reverse primers specific for the wild-type (P4, 5'−CCA AGG ATA TAA CAG ACA CCA TT−3') and mutant (P2, 5'-CGC CTC CCC TAC CCG GTA GAA TTC-3') alleles. The GFAP-T-IGF-IR mice showed lower levels of IGF-IR in their brains after tamoxifen injection. The mice were housed in standard cages (48 × 26 cm2) with 4 - 5 animals per cage, kept in a room with controlled temperature (22 °C) under a 12-12h light-dark cycle, and fed with a pellet rodent diet and water ad libitum. All experimental protocols were performed during the light cycle between 14 pm - 18 pm. The animal procedures followed European guidelines (2010/63/EU) and were approved by the local Bioethics Committee (Madrid Government). ***qPCR:*** Total RNA isolation from their brain tissue was carried out with Trizol. One mg of RNA was reverse transcribed using a high-capacity cDNA reverse transcription kit (ThermoFisher Scientific, USA) according to the manufacturer's instructions. cDNA (62.5 ng) was amplified using TaqMan probes for mouse IGF-I receptor (IGF-IR), and rRNA 18S as the endogenous control (ThermoFisher Scientific). Each sample was run in triplicate in 20 µl of reaction volume using a TaqMan Universal PCR Master Mix according to the manufacturer's instructions (ThermoFisher

Scientific). All the reactions were performed in a 7500 Real Time PCR system (ThermoFisher Scientific). Quantitative real time PCR analysis was carried out as described (Pfaffl, 2001). The results were expressed as relative expression ratios on the basis of group means for target transcripts versus the reference 18S transcript.

For the behavioural tests, both sexes were used and balanced between experimental groups. Since the wild type mice and their control littermates performed similarly in the tests, they were pooled and presented as a single control group. We performed the following three behavioural tests:

(1) Gap-crossing test. To assess sensory perception through the whiskers we used the gap-crossing test that consists of a series of trials in which the mouse has to cross a gap with increasing distances (Hutson and Masterton, 1986; Barnéoud et al., 1991). Each mouse was placed in the centre of an elevated platform (5 cm wide, 9 cm long) connected to a safe black cylindrical tube (8 cm diameter, 9 cm long). The gap distance between the platform and the cylindrical tube ranged from 0 to 8 cm in 1 cm increments. The test was performed under infrared lighting, the trials were recorded with a video camera and the maximum distance crossed by each animal was measured.

(2) Y-maze spontaneous alternation test. Working memory was assessed by recording spontaneous activity while exploring a Y-maze (Sarter et al., 1988). The maze was made of black-painted wood and each arm was 25 cm long, 14 cm high, 5 cm wide, and positioned at equal angles. The trials lasted 8 min each. After each trial, the maze was cleaned with 70 % ethanol to remove any olfactory cues. An offline analysis of the videos was carried out to obtain the sequence of entries during the test. Alternate behavior was calculated as the percentage of real alternations (number of triplets with non-repeated entries) versus total alternation opportunities (total number of triplets).

(3) Whisker discrimination test. To assess the ability of the mice to discriminate different textures with their whiskers, we adapted the two-trial Y-maze test described in previous work (Dellu et al., 2000; Hausrat et al., 2015). The apparatus was constructed of black-painted wood with three arms, each 25 cm long, 5 cm wide, and 14 cm high. The walls of the maze arms were covered with two different grades of black sandpaper. While two arms (familiar) were covered with a 500 grit sandpaper, the third (novel) was covered with 220 grit sandpaper. Because the three arms of the maze were identical, and there are no extra-maze cues, the discrimination of novelty vs familiarity relies only on the different textures that the mouse can perceive with its whiskers. The experiments were conducted in a room with dim illumination (6 lux). During the acquisition phase the mice were placed at the end of one of the familiar arms (in a random order) and were allowed to explore both familiar arms (500 grit sandpaper) for 5 min while the third arm (novel; 220 grit sandpaper) was closed with a guillotine door. At the end of the first trial the mice were returned to their home cage for 5 min. In the retrieval phase the mice were again placed at the end of the same arm where they started the acquisition phase and allowed to freely explore all three arms for 5 min. To remove any possible olfactory cues, the maze was cleaned with 70 % ethanol between the trials. The time spent in each of the arms was recorded using a video camera, and the discrimination index [novel arm/ (novel+familiar arms)] × 100 calculated.

Data analysis: Electrophysiological data analysis was carried out in a Clampfit 10 (Axon Instrument) and an ImageJ (NIH, Bethesda, Maryland, USA) was used for the calcium imaging. The graphs were drawn in SigmaPlot 11 (Systat Software Inc, San Jose, CA, USA). The fluorescence values are given as $\Delta F/F_0$ ($\Delta F/F_0 = 100 \times (F-F_0)/F_0$), where F_0 is the pre-stimulus fluorescence level when cells were at rest, and F is the fluorescence at different times during activity. Data normality was tested using a

329 Kolmogorov–Smirnov test. We used the Student's two-tailed *t*-tests for unpaired or
330 paired data as required in the electrophysiology experiments. Data are presented as
331 means ± SEM. The threshold for statistical significance was $P< 0.05$(*); $P< 0.01$ (**)
332 and $P< 0.001$ (***) for the Student's test. Statistical analysis was performed using
333 GraphPad Prism 5.0 (La Jolla, CA, USA). To compare differences between two groups
334 and compare multiple variables, two, one-way ANOVA (parametric test) or kruskal-
335 wallis test (non-parametric test) was used, followed by a Holm-Sydak and Dunn (for
336 parametric and non-parametric test respectively) or Bonferroni post hoc test to compare
337 replicate means. Statistical differences were considered when $p < 0.05$. The results are
338 presented as the mean ± SEM of five independent experiments. (#$p< 0.05$, ##$p < 0.01$,
339 ###$p < 0.001$).
340

RESULTS

IGF-I induces LTP of PSPs (LTP_{IGFI}) at layer II/III pyramidal neurons

The experience-dependent plasticity of Barrel cortex layer IV neurons is largest when the experience is manipulated early (before P7), but the plasticity of layer II/III responses persists beyond the first week of life (Fox, 1992). During the second postnatal week, the layer IV map serves as a template for the growth and Hebbian refinement of the cortical circuitry underlying the layer II/III map, and layer IV to layer II/III feedforward excitatory projections are confined almost exclusively to one Barrel column (Lübke et al., 2000). Therefore, we investigated whether IGF-I modulates the efficacy of these synapses. We recorded the PSPs at layer II/III pyramidal neurons evoked by the stimulation of layer IV (**Figure 1A)**. After recording 5 minutes of stable baseline of the IPSCs, IGF-I was added to the bath for 35 minutes in an attempt to mimic the long lasting increase in serum IGF-I observed after a long-term resistance exercise (Tsai et al., 2015). After 5 min of stable recording, 10 nM IGF-I was applied for 35 min. The addition of IGF-I increased the number of synaptic stimuli that evoked action potentials (APs) (from 6.5 ± 2.1 in basal to 32.6 ± 4.5 number of APs after 15 min of IGF-I; ###p=0.0004, **Figure 1B**). In addition, it also induced a potentiation of the PSPs that remained for 30 min after the IGF-I washout (from 100.70 ± 1.58 in basal to 146.80 ± 9.06 % of peak amplitude at 60 min after IGF-I application; **p=0.0014, **Figure 1B**). These IGF-I-induced effects were prevented by bath perfusion of the IGF-IR selective inhibitor NVP-AEW 541 (from 6.4 ± 0.5 in basal to 12.4 ± 2.1 number of APs at 15 min after IGF-I application (p =0.101) and from 97.00 ± 1.87 in basal to 103.60 ± 12.27 % of peak amplitude at 60 min after IGF-I application; (p =0.6472), **Figure 1B**), indicating that were mediated by IGF-IR activation. Therefore, activation of IGF-IR induces a LTP of the PSPs (termed $LTP_{IGF\text{-}I}$).

In order to test whether the LTP can be also induced *in vivo*, we recorded the extracellular local field potentials (LFPs) at layer II/III of the Barrel cortex evoked by low-frequency deflections delivered at the principal whisker (**Figure 1C**). Following 15 minutes of control whisker-evoked LFP recording, IGF-I was applied in six IGF-I applications, one injection of 84 nL IGF-I (0.076mg/ml) every 5 minutes via a Hamilton syringe placed close to the recording eletrode (See methods). IGF-I application induced a long-lasting enhancement of the LFP (from 99.45 ± 1.80 in basal to 177.60 ± 21.00 % of LFP area at 80 min after IGF-I application; ***p =0.0002, **Figure 1D).** This enhancement in the synaptic efficacy was absent in the controls using a saline injection (from 99.94 ± 0.33 in basal to 90.98 ± 10.74 % of LFP area at 80 min after IGF-I application; p =0.4317, **Figure 1D**). Therefore, these results suggest that IGF-I induces the LTP of the synaptic potentials at layer II/III pyramidal neurons both *ex vivo* and *in vivo*.

Activation of IGF-IR in astrocytes induces presynaptic LTD of IPSCs (LTD_{IGFI})

The increase in the efficacy of the synaptic potentials could be due to the modulation of the inhibitory and/or excitatory synaptic transmission. To elucidate this issue, we first investigated whether IGF-I reduces the efficacy of inhibitory synaptic transmission. Inhibitory postsynaptic currents (IPSCs) were isolated in the presence of the glutamate receptor antagonists 20 μM CNQX and 50 μM AP5. After 5 min IPSC recording, IGF-I was bath applied for 35 min and then washed out. The IGF-I induced a long-term depression (LTD) of the IPSCs peak amplitude (termed LTD_{IGFI}) that persisted after IGF-I washout (from 100.60 ± 1.42 in basal to 60.73 ± 5.08 % of peak amplitude at 60 min after IGF-I application; ***p =0.0002, **Figure 2A, B**). The IGF-I-induced effects were prevented by NVP-AEW 541 (from 98.72 ± 0.50 in basal to 96.15 ± 3.59 % of

peak amplitude at 60 min after IGF-I application; p =0.9881, **Figure 2A**), indicating that they were mediated by IGF-IR activation. Because postsynaptic calcium increases are known to be required for the induction of long-term synaptic plasticity (Zucker, 1999), we then tested whether LTD_{IGFI} induction required cytosolic calcium elevation in the recorded neuron. Therefore, we included the Ca^{2+}chelator BAPTA (40 mM) in the patch pipette to prevent neuronal calcium elevations. However, a similar LTD_{IGFI} was induced in both the control and the neuron BAPTA-loaded conditions (from 100.08 ± 0.60 in basal to 68.67 ± 6.76 % of peak amplitude at 60 min after IGF-I application; **p =0.0066, **Figure 2C, BAPTAn**). To test the possibility of a presynaptic origin for the effect of IGF-I, we recorded the IPSCs evoked by paired-pulse stimulation (50-ms delay), and analyzed changes in paired-pulse responses (PPRs). The PPRs were increased during LTD_{IGFI} recorded both in the control condition and during the BAPTA loading in neurons (from 100.00 ± 3.23 in basal to 120.37 ± 5.33 % of PPR (***p =0.0002) in control and from 100.00 ± 3.40 in basal to 114.34 ± 4.59 % of PPR at 60 min after IGF-I application (**p =0.0015), in postsynaptic BAPTA condition, **Figure 2D, control and BAPTAn**) indicating the presynaptic origin of the LTD_{IGFI}. This increase in the PPR was prevented by NVP (from 100.00 ± 3.23 in basal to 99.95 ± 14.00 % of PPR at 60 min after IGF-I application; p =0.9636, **Figure 2D, NVP**). Taken together, these data suggest that IGF-I induces a presynaptic LTD of IPSCs, which do not require cytosolic calcium elevations in the recorded postsynaptic neuron.

Astrocytes are emerging as important cells involved in the regulation of synaptic transmission and plasticity (Araque et al., 2014) that are able to regulate inhibitory synaptic transmission in the hippocampus (Kang et al., 1998). Therefore, we investigated whether LTD_{IGFI} requires cytosolic calcium elevations in astrocytes. The application of IGF-I induced an increase in the frequency of astrocyte calcium

elevations (from 0.71 ± 0.09 in basal to 1.35 ± 0.12 after IGF-I application; **p =0.0023, **Figure 3A, control**) that was absent under NVP (from 0.98 ± 0.10 in basal to 1.00 ± 0.05 after IGF-I application; p =0.8909, **Figure 3A, NVP**). We then tested whether the IGFI-induced enhancement of the astrocyte calcium signal contributed to the LTD_{IGFI}. We prevented the Ca^{2+} signal selectively in astrocytes by recording a cortical astrocyte with a patch pipette containing 40 mM BAPTA. Since cortical astrocytes are gap-junction connected, injecting BAPTA into a single astrocyte can diffuse through a large extension of the gap junction-coupled astrocytic network (Navarrete and Araque, 2010). However, BAPTA-loading the astrocytic network, IGF-I failed to modulate the IPSCs (from 99.79 ± 0.62 in basal to 92.75 ± 6.20 % of peak amplitude at 60 min after IGF-I application; p =0.3222, **Figure 2C, BATPAa**) or the PPR (from 100.00 ± 3.86 in basal to 98.01 ± 2.48 % of PPR at 60 min after IGF-I application; p =0.5896. **Figure 2D, BAPTAa**). Therefore, these data suggest that cytosolic Ca^{2+} increases in the astrocytes, not in the postsynaptic neurons, are essential for the induction of LTD_{IGFI}.

Although LTD_{IGFI} requires astrocyte calcium elevations, astrocyte stimulation by IGF-I could occur directly either through the activation of astrocytic receptors, or indirectly through the activation of neuronal receptors that trigger an indirect signalling pathway. To discriminate between these two possibilities, we deleted IGF-IR specifically in astrocytes using a combined viral and genetic approach. Mice with floxed IGF-IR gene ($IGF\text{-}IR^{f/f}$ mice) were injected in the Barrel cortex with the virus AAV8-GFAP-Cre-mCherry (**Figure 3B, bottom images)** (or AAV8-GFAP-mCherry as a control. **Figure 3B, top images**) that included the Cre-recombinase under the astroglial promoter GFAP, and mCherry as a reporter**.** In $IGF\text{-}IR^{f/f}$ mice injected with AAV8-GFAP-Cre-mCherry, mCherry expression was largely reduced and confined to the soma (**Figure**

2F, mCherry**,** IGF-IR$^{-/-}$) compared to the higher and more spread signal in control littermates (**Figure 2F,** mCherry, IGF-IR$^{+/+}$). As expected, in the IGF-IR$^{+/+}$ mice, IGF-I elevated astrocyte calcium levels (from 1.60 ± 0.03 in basal to 2.93 ± 0.05 frequency of calcium event after IGF-I application; p =0.0054, **Figure 3C, H, IGF-IR$^{+/+}$**) inducing the LTD_{IGFI} and increasing the PPR (from 99.89 ± 0.80 in basal to 74.74 ± 4.97 % of peak amplitude (*p =0.0108) at 60 min after IGF-I application and from 100.00 ± 2.64 in basal to 120.92 ± 1.31 % of PPR at 60 min after IGF-I application; **p =0.0034, **Figure 2C, D**, **IGF-IR$^{+/+}$**). By contrast, in the IGF-IR$^{-/-}$ mice, IGF-I did not modify the frequency of calcium elevations in astrocytes (from 1.23 ± 0.02 in basal to 1.04 ± 0.004 frequency of calcium event after IGF-I application; p =0.2128) or alter the IPSC amplitude and PPR (from 100.27 ± 1.55 to 107.16 ± 5.62 % of peak amplitude (p =0.3106); from 100.00 ± 5.36 to 102.92 ± 5.05 % of PPR (p =0.7014) in basal and at 60 min after IGF-I application respectively, **Figure 2C, D and Figure 3D IGF-IR$^{-/-}$**). These results further support the idea that LTD_{IGFI} depends on IGF-IR activation in the astrocytes.

LTD_{IGFI} requires A_{2A} adenosine but not mGluR receptor activation

Astrocytic activation stimulates the release of glutamate that regulates synaptic transmission by acting on metabotropic glutamate receptors (mGluRs) (Perea and Araque, 2007). We therefore checked whether the LTD_{IGFI} is mediated by the activation of metabotropic glutamate receptors (mGluRs). However, LTD_{IGFI} was unaffected (from 98.89 ± 0.90 in basal to 60.78 ± 7.39 % of peak amplitude at 60 min after IGF-I application; **p =0.0022, **Figure 2C, MPEP+LY**) by treatment with the antagonists of group I metabotropic glutamate receptors MPEP and LY367385 and the increase observed in the PPR induced by IGF-I was preserved (from 100.00 ± 4.06 in basal to

120.52 ± 7.66 % of PPR, at 60 min after IGF-I application; *p =0.024, **Figure 2D, MPEP+LY**). Moreover, we observed that IGF-I evoked an increase in calcium event frequency in the presence of MPEP + LY367385 (from 0.51 ± 0.12 in basal to 1.07 ± 0.14 frequency of calcium event after IGF-I application; **p =0.0066, **Figure 3A, MPEP + LY**). Although astrocytes are able to release glutamate, these data rule out the requirement of mGluR activation in the induction of $LTD_{IGFI.}$

Astrocytes may also release ATP which after being converted to adenosine may regulate synaptic transmission (Panatier et al., 2011; Araque et al., 2014). Therefore, we tested whether astrocytic ATP/Adenosine (ATP/Ado) was responsible for the LTD_{IGFI}. The LTD_{IGFI} was abolished by the antagonist of adenosine A_{2A} receptors, antagonist SCH 58261 (from 100.02 ± 0.63 in basal to 94.98 ± 2.43 % of peak amplitude at 60 min after IGF-I application; p =0.0916, **Figure 2C, SCH**), but not by the antagonist of adenosine A1 receptors, CPT (from 98.72 ± 1.75 in basal to 55.74 ± 7.08 % of peak amplitude at 60 min after IGF-I application; ***p =0.0008, **Figure 2C, CPT**). Moreover, the increase in PPR induced by IGF-I was preserved in the presence of CPT but absent in SCH 58261 (from 100.00 ± 2.09 to 99.76 ± 1.85 (p =0.8967) and from 100.00 ± 5.90 to 118.42 ± 4.95 % of PPR (**p =0.0023), in basal and at 60 min after IGF-I application in SCH and CPT respectively, **Figure 2D SCH and CPT**). To test whether adenosine-receptor activation occurs downstream of astrocytic calcium activity, we analyzed the effects of A_{2A} and A_1 receptor antagonists on astrocytic calcium signals. We observed that IGF-I still evoked an increase in calcium event frequency in the presence of SCH and CPT (from 0.67 ± 0.12 to 1.40 ± 0.17 (*p =0.0194) and from 0.59 ± 0.10 to 1.20 ± 0.13 (***p =0.0008) frequency of calcium event in basal and after IGF-I application in SCH and CPT respectively, **Figure 3A, SCH and CPT**). Taken together these results

suggest that IGF-I stimulates the astrocytic release of ATP that induces LTD_{IGFI} acting downstream of astrocytic calcium activity.

Therefore, we tested whether IGF-I was capable of stimulating the release of ATP from astrocytes. We used an ATP Assay Kit (see Materials and Methods) to monitor ATP levels in cultured astrocytes before and after 1 h treatment with IGF-I (10 nM). We found that IGF-I elevated the extracellular levels of ATP (1.1 ± 0.04 nmol/µg; ***p<0.001, **Figure 3E control**), an effect that was prevented when cultures where simultaneously treated with NVP (0.25 ± 0.05 nmol/µg, **Figure 3E NVP**). Furthermore, the IGF-I-induced release of ATP was absent when astrocyte calcium signalling was prevented by treating cultures with BAPTA-AM (0.45 ± 0.04 nmol/µg, **Figure 3E, BAPTA-AM**). Moreover, ATP was not released when astrocytes were obtained from mice lacking IGF-IR. (1.1 ± 0.02 nmol/µg and 0.3 ± 0.01 nmol/µg in $IGF\text{-}IR^{+/+}$ and $IGF\text{-}IR^{-/-}$ respectively. **Figure 3E $IGF\text{-}IR^{-/-}$ and $IGF\text{-}IR^{+/+}$**). These data suggest that IGF-I, acting through the IGF-IR in astrocytes, stimulates the calcium-dependent release of ATP, which leads to the activation of A_{2A} receptors, thus inducing LTD_{IGFI}.

IGF-IR activation induces a short term potentiation of the EPSCs.

The next step was to analyse whether IGF-I could also modulate the efficacy of excitatory synaptic transmission. In order to measure the effect of IGFI in excitatory synaptic transmission when the inhibition is present, we repeated the experiment illustrated in Figure 1B but instead of recording the PSPs, we recorded the EPSCs at the reversal potential of the inhibitory synaptic transmission (-70mV). In these experiments, as in Figure 1B, IGF-I still increased the number of synaptic stimuli that evoked APs (from 8.0 ± 2.5 in basal to 30.5 ± 4.8 number of APs at 15 min after IGF-I application; ##p =0.0044, **Figure 4A**). Moreover, IGF-I induced a significant short-term

potentiation (STP) of the ESPCs (termed STP_{IGF-I}, from 102.00 ± 2.16 in basal to 141.32 ± 9.10 % of peak amplitude at 20 min after IGF-I application; **p =0.0011. **Figure 4A, B)**. This effect was prevented by the IGF-IR selective inhibitor NVP-AEW 541 (from 4.4 ± 2.3 in basal to 5.2 ± 3.5 number of APs at 15 min after IGF-I application (p=0.1806) and from 99.10 ± 0.79 in basal to 103.0 ± 3.02 % of peak amplitude at 20 min after IGF-I application; p =0.2338. **Figure 4A, C**). Since an increase in cytosolic Ca^{2+} in astrocytes is required for the induction of LTD_{IGFI} (see above), we explored whether the induction of STP_{IGFI} also depended on the activation of astrocytes. After BAPTA-loading into the astrocytic network, IGF-I was not able to modulate the EPSCs (from 95.10 ± 1.46 in basal to 108.21 ± 10.51 % of peak amplitude at 20 min after IGF-I application; p =0.2571, **Figure 4C, BAPTAa**). These results confirm that the STP_{IGFI} not only depends on IGF-IR activation in astrocytes, but also on calcium elevations in their cytosol.

We then checked whether the potentiation of EPSCs by IGF-I requires ATP released by astrocytes. The EPSC modulation by IGF-I was abolished by the A_{2A} receptors antagonist, SCH (from 98.42 ± 2.41 in basal to 90.20 ± 3.36 % of peak amplitude at 20 min after IGF-I application; p =0.2084, **Figure 4C, SCH**). In addition, IGF-I did not increase the number of APs during SCH or when BAPTA was loaded into the astrocytic network (from 3.8 ± 1.1 to 3.6 ± 1.4 (p =0.9941) and from 6.0 ± 1.2 to 8.0 ± 2.9 (p =0.994) number of APs in basal and at 15 min after IGF-I application in BAPTAa and SCH, respectively, **Figure 4D**). To test the pre or postsynaptic origin of the effect of IGF-I on the EPSCs we constructed $1/CV^2$ plots (see experimental procedures). We observed that the increase in the mean EPSC peak amplitude was not paralleled by an increase in the $1/CV^2$ parameter, which suggests a post-synaptic origin of the EPSC potentiation (R^2 = 0.0107, **Figure 4E, control**). These data suggest that

potentiation of the EPSCs induced by IGF-I was mediated by a postsynaptic mechanism that was absent in the recordings under BAPTA in astrocytes (**Figure 4E**). These combined results indicate that IGF-I induces an increase in calcium levels in astrocytes, which leads to the activation of A_{2A} receptors thus inducing a postsynaptic STP of the EPSCs, and a presynaptic LTD of the IPSCs, that would result in the LTP of the PSPs.

IGF-I controls the induction threshold of Hebbian LTP (LTP_H)

Because IGF-I induces the LTP of the PSPs, it may have a key role in the modulation of Hebbian LTP (LTP_H), decreasing its induction threshold. Therefore, we tested the impact of IGF-I on the LTP_H induced by STDP. Firstly, we analyzed the LTP_H induction threshold under the control conditions. We used STDP protocols consisting of a subthreshold PSP followed by a back-propagating action potential (BAP) at delays of 10 ms repeated 10, 20 and 50 times at 0.2Hz. While 10 and 20 pairings were unable to induce the LTP_H of the PSPs (from 99.89 ± 0.21 to 100.03 ± 4.17 ($p = 0.9758$) and from 101.88 ± 0.71 to 101.51 ± 2.79 ($p = 0.8339$) % of peak amplitude in basal and at 40 min after 10 and 20 pairings respectively, **Figure 5A** blue squares and circles respectively), 50 pairings induced a robust LTP of PSPs (from 96.01 ± 4.12 in basal to 137.62 ± 8.33 % of peak amplitude at 40 min after 50 pairings; ***$p = 0.0007$, **Figure 5A** white triangles). We then performed similar experiments but in slices, in which IGF-I was bath applied previously. We allowed 25 minutes of IGF-I washout and a pyramidal neuron was 'patched' and the number of pairings required to induce LTP_H by STDP was tested. In these experiments, repeating 10 pairings were unable to induce the LTP_H of the PSPs (from 99.39 ± 1.46 in basal to 99.79 ± 3.88 % of peak amplitude at 40 min after 10 pairings; $p = 0.9271$, **Figure 5B** red squares). However, 20 and 50 pairings induced a robust LTP_H of PSPs (from 99.36 ± 1.08 to 141.59 ± 4.38 (***p

=0.00008) and from 99.36 ± 0.89 to 169.13 ± 6.36 (***p =0.0001) % of peak amplitude in basal and at 40 min after 20 and 50 pairings respectively. **Figure 5B** red circles and white triangles). These results suggest that these pyramidal neurons had been facilitated by IGF-I, showing a reduction of the induction threshold of the LTP_H (**Figure 5C)**.

Repetitive whisker stimulation at the frequency used to explore the environment (5 Hz) induces a long-lasting increase in synaptic efficacy at layer II/III neurons depending on the activation of NMDA receptors (Barros-Zulaica et al., 2014). To test for a similar reduction in the LTP_H induction threshold *in vivo*, we analyzed the effect of IGFI on this NMDAR dependent LTP induced by repetitive stimulation of the vibrissae (**Figure 5D**). In the control conditions using saline injections, 30 stimuli of the vibrissae at 5 Hz stimulation induced a LTP of the LFP (150.83 ± 12.20 LFP area (%) of the baseline; *p =0.0248, **Figure 5F and G**), whereas 10 stimuli at 5 Hz train was not sufficient to modulate the LFP (from 99.94 ± 0.33 in basal to 101.76 ± 8.01 % of LFP area at 30 min after 5Hz train (p = 0.5055) and from 100.16 ± 0.57 in basal to 99.29 ± 3.34 % of LFP amplitude (p =0.8699) at 30 min after 5Hz train, **Figure 5E and G**). However, 10 stimuli at 5 Hz induced a LTP of the LFP (from 99.45 ± 1.80 in basal to 201.66 ± 23.49 % of LFP area (###p < 0.0001) at 30 min after 5Hz train and from 99.41 ± 0.55 in basal to 223.53 ± 22.56 % of LFP amplitude (###p < 0.0001) at 30 min after 5Hz train, **Figure 5E and H, IGF-I**) in mice in which LTP_{IGFI} was previously induced by intracranial IGF-I injection (see above, **Figure 1C and D**). This reduction in the threshold of induction of LTP was abolished when IGF-I was applied to mice in which the A_{2A} adenosine receptor antagonist SCH had previously been intraperitoneally injected (from 92.82 ± 1.21 in basal to 96.74 ± 10.34 % of LFP area (p =0.6545) at 30 min after 5Hz train and from 97.61 ± 2.39 in basal to 106.62 ± 9.12 % of LFP amplitude (p =0.5076) at 30 min after 5Hz train, **Figure 5 E and H, SCH+IGF-I**) or when IGF-I

was applied to the mice in which IGF-IR had been removed specifically from astrocytes (from 101.51 ± 2.05 in basal to 91.48 ± 11.85 % of LFP area (p =0.9999) at 30 min after 5Hz train and from 99.68 ± 0.31 in basal to 106.40 ± 10.15 % of LFP amplitude (p =0.8977) at 30 min after 5Hz train in IGF-IR$^{-/-}$ mice. Interestingly, we observed a LTP of the LFP from 99.64 ± 0.43 in basal to 241.08 ± 15.07 % of LFP area (***p < 0.0001) at 30 min after 5Hz train and from 98.89 ± 1.02 in basal to 206.60 ± 17.31 % of LFP amplitude (***p < 0.0001) at 30 min after 5Hz train in IGF-IR$^{+/+}$ mice, **Figure 5E and H, IGF-IR$^{-/-}$ and IGF-IR$^{+/+}$**). Therefore, these results indicate that IGF-I favours the induction of a LTP by reducing the threshold of its induction both *ex vivo* and *in vivo* (**Figure 6**).

The performance of a whisker discrimination task is impaired in the astrocyte-specific IGF-IR$^{-/-}$ mice

Finally, we tested whether the activation of IGF-IR on astrocytes is involved in the performance of a dependent discrimination task. We used a test based on the ability of the mice to discriminate different textures in the arms of a Y maze (**Figure 6B**). We compared the ability to perform this task on the astrocyte-specific IGF-IR$^{-/-}$ mice (##p = <0.01; **Figure 7A**) with their control littermates (IGF-I$^{+/+}$ mice). No differences were observed between the IGF-IR$^{-/-}$ mice and their control littermates in the number of visits to the arms (p=0.1142, **Figure 7B**), indicating normal deambulatory activity. Interestingly, the IGF-IR$^{-/-}$ mice spent less time examining the arm with the novel texture (#p =0.0336, **Figure 7C** left), indicating impaired texture discrimination (##p =0.0059, **Figure 7C** right). Conversely, whisker perception in the IGF-IR$^{-/-}$ mice was preserved, as indicated by their normal performance in the gap-crossing test (p =0.6199, **Figure 7D** left). In addition, the working memory was also normal in the IGF-IR$^{-/-}$ mice, as indicated by preserved performance in the Y maze alternation test (p = 0,7474,

Figure 7D right). Taken together these results indicate that the activation of IGF-IR on astrocytes increases the performance of the texture discrimination in mice, which suggests a crucial role in the learning and memory processes dependent on the Barrel cortex activity.

DISCUSSION

The data in this study challenges the standard view that astrocytes play a key role in the induction of LTP or LTD mainly on excitatory synapses in cortical circuits and expands the view of the IGF-I action in the brain. Instead, we now provide conclusive evidence that astrocytes are mandatory intermediates for the LTD of inhibitory synapses in the Barrel cortex, which relies on astrocytic release of ATP as a result of the IGF-IR activation. Although the observation that IGF-I action on the brain is crucial for learning and memory is well known, here we are proposing a reinterpretation of the classical IGF-I mechanism, from the IGF-I activation of neurons to its action on astrocytes. Therefore, the results presented in this study support the initial hypothesis that astrocytic IGF-IR is key player in the IGF-I action related to learning and memory.

The inhibitory transmission controls the operation of cortical circuits and the modulation of synaptic inhibition plays an important role in the induction of cortical plasticity. Our results show that IGF-I induces a long-lasting depression of the IPSCs that would result in a LTP of the PSPs that we have termed LTP_{IGFI}. Moreover, we further analyzed how IGF-IR activation is able to favour the induction of NMDAR dependent LTP and improves the performance of the texture discrimination in mice. We have shown that the activation of IGF-IRs in astrocytes triggers the calcium-dependent release of ATP/Ado and the activation of A_{2A} adenosine receptors results in the LTD_{IGFI}

by decreasing the probability of GABA release at the inhibitory terminals (see mechanistic diagram in **Figure 2B**). In addition, the ATP/Ado also induced a short-term increase in the efficacy of the excitatory synaptic transmission. The lack of LTD_{IGFI} in mice in which IGF-IR has been selectively ablated in astrocytes suggests that IGF-IR activation in astrocytes is crucial to the induction of this novel form of long-lasting down regulation of cortical synaptic inhibition.

IGF-I elicits a long-lasting depression of GABA release by cerebellar Purkinje cells in response to glutamate, indicating that IGF-I may act as a modulator of glutamatergic transmission in the adult rat olivo-cerebellar system (Castro-Alamancos and Torres-Aleman, 1993; Castro-Alamancos et al., 1996). Also, IGF-I has been shown to modulate GABAergic transmission in the olfactory bulb (Liu et al., 2017). However, this is the first time that an IGF-I mediated long lasting depression of fast GABAergic synaptic transmission in the neocortex been described. We have demonstrated that IGF-I induces the release of ATP from cortical astrocytes that generates the long-lasting depression of GABA release. Although IGF-IRs may be present in the inhibitory GABAergic terminals, there are no clear data showing a direct action of IGF-I acting on presynaptic IGF-IRs. The only evidence suggesting this mode of action is seen in the hippocampus where IGF-I, possibly acting via GABAergic neurons, can induce the release of GABA to regulate endogenous ACh release (Seto et al., 2002). Nevertheless, our observations contribute to the notion that IGF-I can modulate both excitatory and inhibitory synaptic activity throughout the CNS.

Although there is evidence showing that insulin signalling in astrocytes mediates tyrosine phosphorylation of Munc18cand syntaxin-4–dependent ATP exocytosis, which in turn modulates presynaptic dopamine release (Cai et al., 2018), we have not found

any previous evidence for any stimulatory action of IGF-I on ATP release by astrocytes. In this regard, it has been shown previously that metabotropic Protease-activated receptor 1 (PAR-1) induces exocytosis of ATP from cortical astrocytes, which leads to a short term downregulation of inhibitory synaptic currents in layer II/III pyramidal neurons (Lalo et al., 2014). In contrast with the presynaptic LTD described herein, this short-term modulation is mediated by a postsynaptic mechanism in which Ca^{2+}-entry through the neuronal P2X purine receptor leads to a phosphorylation-dependent down-regulation of $GABA_A$ receptors. Therefore, astrocytes can down- or up-regulate inhibitory synaptic transmission by a calcium-dependent release of ATP depending on whether IGF-IR or PAR1R respectively, are activated.

Astrocytes have been shown to respond to both glutamate and GABA (Kang et al., 1998; Guthrie et al., 1999; Navarrete and Araque, 2008; Navarrete and Araque, 2010; Pasti et al., 2018), which allows them to sense the activity of excitatory and inhibitory neurons. In response to these neurotransmitters they can release both glutamate and ATP/Ado (Min et al., 2012; Araque et al., 2014; De Pittà et al., 2016; Guerra-Gomes et al., 2018; Savtchouk and Volterra, 2018). In fact, hippocampal interneuron activity leads to $GABA_BR$-mediated release of glutamate from astrocytes that potentiates both inhibitory (Kang et al., 1998), and excitatory (Perea et al., 2016) synaptic transmission. In addition to glutamate, hippocampal astrocytes may also release ATP, which is converted to adenosine that depresses (Pascual et al., 2005; Serrano, 2006; Andersson et al., 2007; Chen et al., 2013) or potentiates excitatory synaptic transmission (Panatier et al., 2011). Moreover, ATP released by astrocytes can depress excitatory synapses from basolateral amygdala and enhance inhibitory synapses from the lateral subdivision of the central amygdala via the activation of A_1 and A_{2A} adenosine receptors respectively (Martin-Fernandez et al., 2017). Furthermore, cortical

astrocytes have been shown to induce a short-term depression of inhibitory synaptic currents (Lalo et al., 2014). However, to our knowledge, our research is the first evidence that IGF-IR activation in astrocytes can induce a long-lasting depression of inhibitory cortical synaptic transmission through the release of ATP/Ado via a presynaptic mechanism. Therefore, we are presenting novel data showing that not only the astrocyte-mediated LTP_{IGFI} and its physiological consequence favours the associative synaptic plasticity described by Donald Hebbs, but also a novel mechanism in which IGF-I would induce cortical plasticity through the activation of adenosine receptors.

At the circuit level, interneurons control the flow of information and synchronization in the cerebral cortex. Synaptic inhibition is involved in the emergence of fast brain rhythms (Csicsvari et al., 2003), and in the induction of synaptic plasticity (Hensch, 2005), that jointly contribute to cognitive functions. Indeed, disruption of astrocytic vesicular release has been found to be crucial for gamma oscillatory hippocampal activity with a significant impact on recognition memory tasks (Lee et al., 2014). On the other hand, cortical astrocytes respond to sensory inputs and control sensory-evoked cortical neuronal network activity, indicating that astrocytes are actively involved in cortical sensory information processing (Lines et al., 2020). Our results demonstrate that the IGF-I receptor on astrocytes improves the performance of the texture discrimination in mice, suggesting that the LTD_{IGFI} is essential in this dependent task. The release of ATP/Ado from astrocytes and the LTD_{IGFI} described here play a relevant role in this cognitive function by controlling brain rhythms and favouring the induction of Hebbian synaptic plasticity. Decreased synaptic inhibition would facilitate the backpropagation of action potentials into the dendrites and the induction of spike timing-dependent plasticity. Furthermore, by changing the ratio between synaptic

excitation and inhibition, neuronal membranes can rapidly reach the threshold for action-potential generation, and enhanced cortical activity is expected when cortical levels of IGF-I increase. Indeed, this increase in cortical activity would activate the neurotrophic coupling mechanism for the entrance of IGF-I from the plasma into the brain (Nishijima et al., 2010).

IGF-I is able to enhance glutamatergic synaptic transmission in the hippocampal slices of juvenile rats through a mechanism that involves α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) but not N-methyl-D-aspartate (NMDA) postsynaptic receptors (Ramsey, 2005). However, IGF-I significantly increases both AMPA and NMDA-mediated synaptic transmission by a postsynaptic mechanism in young adults and mature rats, (Molina et al., 2012). Additionally, the increase in the expression levels of the NMDA receptor subunits 2A and 2B at the hippocampus by IGF-I in aged rats (Sonntag et al., 2000), may facilitate long-term potentiation (LTP) induction. As in the hippocampus, we have shown that IGF-I increases the efficacy of excitatory synaptic transmission. The short-term potentiation of the EPSCs by IGF-I would establish a temporal window in which the threshold for inducing the Hebbian LTP would be the lowest because of the modulation of both excitation and inhibition by IGF-I. According to this scenario, another question arises as to how the IGF-IR activation on astrocytes is linked with the release of ATP. However, these questions should be the subject of analyses of further details in future articles.

In summary, our findings reveal novel mechanisms and functional consequences of IGF-I signalling in the cortex. It induces the long-lasting depression of inhibition and short enhancement of the excitation opening a temporal window to favour the generation of associative memories that impact on the behavioural performance of

Barrel cortex-related texture discrimination tasks, through the activation of cortical astrocytes.

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968 **FIGURE LEGENDS**

969 **Figure 1. IGF-I induces LTP of the PSPs at layer II/III pyramidal neuron of Barrel**
970 **cortex. A**. DIC image showing the recording (rec.) and the stimulation (stim.)
971 electrodes in a slice (scale bar 100 µm). **B**. *Top.* Representatives PSPs in control (black,
972 left, (1)) and during IGF-I exposure (red, right, (4)). In the middle, representative AP
973 responses before (black (2), 6 out of 60 stimuli) and during IGF-I (red (3), 32 out of 62
974 stimuli). *Bottom. Left*, Time course of the PSP before, during and after washing out the
975 IGF-I in ACSF (full circles; n= 8 cells/3 animals; control *vs* IGF-I **p<0.01, and in the
976 presence of NVP-AEW 541 (empty circles n= 5 cells/2 animals; NVP *vs* NVP+IGF-I,
977 ns (non-significant). Two-tail Student´s paired t-test. *Right.* Number of APs every 5

minutes in before and during the first 15 minutes of IGF-I exposure in ACSF (filled bars n= 8 cells/3 animals control *vs* IGF-I ##p<0.01, ###p<0.001; $F_{(3,28)}$ =8.757) and in the presence of NVP-AEW 541 (empty bars n= 5 cells/2 animals; NVP *vs* NVP+IGF-I, ns; $F_{(3,16)}$ =2.687) One-way ANOVA. **C**. *Top*. Time course scheme showing the local injections of saline or IGF-I (arrows) during LFP recordings in layer II/III of the barrel cortex *in vivo. Bottom.* Drawing illustrating the recording electrode and Hamilton syringe location, and air puff stimulation. **D.** *Left.* Time course of LFP recorded in mice injected (arrows) with IGF-I (red n=10 animals, control *vs* IGF-I, ***p<0.001) and saline (black n=9 animals, control *vs* saline, ns) Two-tail Student´s paired t-test. *Right.* Representative superimpossed LFPs recorded in the control (black (1)) and after 80 minutes of the injections (grey (2)) in mice injected with saline (top, LFP saline) and mice injected with IGF-I (bottom, LFP IGF-I). Note that IGF-I induce LTP of the synaptic potentials recorded at layer II/III of the barrel cortex both in vivo and in the slices.

Figure 2. Astrocytic IGF-IR activation induces adenosine mediated LTD of the IPSCs. A. Time course of the IPSC peak amplitude before, during and after washing out of IGF-I in ASCF (control; empty circles, n= 7 cells/4 animals; control *vs* IGF-I **-p<0.001), and in the presence of NVP-AEW 541 (NVP-AEW 541; grey circles, n= 7 cells/3 animals; NVP *vs* NVP+IGF-I, ns (non-significant)). Two-tail Student´s paired t-test. *Right.* Representative IPSCs in control (grey) and after 20 minutes of IGF-I washout (red). **B**. Cartoon showing that the LTD of the IPSCs induced by IGF-I in layer II/III pyramidal neurons (green cell) depends on the IGF-IR activation on astrocytes (orange cell with blue processes) and the adenosine receptor mediated reduction of GABA release form the GABAergic interneurons (grey cell). **C.** Effect of IGF-I on the IPSC peak amplitude (red bar), as percentage of the control (grey bar), in different

experimental conditions: ACSF, NVP, BAPTA in the postsynaptic neuron (BAPTAn, n= 6 cells/3 animals, control *vs* IGF-I **p<0.01), BAPTA loaded astrocyte (BAPTAa n= 6 cells/3 animals, control *vs* IGF-I **p<0.01), ACSF in the IGF-IR$^{-/-}$ (n= 5 cells/2 animals, control *vs* IGF-I, ns) mice and its littermates (IGF-IR$^{+/+}$, n= 5 cells/2 animals, control *vs* IGF-I *p<0.05), MPEP+LY 367385 (n= 6 cells/3 animals, MPEP+LY *vs* MPEP+LY+IGF-I , **p<0.01), SCH (n= 8 cells/4 animals, SCH *vs* SCH+IGF-I , ns) and CPT (n= 7 cells/2 animals, CPT *vs* CPT+IGF-I , ***p<0.001). Two-tail Student´s paired t-test. Note that the LTD of the IPSCs by IGF-I is prevented when IGF-IR are absent on astrocytes or when they are filled with BAPTA, and when adenosine A2 receptors or IGF-I receptors are blocked by CPT and NVP respectively. **D.** *Left*, Effect of IGF-I on the IPSC PPR (red bar), as percentage of the control (grey bar), in the same different experimental conditions of C. *Right*, Representative IPSCs evoked by paired pulses recorded before (grey trace, control) and during IGF-I (red trace, IGF-I). Note that IGF-I increases the PPR (R2/R1) because it reduces more the first IPSC (R1) than the second IPSC (R2).

Figure 3. Astrocytic IGF-IR activation stimulates the calcium-dependent release of ATP. A. Effect of IGF-I on the spontaneous Ca^{2+} event frequency of the astrocytes in different experimental conditions: in ACSF (control, n= 55 astrocytes/4 animals, control *vs* IGF-I **p<0.01), NVP (n= 65 astrocytes/3 animals, NVP *vs* NVP+IGF-I, ns), MPEP+LY (n= 44 astrocytes/3 animals, MPEP+LY *vs* MPEP+LY+IGF-I **p<0.01), SCH (n= 48 astrocytes/4 animals, SCH *vs* SCH+IGF-I *p<0.05) and CPT (n= 72 astrocytes/2 animals, CPT *vs* CPT+IGF-I ***p<0.001). Two-tail Student´s paired t-test. **B.** Representative images of locally targeting astrocytes with GFAP-GCaMP6f-mCherry for IGF-IR$^{+/+}$ (top images) and GFAP-GCaMP6f-mCherry-CRE for IGF-IR$^{-/-}$ (bottom images) scale bar, 50 µm. **C**. Pseudocolour Ca^{2+} images showing the intensities

of GCaMP6f expressing astrocytes in the Barrel cortex, in the basal condition and during IGF-I exposure in IGF-IR$^{+/+}$ (top images) and IGF-IR$^{-/-}$ astrocytes (bottom images; scale bar, 50 µm). *Right.* Representative Ca^{2+} traces in the astrocytes from IGF-IR$^{+/+}$ (top traces) and the IGF-IR$^{-/-}$ astrocytes (bottom traces). **D.** Spontaneous Ca^{2+} event frequency in the processes of the astrocytes in the basal condition and during IGF-I exposure in IGF-IR$^{+/+}$ (n= 135 astrocytes/2 animals, control *vs* IGF-I **p<0.01) and IGF-IR$^{-/-}$ (n= 67 astrocytes/2 animals, control *vs* IGF-I, ns) astrocytes. Two-tail Student´s paired t-test. E. Bar plot of ATP concentration in a culture of astrocytes before (black bar) and during IGF-I exposure (red bar) in different experimental conditions: ACSF (n=6, basal *vs* IGF-I ***p<0.001), NVP (n=6, IGF-I *vs* NVP+IGF-I ***p<0.001), BAPTA-AM (n=6, IGF-I *vs* BAPTA AM+IGF-I **p<0.01) and in the astrocytes from the IGF-IR$^{+/+}$ and IGF-IR$^{-/-}$ mice (n=6, IGF-I$^{+/+}$ vs IGF-I$^{-/-}$ **p<0.01). Two-way ANOVA with post hoc Bonferroni multiple comparisons test.

Figure 4. IGF-I generates a transient potentiation of the EPSCs. A. *Top.* Representative EPSCs responses before (black, (1)), during IGF-I (red, (4)) and during IGF-I washout (grey, (5)). IGF-I increases the number of AP responses recorded every 60 stimuli (green box), from 8 before IGF-I (black trace, (2)) to 30 during IGF-I (red trace, (3)). *Bottom. Left,* time course of the EPSC peak amplitude before, during IGF-I and during IGF-I washout in ACSF (full circles), and in the presence of NVP-AEW 541 (empty circles). Note that during the green box, EPSC peak amplitude values are missed because we recorded the effect of IGF-I on the number of stimuli evoking AP responses. *Right,* number of APs recorded every 5 minutes before (black) and during IGF-I (red) in ACSF (filled bars, n= 8 cells/3 animals, control *vs* IGF-I #p<0.05, ##p<0.01; $F_{(3,28)}$ =5.474) and in NVP (empty bars. n= 5 cells/2 animals; NVP *vs* NVP+IGF-I, ns; $F_{(3,16)}$ =0.2402) One-way ANOVA. **B.** Cartoon showing that the STP

of the EPSCs induced by IGF-I depends on the IGF-IR activation on astrocytes and the adenosine receptor mediated increase of the release of glutamate from the glutamatergic terminal (blue terminal). **C.** Effect of IGF-I on the EPSC peak amplitude (green bar), as percentage of the control (grey bar), in different experimental conditions: ACSF, NVP, BAPTAa (n= 5 cells/3 animals; control *vs* IGF-I, ns) and SCH (n= 6 cells/3 animals; SCH *vs* SCH+IGF-I, ns) Two-tail Student´s paired t-test. Note that the modulation of the EPSCs by IGF-I is prevented in the same conditions in which the modulation of the IPSCs by IGF-I was abolished. **D.** As in A right bottom but in BAPTA loaded astrocyte (n= 5 cells/3 animals; control *vs* IGF-I, ns; $F_{(3,16)}$ =0.2457) and SCH (n= 6 cells/3 animals; SCH *vs* SCH+IGF-I, ns; $F_{(3,20)}$ =0.1302). One-way ANOVA. **E.** Plot of the variance ($1/CV^2r$) as a function of the mean EPSC peak amplitude (M) in the control, BAPTA loaded astrocyte and SCH. Note that the control condition does not follow a diagonal, suggesting a postsynaptic origin of the transient potentiation.

Figure 5. IGF-I favours Hebbian LTP *ex vivo* and *in vivo*. A. *Top.* Representative PSP followed by an AP with a 10 ms delay and a frequency of 0.2 Hz. *Bottom, upper.* Representative PSPs in the control and 40 minutes after 10, 20 and 50 pairings for the STDP induction. *Lower.* Time course of the PSPs with 10 pairings (n=5 cells/2 animals basal *vs* after 40min of STDP, ns), 20 pairings (n=6 cells/3 animals basal *vs* after 40min of STDP, ns) and 50 pairings (n=7 cells/3 animals basal *vs* after 40min of STDP, ***p<0.001) for the STDP induction (black arrow). Two-tail Student´s paired t-test. **B.** *Top.* Time course scheme showing the IGF-I exposure, washout and STDP induction (black arrow). *Bottom, upper.* Representative PSPs in the control and 40 minutes after 10, 20 and 50 pairings for the STDP induction after IGF-I exposure and washout. *Lower.* Time course of the PSPs with 10 pairings (n=5 cells/2 animals basal *vs* after IGF-I, ns), 20 pairings (n=6 cells/2 animals basal *vs* after IGF-I, ***p<0.001), and 50

pairings (n=6 cells/3 animals basal *vs* after IGF-I, ***p<0.001) for the STDP induction (black arrow) after IGF-I exposure and washout. Two-tail Student´s paired t-test. **C.** Cartoon showing that IGF-I exposure induce an LTP of the PSPs (LTP_{IGF-I}) that increase the magnitude of LTP by STDP and facilitates its induction. **D.** *Top.* Scheme showing the time course of local injection (arrows) and whisker stimulation (5 Hz, 10 stimuli, blue arrow) used in the in-vivo experiments. *Bottom.* Drawing to illustrate the recording electrode and Hamilton syringe location, and air puff stimulation in the anesthetized mouse. **E.** *Top.* Representative LFPs in the control (black, (1)), after 80 minutes IGF-I and saline injections in the wild type mouse (dark grey, (2)) and after 5 Hz train whisker stimulation (grey, (3)). *Bottom.* Time course of LFP area with IGF-I injection (arrows) in the wild type mouse (red triangle, n=10 animals, basal *vs* after 80 min injection ##p<0.01, basal *vs* after 5Hz train ###p<0.001, after 80 min injection vs after 5HZ train ##p<0.01; $F_{(2,26)}$ =20.65), previous intraperitoneally injected SCH (2 mg/Kg, purple square, n=7 animals, basal *vs* after 80 min injection ns, basal *vs* after 5Hz train ns, after 80 min injection vs after 5HZ train, ns. Non-parametric test; H =1.983), IGF-$IR^{-/-}$ (green diamond n=6 animals, basal *vs* after 80 min injection ns, basal *vs* after 5Hz train ns, after 80 min injection vs after 5HZ train, ns. Non-parametric test; H =0.3702) and IGF-$IR^{+/+}$ (dark green triangle n=7 animals, basal *vs* after 80 min injection ##p<0.01, basal *vs* after 5Hz train ###p<0.001, after 80 min injection vs after 5HZ train ##p<0.01; $F_{(2,18)}$ =27.94) and saline injection (black circle n=9 animals, basal *vs* after 80 min injection ns, basal *vs* after 5Hz train ns, after 80 min injection vs after 5HZ train, ns; $F_{(2,23)}$ =1.766) One-way ANOVA. **F**. Top. Scheme showing the time course of the saline local injection (arrows) and the following 5 Hz train whisker stimulations. **G**. Time course of LFP area with IGF-I injection (arrows) in the wild type mice with local saline injection (black arrows) and 10 (yellow arrow) and 30 (blue arrow) stimuli trains

whisker stimulation at 5Hz. Note that 30 stimuli induced the LTP of the LFP but 10 stimuli did not change the LFP amplitude (n=7 animals, 10 stim. train vs 30 stim. train *p<0.05). **H.** LFP amplitude in the control, 80 minutes after injections and after 5 Hz train whisker stimulation in different experimental conditions: the wild type mice with IGF-I injection (red, n=10 animals, basal *vs* after 80 min injection ##p<0.01, basal *vs* after 5Hz train ###p<0.001, after 80 min injection vs after 5HZ train #p<0.05; $F_{(2,24)}$ =18.97), saline injection (grey, n=9 animals, basal *vs* after 80 min injection ns, basal *vs* after 5Hz train ns, after 80 min injection vs after 5HZ train, ns; $F_{(2,24)}$ =0.4747), previous intraperitoneally injected SCH (purple, n=7 animals, basal *vs* after 80 min injection ns, basal *vs* after 5Hz train ns, after 80 min injection vs after 5HZ train, ns. Non-parametric test; H =1.967), and the IGF-$IR^{-/-}$ mice (green, n=6 animals, basal *vs* after 80 min injection ns, basal *vs* after 5Hz train ns, after 80 min injection vs after 5HZ train, ns. Non-parametric test; H =0.3702) and its littermates the IGF-$IR^{+/+}$ mice (dark green n=7 animals, basal *vs* after 80 min injection ##p<0.01, basal *vs* after 5Hz train ###p<0.001, after 80 min injection vs after 5HZ train ##p<0.01; $F_{(2,18)}$ =23.35) one-way ANOVA.

Figure 6. Model showing the actions of IGF-I. Schematic diagram showing, from bottom to the top, that the activation of layer II/III astrocytes (1) (orange cell in the bottom) by IGF-I (red circles) promotes the release of ATP/adenosine (Ado) (2). Ado acts on both the excitatory (3) (blue) and inhibitory (3) (grey) synapses inducing STP of the EPSCs (4), and LTD of the IPSCs (5) respectively (black and red superimposed responses are those recorded before and during the application of IGF-I). The net effect of this synaptic modulation is the $LTP_{IGF\text{-}I}$, a LTP of the PSP recorded from the soma of the layer II/III pyramidal neuron. The $LTP_{IGF\text{-}I}$ facilitates the induction of the Hebbian LTP by STDP protocols and increases its magnitude.

Figure 7. The performance of a whisker-based discrimination task is impaired in the astrocyte-specific IGF-IR$^{-/-}$ mice. A. *Left.* Schema of the transgenic mice generation with tamoxifen-regulated deletion of IGF-IR in astrocytes (IGF-IR$^{-/-}$ mice). *Right.* The brain levels of IGF-IR mRNA, as determined by qPCR, are reduced in the IGF-IR$^{-/-}$ mice after tamoxifen injection, when compared with the vehicle-injected control mice (n=5 animals tamoxifen vs control **p<0.01). **B.** *Left.* Schema of Y maze whisker dependent texture discrimination test. Two arms (familiar) were covered with 500 grit sandpaper, the third (novel) was covered with 220 grit sandpaper. Because the three arms of the maze are identical and there are no extra-maze cues, discrimination of novelty vs familiarity relies only on the different textures that the mice can perceive with their whiskers. *Right.***.** IGF-IR$^{-/-}$ and the control mice show no difference in their entrance to the different arms (n=13 animals tamoxifen *vs* control ns). Two-way ANOVA with post hoc Bonferroni multiple comparisons test **C.** *Left.* However, the control mice spent more time exploring the novel arm (220 grit sandpaper) than the IGF-IR$^{-/-}$ mice (n=13 animals tamoxifen *vs* control *p<0.05). *Right.* The percentage time spent in the novel-texture arm was higher in he controls than in the IGF-IR$^{-/-}$ mice (n=13 animals tamoxifen *vs* control **p<0.01). Two-way ANOVA with post hoc Bonferroni multiple comparisons test. **D**. Left. The control and the IGF-IR$^{-/-}$ mice showed a similar performance in the Gap-crossing test, indicating that there were no alterations in their sensory perception (n=5 tamoxifen vs control, ns). *Right.* No differences were observed between the control and the IGF-IR$^{-/-}$ mice in the Y-Maze Spontaneous Alternation Task, indicating a similar working memory (n=5 tamoxifen vs control, ns). Two-way ANOVA with post hoc Bonferroni multiple comparisons test.