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Regular Article

A novel function for Wnt signaling modulating neuronal firing activity and the temporal structure of spontaneous oscillation in the entorhinal–hippocampal circuit

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ABSTRACT

During early and late postnatal developments, the establishment of functional neuronal connectivity depends on molecules like Wnt that help the recently formed synapses to establish and consolidate their new cellular interactions. However, unlike other molecules, whether Wnt can modulate the firing properties of cells is unknown. Here, for the first time we explore the physiological effect of the canonical and non-canonical Wnt pathways on a circuit that is currently generating oscillatory activity, the entorhinal cortex–hippocampal circuit. Our results indicate that Wnt pathways have strong influence in the circuital and cellular properties depending on the Wnt protein isoforms, concentration, and type of neuronal circuit. Antibodies against canonical and non-canonical ligands, as well as WASP-1 and sFRP-2, demonstrate that constitutive release of Wnts contributes to the maintenance of the network and intrinsic properties of the circuit. Furthermore, we found that the excess of Wnt3a or the permanent intracellular activation of the pathway with BIO-6 accelerates the period of the oscillation by disrupting the oscillatory units (Up states) in short units, presumably by affecting the synaptic mechanisms that couples neurons into the oscillatory cycle, but without affecting the spike generation. Instead, low doses of Wnt5a increase the period of the oscillation in EC by incorporating new cells into the network activity, probably modifying firing activity in other places of the circuit. Moreover, we found that Wnt signaling operates under different principles in the hippocampus. Using pyrvinium pamoate, a Wnt/β-catenin dependent pathway inhibitor, we demonstrated that this pathway is essential to keep the firing activity in the circuit CA3, and in less degree of CA1 circuit. However, CA1 circuit possesses homeostatic mechanisms to up-regulate the firing activity when it has been suppressed in CA3, and to down-modulate the cellular excitability when exacerbated circuital activity has dominated. In summary, the amount of Wnt that is being released can exert a fine tuning of the physiological output, modulating firing activity, improving reliability of communication between neurons, and maintaining a continuous self-regulatory cycle of synaptic structure–function that can be present during all postnatal life.

Introduction

Neuronal activity during early postnatal life has a potent trophic role, not only favoring neuronal survival (Golbs et al., 2011) but also inducing the maturation of molecular, cellular and structural features of neuronal circuits (Owens and Kriegstein, 2002) that contribute to activity-dependent circuit wiring (Garaschuk et al., 1998). This early spontaneous firing activity in the form of action potentials has been shown to play a fundamental role in the fine tuning of connectivity in many regions of the brain (Ben-Ari et al., 1989; Feller et al., 1996; Garaschuk et al., 1998; Moody and Bosma, 2005; Yuste et al., 1992), later leading to the emergence of highly organized patterns of neuronal activity that underlie complex functions such as perception, cognition and memory formation (Egorov and Draguhn, 2013). The neuronal firing activity arises from the interaction between the synaptic activity and the intrinsic firing properties of the neurons. However, during the establishment and refinement of circuit connectivity, neurons must use several mechanisms to adjust their firing to the enormous incoming but unstable activity and to periods of activity and inactivity to keep brain activity balanced (Turrigiano, 2011). Interestingly, molecules

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that are involved during trophic neuronal development like the tumor necrosis factor α (TNF-α) or the brain-derived neurotrophic factor (BDNF), both participate adjusting the strength of synaptic excitatory activity, but only BDNF participates in adjusting the intrinsic excitability of cells to boost neuronal excitability in response to reduced general activity, thereby stabilizing cortical firing rates when needed (Desai et al., 1999; Kaneko et al., 2008; Magby et al., 2006; Rutherford et al., 1998; Stellwagen and Malenka, 2006; Turrigiano, 2011).

Recently it has been demonstrated that BDNF is released by a Wnt ligand or by an activator of Wnt in retina cells, suggesting that BDNF is a direct target gene of the Wnt signaling pathway (Yi et al., 2012). However, besides its well-known role during neural development and synaptogenesis, whether Wnt proteins are involved in the regulation of neuronal firing activity has not been yet explored. Wnt signaling components are continuously expressed in the postnatal brain (Shimogori et al., 2004), and their role in the development of neuronal circuits and plasticity has been established (Ciani and Salinas, 2005; Inestrosa and Arenas, 2010). On the presynaptic compartment, Wnt leads to rapid modification of the presynaptic machinery, regulating the clustering of synaptic proteins and the cycling of synaptic vesicles, while at the postsynaptic compartment Wnt modulates the assembly and function of the postsynaptic apparatus the dendritic spine arborization and morphogenesis (Ciani and Salinas, 2005; Inestrosa and Arenas, 2010; Miech et al., 2008; Speese and Budnik, 2007). The activation of Wnt signaling modulates the efficacy of the excitatory and inhibitory synaptic transmission (Cerpa et al., 2011; Chen et al., 2006; Cuitino et al., 2010; Inestrosa and Arenas, 2010; Wisniewska, 2013), and has been involved in synaptic plasticity in the adult brain. Wnt is present or constitutively released during basal synaptic activities in vitro (Cerpa et al., 2011; Rosso et al., 2005; Varela-Nallar et al., 2010), maintaining the basal tone of synaptic activity (Varela-Nallar et al., 2010) and specifically regulating the function of NMDAR (Cerpa et al., 2011). In vivo studies demonstrated that Wnt pathway is involved in both hippocampal-long term memory and amygdala-fear memory consolidation (Fortress et al., 2013; Maguschak and Ressler, 2011; Tabatadze et al., 2012; Vargas et al., 2014). Additionally, in vivo evidence suggests that basal Wnt is required in mossy fiber-CA3 for structural plasticity generated by an enriched ambient, but also in mice housed in control conditions (Gogolla et al., 2009), suggesting that the previous condition of any new experience that generates structural plasticity needs a basal condition in which Wnt signaling might be relevant (Gogolla et al., 2009). Wnt signaling deregulation has been implicated in neurodegenerative disorders and neurological pathologies, including Alzheimer's disease (AD), schizophrenia (SZ), epilepsy and in mood disorders such as depression and bipolar disorder (Oliva et al., 2013a,b; Valvezan and Klein, 2012). Thus, despite that Wnt signaling could constitute a promising therapeutic target for the treatment of certain neurological diseases the information regarding on how Wnt can affect the physiology of the cells remains unclear.

Looking forward to find a handle but physiological model to explore whether exogenous or endogenous Wnt can modulate the properties of cell firing in a neural circuit, we used mouse brain slices containing the entorhinal cortex–hippocampal circuit, a circuit involved in spatial navigation and declarative memory formation (Buzsaki and Moser, 2013) and where the "place cells" described in the hippocampus (O'Keefe, 1976; O'Keefe and Dostrovsky, 1971) and the "grid cells" described in entorhinal cells (Hafting et al., 2005) appear to underlie both memory and navigation mechanisms (Moser et al., 2008). This circuit is one of the first and most affected during Alzheimer's (Braak and Braak, 1991; Detoledo-Morrell et al., 1997; Killiany et al., 2002) and Parkinson's diseases (Goldman et al., 2012) and is where Wnt ligands have been shown to have positive effects of improving synaptic general activity (Cerpa et al., 2010; Toledo and Inestrosa, 2010; Vargas et al., 2014). Using young mouse brain slices that can develop spontaneous oscillatory activity (Tahvildari et al., 2012), we demonstrated that Wnt signaling modulates the firing rate and the temporal structure of the network activity depending on the Wnt protein isoform, concentration, and type of neuronal circuit. Using specific antibodies, activators and inhibitors, we demonstrated that the active circuit is constitutively releasing Wnt to modulate the spiking activity and the synaptic structure that underlies the oscillation. Our evidence suggests a novel physiological role of the Wnt signaling in the brain circuit that contains the mechanisms for spatial positioning and memory representation.

Materials and methods

Preparation of mouse brain slices

All the animals were obtained from a colony of the Animal House Facility at the Pontificia Universidad Católica de Chile. All the procedures for animal handling and the experimental procedure were approved by the Institutional Ethics Committee in accordance with the guidelines for the care and use of laboratory animals adopted by the Society for Neuroscience. Wild type (C57/BL6) male or female mice at the age of P15–P20 were used in this study. We prepared acute horizontal slices that contain the entorhinal cortex and hippocampus. We anesthetized the animals with sodium pentobarbital and killed them by decapitation. The brain was rapidly removed and placed in ice cold (~4 °C) oxygenated (95% O_2 and 5% CO_2), sucrose-substituted artificial cerebrospinal fluid (ACSF) in which sucrose was used as a substitute for NaCl. This solution contains (in mM): 85 NaCl, 75 sucrose, 3 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 10 dextrose, 3.5 $MgSO_4$, 0.5 $CaCl_2$, 3 sodium pyruvate, 0.5 sodium L-ascorbate, and 3 *myo*-inositol (305 mOsm, pH 7.4). Slices were cut (300–350 μm thick) and immediately transferred to an incubation beaker filled with the same solution at 36 °C. An hour after dissection, the slices were transferred to an incubator filled with the recording buffer solution at room temperature (22 °C), containing (in mM): 126 NaCl, 3.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 10 dextrose, 1 $MgSO_4$, 1.2 $CaCl_2$, 3 sodium pyruvate, 0.5 sodium L-ascorbate, and 3 *myo*-Inositol (305 mOsm, pH 7.4). For recording, slices were mounted on a grid (submerged-style chamber in vitro) so that the ACSF solution flowed freely over and under the cortical slice. The temperature was maintained at 32–34 °C during the whole experiment. Under these conditions, we were able to generate and maintain robust and stable spontaneous Up and Down states in medial entorhinal cortex (mEC) and high spontaneous firing activity in the CA3 and CA1 of the hippocampus.

Electrophysiological recordings

Slices were examined under an upright infrared-differential interference contrast (IR-DIC) fluorescence microscope (Eclipse FNI, Nikon) equipped with a 40× water objective and a light-sensitive camera (TOPICA CCD Camera) to visualize neuronal profiles. To record the spikes generated by spontaneous network activity in mEC and hippocampus, we recorded multiunit activity using a borosilicate glass electrode (World Precision Instruments), typically ranging between 0.5 and 1 MΩ as pulled on a P-97 Flaming/Brown Micropipette Puller (Sutter Instruments), filled with external bath solution (Tahvildari et al., 2012). The glass pipette was placed in layer II–III in mEC and in *stratum pyramidale* of CA3 and CA1. To record multiunit activity (MUA), a MultiClamp 700B amplifier (Axon CNS, Molecular Devices LLC) was used. For MUA, the signal was band-pass filtered between 2 kHz–300 Hz, digitally sampled at 30 kHz and recorded using a Digidata-1440A interface (Axon CNS, Molecular Devices) on pCLAMP 10 software (Molecular Devices LLC). Data were analyzed offline using pCLAMP 10 and Spike2 (Cambridge Electronics Design Limited).

Wnt-conditioned media

We generated the conditioned media from stable HEK293 cells transfected with Lipofectamine 2000 (Invitrogen). We used constant

and equal amounts of empty vector pcDNA (for control) or pcDNA containing sequences encoding Wnt3a or Wnt5a sequences, coupled to a sequence that encodes for hemagglutinin (HA) tag, as has been shown previously by our laboratory (Alvarez et al., 2004; Chacon et al., 2008; Cuitino et al., 2010). Wnt7a was a gift from Dr. Patricia Salinas (University College London, UK), Wnt5a was a gift from Dr. Randall T. Moon (University of Washington, Seattle, WA), and Wnt3a was a gift from Dr. Roel Nusse (Stanford University, Palo Alto, CA). Transfected cells were grown up to 85% confluence and maintained in neurobasal medium without supplements for 60 h. Wnt secretion was verified by Western blot using a HA-specific antibody (Millipore). The media containing the Wnt ligands was collected and dialyzed overnight at 4 °C using an "ACSF-like" solution. This solution has the same ionic composition as the solution used for recording, except it does not contain calcium and magnesium to avoid affecting the final concentration of these two divalent ions, which are important during electrophysiological recording. Once we obtained the conditioned, dialyzed media, we prepared different dilutions (from 1:6 to 1:100) using the recording solution, and we added the calcium and magnesium necessary to reach the final concentrations (1 mM $MgSO_4$, 1.2 mM $CaCl_2$). Because of the variability that conditioned media obtained from culture cells can represent, every time we collected and dialyzed the media we diluted them in the whole range of dilutions, and all the experiments conducted by this batch were normalized by the effect caused by the most concentrated dose applied (1:6) in that set of data. The results of several weeks of experiments were averaged and shown here as relative values normalized to the control condition. Of note is the fact that, for the control condition and the wash out period of all our experiments, we used pcDNA-conditioned media obtained from cells transfected with the empty vector. This media was dialyzed and diluted in the same proportion as every conditioned media containing Wnt, being the only difference the type and content of Wnt during the experimental situation.

Spike detection method, data analysis and statistics

To detect spikes in the extracellular recordings we used the event detection/peak find method present in the Analysis measurement main menu of the Spike2 software. We first determined the amplitude of the intrinsic noise, which in our recordings is in average between +20 and −20 μV (±40 μV). Then we manually set the "amplitude" parameter using 10–15% over the noise value to detect all the spikes over and below that amplitude. The setting is completed using other two parameters, the "minimum step" which is the minimum separation between two spikes to consider them as independent events; and the "maximum width" which considers the duration of the wave (see Supplementary Fig. 3). This method was performed to detect spikes in all our recordings. For the analyses we used 1000 s of continuous recording in every experimental condition. The spike rate was calculated as the number of detected spikes in 1000 s. The mean frequency was calculated using 1 s bins. For the event correlation plot, we used a bin size of 0.001 s in each condition. The interspike interval (ISI) histogram was calculated using a width of 0.1 s and a bin size of 0.0001 s. Analyses were conducted with pCLAMP 10.0 and Spike2 and the graphs and statistical analysis were performed with GraphPad Prism 5.0 and OriginPro 8. Differences between means were analyzed with non-paired Student's t tests (for comparison of two groups) or ANOVAs (for comparison of multiple groups), and non-normally distributed data were analyzed using the Mann–Whitney U or Kruskal–Wallis test; $p < 0.01$ (*) and $p < 0.001$ (**) were considered significant. The data are expressed as the mean ± standard error.

Chemical compounds

The antibodies used were anti-Wnt7a (K-15) goat polyclonal IgG (sc-26361; Santa Cruz Biotechnology, Inc., Santa Cruz), anti-Wnt3 (C-15) goat polyclonal IgG (sc-5210; Santa Cruz Biotechnology), and anti-Wnt5a (H-58) rabbit polyclonal IgG (sc-30224; Santa Cruz Biotechnology, Inc., Santa Cruz). WASP-1 (2-(2,7-diethoxy-9H-fluoren-9-ylidene) hydrazinecarboximidamide) and sFRP-2 (soluble Frizzled-related protein) were obtained from R&D Systems (Minneapolis, MN).

Results

Effects of Wnt analogues on spontaneous multiunit activity

To study the effect of Wnt analogues we first developed an in vitro slice preparation where we can record the spontaneous oscillations in three regions of a well-preserved entorhinal–hippocampal circuit: medial-EC (mEC), CA3 and CA1 (Fig. S1, see Materials and methods and Supplementary information). We used extracellular electrodes to record the multiunit activity (MUA) which represents the spiking that is spontaneously generated by local neurons. We recorded in layer II or III in mEC and we showed the development of spontaneous activity that resembles what occurs in vivo during deep anesthesia and during slow wave sleep (Figs. S1 B1–B2), reason why it has been called "slow oscillation" in the form of "Up-Down" states (Sanchez-Vives and McCormick, 2000; Steriade et al., 1993; Tahvildari et al., 2012). During this time, the cells fire mostly during Up states and remain silent during Down states. It has been shown that this slow oscillation in the cortex is a balance between excitation and inhibition, and the synaptic interaction between excitatory and inhibitory cells in a network causes the periodic activation that generates oscillatory activity. In our preparation this cycle is repeated one after the other with a frequency of 0.07–0.14 Hz, where every Up state has an average duration of 3.6 ± 0.9 s, every Down state 13 ± 2 s and an average of 44 ± 5 spikes/Up state (Figs. S1 B1–B2), similar to what has been recently reported (Tahvildari et al., 2012). In a novel approach, we also recorded simultaneously the MUA from region CA3 to CA1 in the hippocampus during the spontaneous emerging oscillation (Figs. S1 C1–D2), where we recorded tonic firing activity at a basal rate of 34 ± 6 spikes/s in CA3 and of 30 ± 4 spikes/s in CA1.

To determine the effect that the direct application of natural Wnt proteins has on spontaneous network activity, we perfused recording solution containing Wnt3a or Wnt5a obtained from conditioned media. Wnt3a has been classified as an activator of the canonical Wnt/β-catenin dependent pathway, involved in maturation and function of presynaptic terminals (MacDonald et al., 2009), whereas Wnt5a is a traditional non-canonical Wnt/Ca^{2+} pathway activator and it has been shown to be involved in structural and functional assembly of the postsynaptic region (Cuitino et al., 2010; Inestrosa and Arenas, 2010; Varela-Nallar et al., 2010). We monitored the changes in spiking activity in the presence of each ligand for at least 60 min. Figs. 1A–B show a real time experiment that exemplifies the effects of the most concentrated dilution of Wnt3a and Wnt5a in the recorded areas. The application of Wnt3a or Wnt5a causes a fast increase in spiking activity within 80–100 s and an evident change in the instantaneous and mean frequency over the basal levels (Figs. 1A–B). Specifically, Wnt5a induces a strong initial peak in the spiking activity (Fig. 1B), which we presumed is due to the entrance of calcium into the cells, as has been shown in cultured hippocampal cells exposed to Wnt5a (Varela-Nallar et al., 2010). Using different dilutions of the conditioned media (from 1:6 to 1:100, see Materials and methods), we determined that the effects of Wnt analogues were strongly dose-dependent (Figs. 1C–F). A detailed analysis by region shows that, in mEC, Wnt3a increases the frequency of regular-Up states only at the most concentrated dilutions (76 ± 18% over control at 1:6, Fig. 1C, black bars). However, over almost the entire dose-range (>1:50) Wnt3a also induces the firing of new episodes of what we called "short-Up states" (see asterisks, Fig. 1C, gray bars). These short-Up states are shorter in duration (< than 2 s in duration versus regular-Up states > 3 s; see Fig. S2), they emerge during Down states (or the period where usually there is no firing) and in 75%

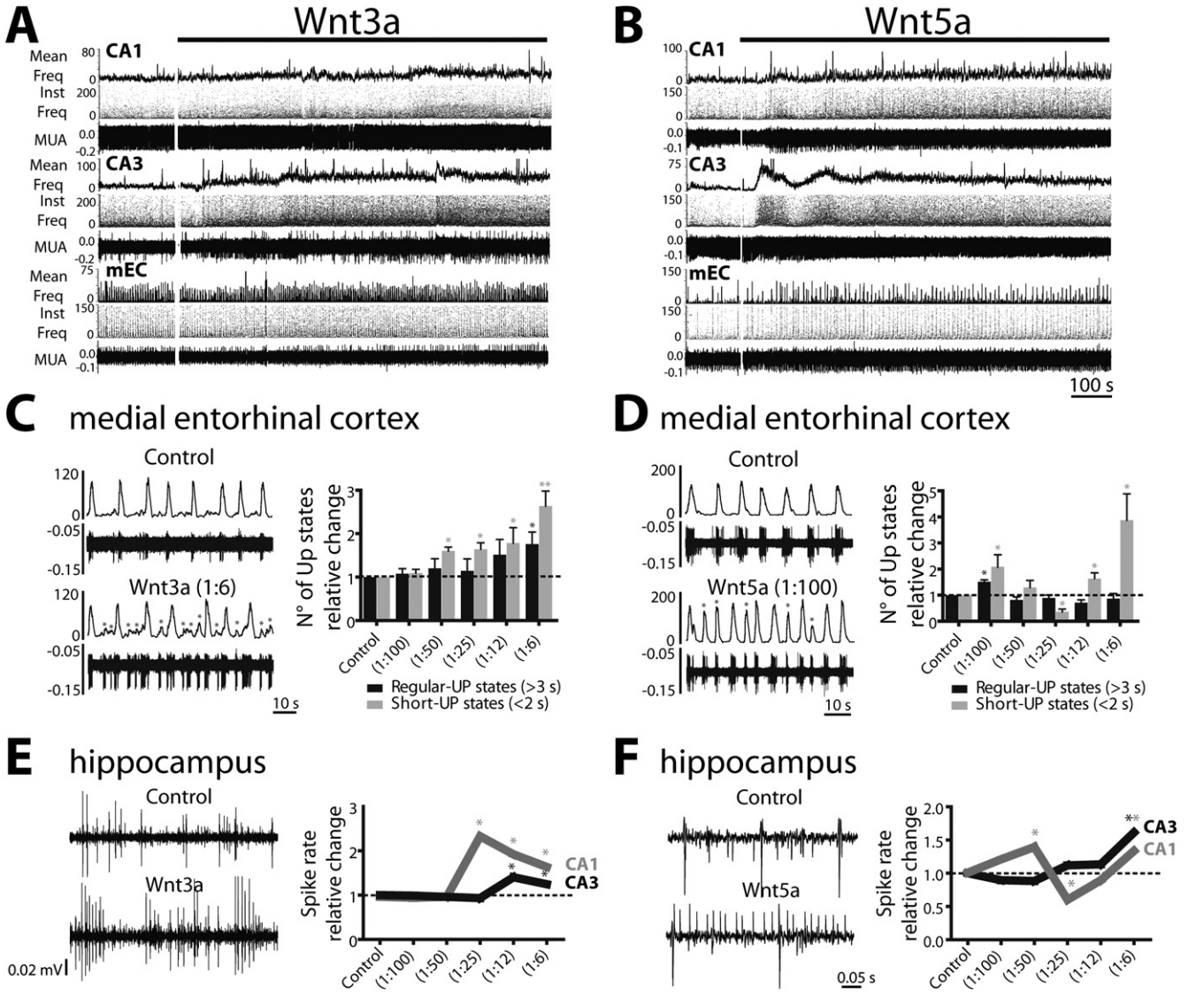

Fig. 1. Effect of Wnt on the spontaneous multiunit activity. The effect of Wnt3a and Wnt5a on the neuronal activity was evaluated at different doses. A–B, Two sets of representative recordings in the three areas simultaneously recorded, indicating the moment when Wnt3a (A) or Wnt5a (B) was applied in the recording solution. From below to top are mEC, CA3 and CA1, with their respective analyses (*InstFreq mEC*: Control = 8–40 Hz, Wnt3a = 45 Hz, Wnt5a = 25–47 Hz; *CA3*: Control = 40 Hz, Wnt3a = 85–100 Hz, Wnt5a = 50–130 Hz; *CA1*: Control = 15–90 Hz, Wnt3a = 80–110 Hz, Wnt5a = 35–60 Hz). C–F, Analyses per region. C–D, Dose-dependent effect of Wnt conditioned media on the slow oscillation in mEC. Left panels show the MUA and the mean frequency in control condition and after perfusion with Wnt3a (C) and Wnt5a (D). Asterisks in mean frequency indicate the short-Up states (duration = less than 2 s). Both bar graphs show the relative change in the number of regular-Up states (Up duration = more than 3 s, black bars in C and D) and in the number of short-Up states (Up duration = less than 2 s, gray bars in C and D) at different doses of Wnt3a and Wnt5a. E–F, Dose-dependent effect of Wnt conditioned media on hippocampal activity. At the left, a representative example recorded in CA3 (E) and CA1 (F) shows the effect of Wnt3a (E) or Wnt5a (F) conditioned media. Right, each graph summarizes the spike rate normalized to the control period (black curve = CA3; gray curve = CA1). For Wnt3a: n = 15 and for Wnt5a: n = 12 experiments at each dose. Data are expressed as the mean ± standard error, where $p < 0.01$ (*) was considered as significant. MUA = multiunit activity; InstFrq = instantaneous frequency; Mean Frq = mean frequency.

of the experiments they appear only when we add Wnt into the recording solution (Fig. 1C). To determine the origin of short-Up states, we counted the spikes generated only during regular-Up states in the whole experiment and we observed that the number of spikes during regular-Up states decreases during Wnt3a perfusion, whereas the number of short-Up states increases. This suggests that the short-Up states are the de-fragmented product of the regular-Up states, caused by Wnt3a (Fig. S4). Opposite to Wnt3a is the effect of Wnt5a. Wnt5a also increases the frequency of regular-Up states but surprisingly only at the lowest dose (52 ± 7% over control at 1:100, Fig. 1D, black bars), and also increase the number of short-Up states. Interestingly, we counted the total number of spikes generated during regular-Up states before, during and after Wnt5a exposure, and we found that they remain constant throughout the experiment (Fig. S4), suggesting that the short-Up states induced by Wnt5a correspond to the activity of new cells that are being incorporated into the oscillatory activity. Moreover, Wnt5a exerts a bimodal dose-dependent effect on the short-Up states, increasing their firing rate at low and high doses and decreasing it at intermediate doses (Fig. 1D, gray bars). The nature of this bimodality in response to Wnt5a is unknown. Previously, Wnt5a has been shown to exert opposite effects, either stimulating or inhibiting synaptic processes (Davis et al., 2008). Here, we observed an increase and a reduction in the number of short-Up states in the same preparation with different Wnt5a concentrations, suggesting that the generation of the oscillatory period in entorhinal cortex is highly regulated by the amount of released Wnt5a.

In the case of the hippocampus, we found that only high doses of Wnt3a increase the spontaneous firing rate in CA3 (41 ± 8% over control at >1:12, Fig. 1E, black line) and in CA1 (95 ± 33% over control at >1:25, Fig. 1E, gray line). High doses of Wnt5a also increase the firing

rate in CA3 (62 ± 18% over the control at 1:6, Fig. 1F, black line), whereas CA1 responded with a bimodal behavior, increasing the firing rate at low and high doses and decreasing it at intermediate doses (Fig. 1F, gray line). Strikingly, the shape of this curve in CA1 (Fig. 1F, gray line) shows the same bimodality as the curve of the short-Up states induced by Wnt5a in mEC (Fig. 1D, gray bars). Whether Wnt5a is modulating the firing activity of CA1 over the mEC (or vice versa) is a very interesting possibility and needs more experiments to be addressed. Altogether, these results show that natural Wnt analogues can finely tune the firing properties of sub-regions of an active circuit, suggesting that the local release of different amounts of these ligands during natural conditions can exert control over the intrinsic and synaptic properties of the circuit.

Effects of the antibodies against the canonical Wnt/β-catenin dependent-pathway

Now we know that Wnt proteins can modulate the firing properties of the circuit, we would like to learn whether endogenous released canonical Wnt proteins are contributing to the generation and maintenance of coordinated spiking activity in the oscillating slices. To do this we suppressed the extracellular activation of the intracellular cascade caused by the two most common canonical Wnt ligands, by perfusing the slices with the antibodies against Wnt3a and Wnt7a (anti-Wnt3a/7a, 0.2 μg/ml each). Fig. 2A displays the effects of the antibodies perfusion in mEC during slow oscillation. First, within the first 2 min of perfusion, there is a clear reduction in the oscillatory period in mEC. Second, the effect lasts for the time the antibodies are present and it recovers completely 5 min after wash out (Figs. 2A, B). The analysis shows that, during the antibodies exposure, there is a significant reduction in the number of Up states (Fig. 2C, gray bars) despite the increase in the number of spikes/Up state (Fig. 2C, black line). This last effect could represent the homeostatic effect in the firing rate to maintain functional connectivity due to the reduction in the frequency of regular Up states, rather than an effect of the antibodies itself.

Simultaneously, in the hippocampus, the presence of the antibodies transiently reduces the firing activity in CA3 only during the first 30 min,

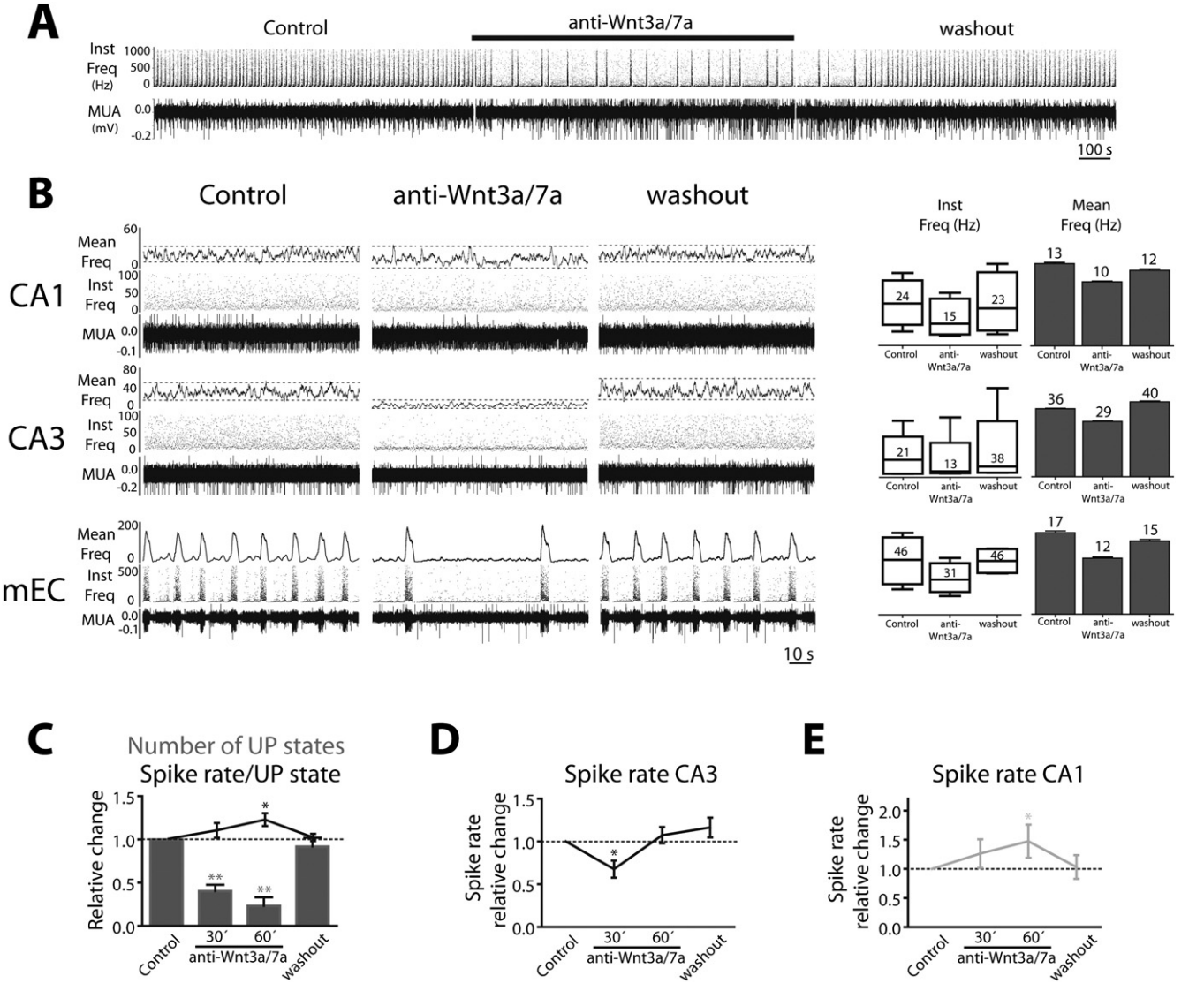

Fig. 2. Antibody anti-Wnt3a and anti-Wnt7a decrease Up states in mEC and it has differential effects in CA3 and CA1. A, A representative recording in mEC showing how fast and strong is the effect of anti-Wnt3a/7a on the slow oscillation. B, Traces from all three areas, showing the MUA, the instantaneous and the mean frequency during control, during 1 h of anti-Wnt3a/7a and during wash out period. The whisker and bar graphs beside show the average of the instantaneous frequency and mean frequency obtained during almost 1000 s of continuous recording. C, The bar graph and the black line on top shows the number of Up states (gray bars) and the corresponding spike rate/Up states (black line) relative changes, respectively, to the corresponding parameter before the application of the antibodies. D–E, Summarizes the spike rate relative change in CA3 (D) and CA1 (E). For all the analysis n = 14 experiments, $p < 0.01$ (*); $p < 0.001$ (**).

after which the cells recover the firing activity while the antibody is still present (Fig. 2D). Meanwhile, the tendency of the cells in CA1 is to fire more (Fig. 2E). The graphs beside the traces (Fig. 2B) are the averaged instantaneous and mean frequency in each condition respectively, showing that both parameters diminish in the presence of the antibody, returning to previous levels after wash out. We did not observe any significant difference between the effects of both antibodies, so we plot the data together. We also used a de-activated antibody (pre-boiled) and we did not observe any of these effects, confirming that this effect depends on the antibody. These results suggest that the antibodies are interfering with the synaptic mechanisms that couples cells into the oscillatory cycle in mEC, which are maintained by a constitutively released canonical Wnt, and that at least partially endogenous Wnt modulates the basal firing activity in CA3.

The pattern of how the firing activity is generated is also relevant for the information processing in the brain. The analysis of the interspike interval (ISI) histogram and the autocorrelation analyses (Fig. S5) show that anti-Wnt3a/7a interferes with the main neuronal features of the circuit, altering not only the number of spikes during short intervals but also modifying the spike patterning as we observed from the correlation analysis. It particularly causes strong effects in mEC and CA3, some of which outlast the blockage itself.

Effects of the antibody against the non-canonical Wnt pathway

Next, we determined whether the non-canonical Wnt pathway is endogenously active and contributing to the circuital activity. Fig. 3A shows that the perfusion with the antibody against Wnt5a (0.2 μg/ml anti-Wnt5a) reduces the period of slow oscillation in mEC within 3 min (Fig. 3A), an effect that completely washes out after 5–7 min (Figs. 3A, B). Quantification of this effect showed a significant reduction in the number of Up states (Fig. 3C, bar graph), though less potent than with anti-Wnt3a/7a. We did not observe any significant change in the number of spikes per Up state during the recording (Fig. 3C, black line), demonstrating, as with anti-Wnt3a/7a, that the antibodies affect the synaptic mechanisms that cause the oscillatory cycle and not the firing properties of the cells in EC. Simultaneously, in the hippocampus, bath application of anti-Wnt5a produces a partial reduction in the firing

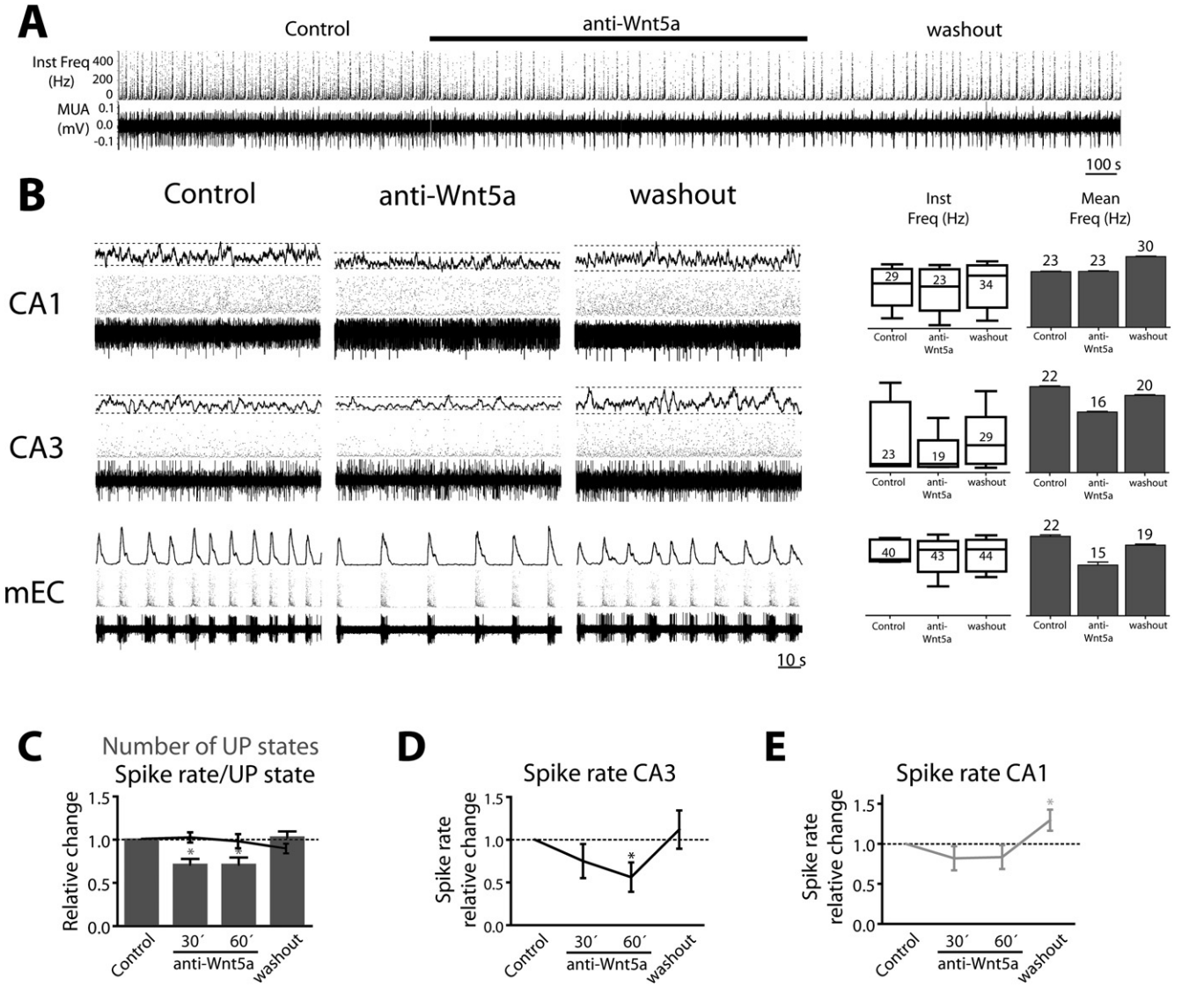

Fig. 3. Antibody anti-Wnt5a also reduces Up states number in mEC and it has differential effects in the hippocampus. A, A representative recording in mEC showing the effect of anti-Wnt5a on the slow oscillation. B, Traces from all three areas, showing the MUA, the instantaneous and the mean frequency during control, during 1 h of anti-Wnt5a and during wash out period. The whisker and bar graphs beside show the average of the instantaneous frequency and mean frequency obtained during almost 1000 s of continuous recording. C, The bar graph summarizes the number of Up states (gray bars) and the spike rate/Up states (black line) relative changes due to the presence of the antibody in mEC. D–E, Resumes the spike rate relative change during the presence of anti-Wnt5a in CA3 (D) and in CA1 (E). For all the analysis n = 12, $p < 0.01$ (*); $p < 0.001$ (**).

activity in CA3 that is significant after 1 h of perfusion (Fig. 3D), which could represent the fraction of the activity that is modulated by Wnt5a and which is at least temporally different from Wnt3a. The CA1 region does not show any significant change in firing rate, despite a tendency to increase after the washout, as it is showed by the instantaneous and mean frequency calculation (whisker and bar graphs, respectively). The ISI histogram and autocorrelation analyses (Fig. S6) show that anti-Wnt5a mainly affects the amount of elicited spikes in short and long intervals, and, to a small degree, the correlation of the activity with a more pronounced effect in mEC. Therefore, our results indicate that the non-canonical Wnt pathway is endogenously active and contributes to the maintenance of rhythmic activity in the cortex, slowly changing the network properties of mEC and the intrinsic firing activity in the hippocampus.

WASP-1 and sFRP-2 mimic the activation and blockage, respectively, of endogenous Wnts

WASP-1 (Wnt-activating small molecule potentiator-1) is a molecule that has been shown to have a strong synergistic effect of excitatory synaptic transmission in the presence of Wnt3a (Beaumont et al., 2007), but in its absence or in the presence of other Wnts, it does not show any significant effect (Beaumont et al., 2007). We hypothesize that if Wnt3a has been released during the spontaneous neuronal activities we are recording, we should observe an effect on the basal activity during WASP-1 application. Our experiments show that, immediately after the perfusion with WASP-1 (200 nM), each brain area shows an increment in the number of spikes and in the mean frequency of spontaneous firing, as we previously observed with Wnt3a (Fig. 4A), supporting the idea that canonical Wnt ligands are being spontaneously released contributing to the firing activity.

To suppress most of the endogenous Wnt released during our recordings that can contribute to these effects, we used the soluble Frizzled-related protein (sFRP), a physiological Wnt-signaling scavenger that binds directly to Wnt because of its similarity with the receptor Frizzled regulating the availability of Wnt proteins (Baarsma et al., 2013; Cruciat and Niehrs, 2013; Finch et al., 1997; Rattner et al., 1997). Despite the lack of a specific sFRP for a particular Wnt, it has been described a selectivity based on affinity in the nanomolar range, where sFRP1-4 binds Wnt3a and sFRP1-2 binds Wnt5a (Cruciat and Niehrs, 2013; Davis et al., 2008; Varela-Nallar et al., 2010). Using sFRP-2 (25-50 nM), we observed a reduction in the basal firing activity in the hippocampus and in the network activity in mEC (Figs. 4B–C). In mEC, there is a strong reduction in the number of Up states (Fig. 4C, gray bars), as we observed with anti-Wnt3a/7a, and no effect on the spike rate per Up state. The effect of sFRP-2 is slower than the effect of the antibodies (reaching the maximum effect within ~10 min of perfusion), reason why we also observed a slower washout effect. In the hippocampus, we observed mixed effects. On one side, in CA3 the effect of sFRP is similar to the effect caused by anti-Wnt3a/7a, where the spike rate transiently decreases during the first 30 min, recovering later even beyond the initial levels (Figs. 4B–C). On the other side, in CA1 sFRP causes a similar effect than the anti-Wnt5a, where the activity tends to decrease while sFRP is present in the recording solution (Figs. 4B–C). The fact that sFRP exerts these mixed effects may be due to that is non-specific and its effect might depend on the context (Baarsma et al., 2013). Despite that, these results show that sFRP is able to scavenge the endogenous Wnt that is released during basal firing activities, and plus the WASP-1 experiment supports the idea that the Wnt released spontaneously helps to keep the fine-tuning of the circuital output.

Activating the canonical Wnt/β-catenin dependent pathway through a GSK-3α/β inhibitor

To elucidate which of the effects on spiking activity and oscillatory activity that we observed can be ascribed to the activation of a particular pathway, we mimicked the intracellular activation of the Wnt/β-catenin dependent pathway by applying BIO-6, a permeable, reversible and ATP-competitive inhibitor of glycogen synthase kinase 3α and β (GSK-3α/β) to the recording solution (Meijer et al., 2004). GSK-3 is central enzyme of the canonical Wnt/β-catenin dependent pathway that is inhibited when the pathway is active, allowing the accumulation of intracellular β-catenin (MacDonald et al., 2009). Then BIO-6 inhibits GSK-3β mimicking the intracellular activation of Wnt/β-catenin pathway. Fig. 5A shows a representative example of a recording in mEC before and during the application of BIO-6 (10 nM). Continuous perfusion of BIO-6 into the recording solution exerts a strong effect changing the pattern of slow oscillation, from regular-Up states to a pattern intermixed with short-Up states, similar to the effect caused by Wnt3a (Fig. 1C, see asterisks). The bar graph summarizes the relative change in the number of Up states in control conditions and during one hour of BIO-6 (Fig. 5B). Surprisingly, there is a strong initial increase in the number of regular Up states that we did not see with Wnt3 (Fig. 5B, black bars), presumably as a consequence of the direct activation of the intracellular pathway. In the case of the hippocampus, most of our recordings in CA3 and CA1 showed an increase in relative spike number (7 of 9 recordings, not shown) after BIO-6 perfusion, suggesting that the intracellular dosage of the enzyme might determine its final effect, as Wnt3a does it at different doses. These results strongly support the idea that the intracellular activation of the canonical Wnt signaling can modulate the temporal organization of the oscillatory activity, transforming the outcome of the circuit into a new temporal dynamic.

Inhibiting the Wnt/β-catenin dependent pathway

To confirm whether the endogenous activation of the Wnt/β-catenin dependent pathway is involved in this phenotype, we used pyrvinium pamoate (Thorne et al., 2010), a potent inhibitor of the Wnt/β-catenin dependent pathway, which selectively potentiates casein kinase-1α (CK-1α) activity, the first kinase in the sequence that phosphorylates β-catenin to degradation (Thorne et al., 2010). We observed that during 60 min of continuous perfusion of pyrvinium pamoate (20 nM) there is a strong and constant reduction in the spiking activity in CA3, and simultaneously, a significant increase in the same parameter in CA1 (Figs. 5C–D). The potency of this effect, the strongest we observed, could be explained by the fact that pamoate keeps the CK-1α active, avoiding any accumulation of β-catenin into the cell (Hernandez et al., 2012; Liu et al., 2002; Thorne et al., 2010). Interestingly, these experiments show that the firing activity in CA3 strongly depends on the activation of Wnt/β-catenin dependent signaling, unlike CA1, which seems to be independent of Wnt/β-catenin pathway (Fig. 5D). The mechanism used by CA1 to increase the firing activity it could represent a novel fine-tuning using homeostatic rules to keep the circuital properties active, especially when CA3 is down-regulated.

Wnt signaling keeps controlled the exacerbated network activity

We have found that Wnt can modify the basal firing activity. However we do not know whether Wnt can exert the same effect during exacerbated firing activity. In order to elucidate this, we induced seizure activity in our slices; this is, the generation of episodes of disbalanced firing that constitutes a model of epileptic activity in vitro (Fig. 6A). To do that we perfused our slices with a recording solution in which Mg^{2+} has been reduced to a nominal free-Mg^{2+} level (Jones and Heinemann, 1988; Tahvildari et al., 2012). By itself this causes the activation of all the excitatory neurons in the circuit, increasing the general firing activity that does not saturate after two hours (Figs. 6A and B). In this condition the firing rate increases up to 3 times over the control in mEC and CA3 and up to 2 times over the control in CA1 (Fig. 6B, gray bars). In the case of mEC the changes involved a complete restructuration of the Up-Down oscillatory structure. Now in this new condition we

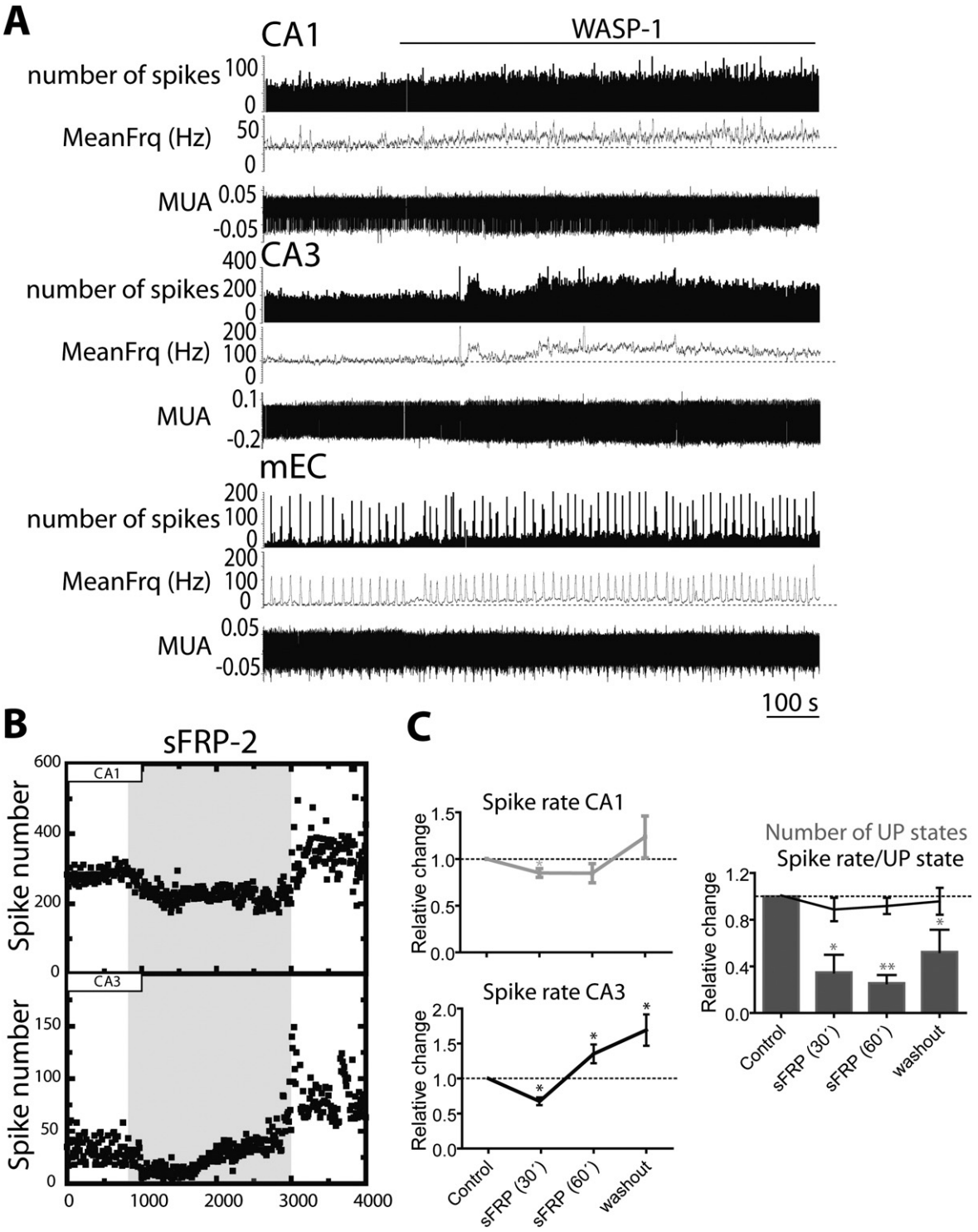

Fig. 4. Effect of WASP-1 and the scavenger sFRP in neuronal activity. A, Bath application of the canonical pathway potentiator WASP-1 (200 nM) changes the number of spikes and the mean frequency in all three recorded areas (n = 9 experiments). B–C, Bath application of scavenger sFRP-2 (25–50 nM) reduces the firing activity in the hippocampus. C, Quantification of the spike rate relative change in all three areas. The plots represent the spike rate relative change in CA3, in CA1, and in mEC before, during and after bath application of sFRP. For clarity, every point represents the output sample rate at 0.1 Hz. For the analysis: n = 8 experiments, $p < 0.01$ (*); $p < 0.001$ (**).

observed that the addition of WASP-1 to the recording solution restores the main Up-Down oscillatory structure, stops the increment in the CA3 firing activity and reduces it to its control levels in CA1 (Fig. 6B), suggesting for the first time that the activations of Wnt signaling also exert a homeostatic effect, stabilizing the overall exacerbated activity in an active network.

Discussion

Here, for the first time we demonstrated that Wnt signaling pathways can modulate the neuronal firing properties of the spontaneous activity in a slice that contain the entorhinal–hippocampal circuit. Moreover, the effect of Wnt could have a physiological relevance since

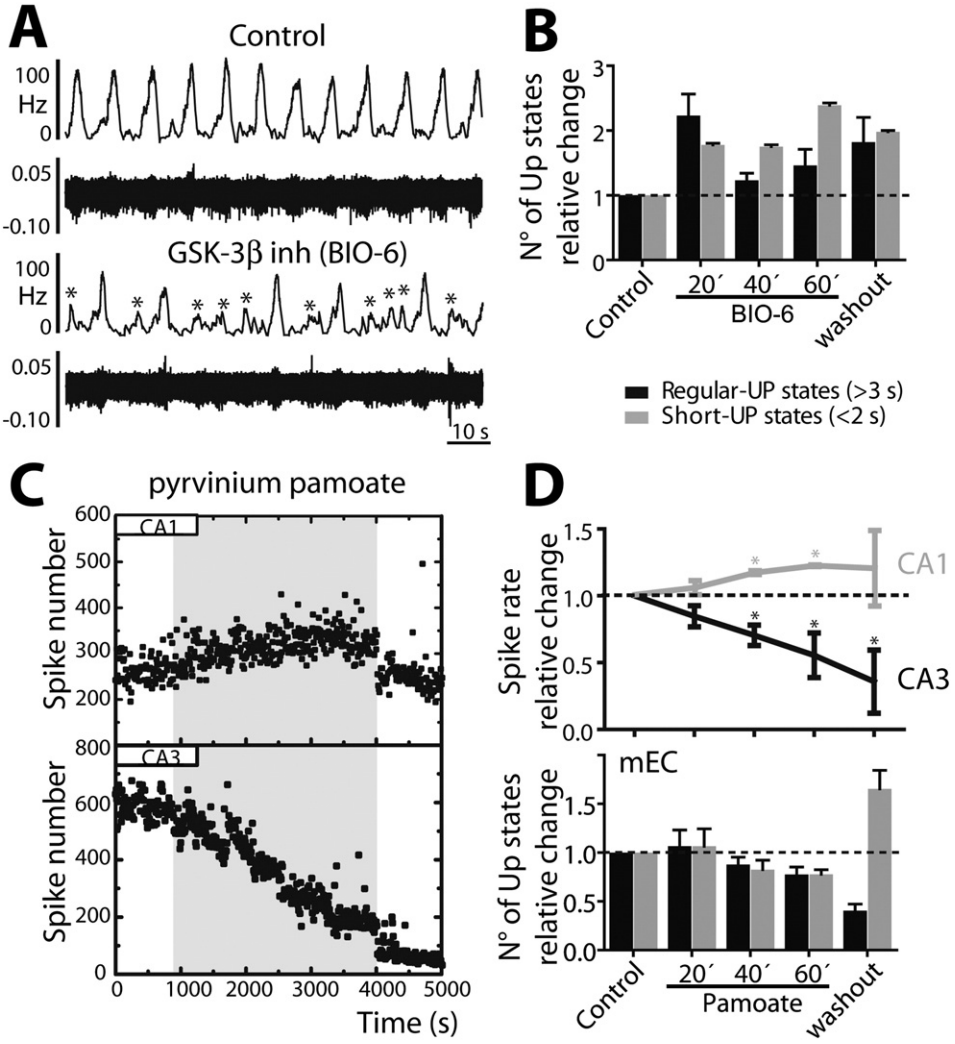

Fig. 5. The effect of GSK-3α/β inhibitor mimics the effect of Wnt3a. A–B, Activation of Wnt/β-catenin dependent pathway using BIO-6. A, Representative example showing the effect of 1 h of bath perfusion with BIO-6 (10 nM), showing the MUA and the mean frequency. Asterisks represent the short-Up states detected. B, Summary bar graph showing the relative change in the number of regular- and short-Up states ($n = 8$ experiments; $p < 0.01$ (*)). C–D, Inhibition of the canonical Wnt pathway with pyrvinium pamoate. C, Representative example showing the spikes detected in CA1 and CA3 regions simultaneously recorded along the experiment, before, during and after bath application of pyrvinium pamoate (20 nM). D, The graphs show the data for both areas in the hippocampus (D, upper) and for mEC (bar graph, below), during 60 min of perfusion with pamoate. For clarity, every point represents the output sample rate at 0.1 Hz. For the analysis: $n = 9$ experiments, $p < 0.01$ (*).

it is able to modify the oscillatory pattern activity in entorhinal cortex and the activity generated simultaneously in the hippocampus, being able to be used as a physiological tool to determine the effect of Wnt proteins on the firing properties of the circuit.

Today we are able to develop in vitro preparations that preserve in a very good shape their properties, very similar to what is in vivo (Huguenard and McCormick, 1992; Llinas and Yarom, 1986; Muhlethaler and Serafin, 1990; Sanchez-Vives and McCormick, 2000; Silva et al., 1991; Yuste and Katz, 1991). The oscillatory activity generated in a brain slice in vitro is the same generated during cognitive processes recognized in vivo, which means that the connectivity has been preserved enough to self-generation of an oscillatory pattern (Hajos et al., 2009; Hajos and Paulsen, 2009). Our recordings mainly from the layer II in mEC, where most of the grid cells are, show the spontaneous generation of recurrent oscillation in the form of Up and Down activity that resembles what occurs in vivo during deep anesthesia and during slow wave sleep (Sanchez-Vives and McCormick, 2000; Steriade et al., 1993). This is, the cells fire mainly during a depolarized step called Up state and remain silent during the hyperpolarized step called Down state. The interplay between excitation and inhibition keeps the cycles in a periodic oscillation (Fig. S1). The information from mEC layer II arrives into hippocampus through the main connectivity with dentate gyrus (DG) and CA3. CA3 is full of recurrent connectivity between the cells in the same area (Li et al., 1994). Through the Schaffer collaterals CA3 projects toward CA1, and then the final output to subiculum and parasubiculum closes the circuit, whose projections arrive back to layer V in mEC. We also recorded stable Up-Down activity from mEC layer III, as has been shown in vivo (Hahn et al., 2012), however because these cells send monosynaptic connections directly into CA1, we did not include them in the analysis.

As a source of Wnt proteins, we used the conditioned media that contain most of the soluble form of specific Wnt (Cuitino et al., 2010; Shibamoto et al., 1998; Varela-Nallar et al., 2010). As has been shown for Wnt3a, this media is enough to up-regulate the level of β-catenin at a 25-fold dilution (Shibamoto et al., 1998), and as we did observe, enough to increase the firing rate at the same dilution (>1:25, Fig. 1). For Wnt5a conditioned media it has been reported that a dilution as low as 1:100 is able to increase the field excitatory postsynaptic potentials (fEPSPs) amplitude in hippocampal slices (Varela-Nallar et al., 2010). We explored a full range of dilutions and we found in the

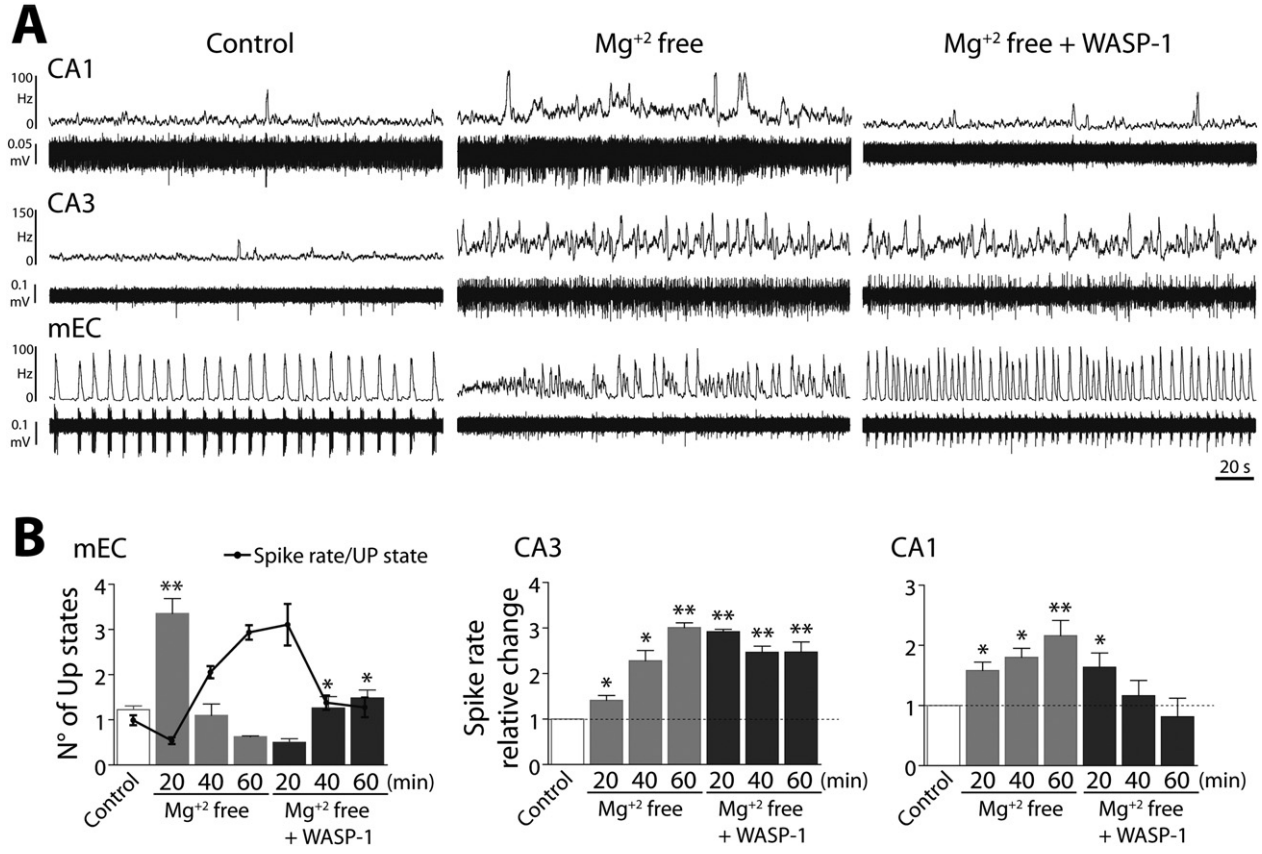

Fig. 6. Exacerbated network activity in the form of seizure activity is attenuated by endogenous Wnt pathway activation. A, Recording solution without Mg^{2+}-free induces seizure activity in the slices by release of excitatory activity. B, Bar graphs show the quantification of the spike rate relative change in the three areas, in Mg^{2+}-free solution (gray bars) and during the addition of WASP-1 (black bars). For the analysis: n = 8 experiments, $p < 0.01$ (*); $p < 0.001$ (**).

majority of the cases a dose-dependent effect, physiologically relevant to our understanding because the Wnt comes from natural sources. The released Wnt protein could act both as an autocrine and as paracrine signal over the neighbor cells and few more cells beyond (Shibamoto et al., 1998). When one cell releases Wnt the effect is presumably local in a gradient that is extended little further away. However, in a slice all the cells are being perfused with the same recording solution containing Wnt at a particular dilution, we assumed all of them are exposed to the same concentration. Then the differences to high or low doses are really the representation of the dose–response effect, of the receptor affinity constants or simply the fact that not all the cells are equally expressing the machinery to respond. On this regard, it has been shown that the Wnt components are expressed in all the brain areas we recorded at the same postnatal age as has been in detail reported (Shimogori et al., 2004).

One of the most striking features we observed in our preparation is that Wnt3a and Wnt5a increase the frequency of the oscillation, augmenting the number of regular-Up states, but also the occurrence of new short-Up states (Fig. S2). Still we do not completely understand the nature of this new activity, but we can speculate whether it comes from the de-synchronization of the pre-existent periodic activity or from new cells that become active in the circuit. Counting the number of spikes generated only during the regular-Up states along the full experiment, before, during and after Wnt3a or Wnt5a perfusion, we found that during the presence of Wnt3a the number of spikes during every regular-Up state decreases; whereas, this number remains stable throughout the experiment with Wnt5a (Fig. S4). Concomitantly, the number of Up states in both cases increases (Fig. S4). This means that Wnt3a causes the de-synchronization of the pre-existent activity and the short-Up states would arise from the de-fragmented regular-Up states. We observed the same effect with BIO-6 (Fig. 5A), strongly suggesting that this feature is caused by the intracellular activation of canonical Wnt pathway. Instead, because the spike number in regular-Up states does not change along the experiment, the new activity induced by Wnt5a would correspond to new cells incorporated in the oscillation. Interestingly, the short-Up states induced by Wnt5a has the same bimodal nature than the effect of Wnt5a in CA1, suggesting that it corresponds to input activity coming from (or influenced by) CA1. In fact, it has been demonstrated a very specific anatomical connectivity between EC-CA1-subiculum (Capogna, 2011; Naber et al., 2001a,b; Soltesz and Jones, 1995; Tamamaki and Nojyo, 1995), and it has also been shown that there is a well-preserved functional connectivity in combined slices such as ours (Barbarosie and Avoli, 1997; Tahvildari et al., 2012). The possibility that Wnt could modulate both synaptic and intrinsic aspects of the circuit exerting effects on the functional coupling between different components of the entorhinal–hippocampal circuit represents a remarkable possibility that relates Wnt with the spatial memory circuit. More experiments are required to further confirm this hypothesis.

On another hand, our experiments with the antibodies anti-Wnt3a/7a and anti-Wnt5a show that both Wnt protein isoforms are spontaneously released by cells and contributing to the general activity. We hypothesized that the antibodies disrupt the period of the oscillatory activity in mEC blocking the extracellular interaction of endogenous Wnt with its receptor, retarding the synaptic processes that sustain the network activity, such as neurotransmitter release and pre- and postsynaptic receptor trafficking. The fact that the ability of cells to fire action potentials is not affected supports this idea. Canonical Wnt ligands like Wnt3a or Wnt7a have been mainly associated with presynaptic action mode, this is, stimulating the recycling of synaptic vesicles

and increasing the neurotransmitter release (Ahmad-Annuar et al., 2006; Cerpa et al., 2008; Hall et al., 2000) and could explain why there is a stronger effect in the presence of anti-Wnt3a/7a, compared to the effect of anti-Wnt5a. Based on these results we can hypothesize that during the oscillatory activity in EC a ligand like Wnt3a or Wnt7a is continuously released to exert a fine regulation over the synaptic mechanisms that keeps the ongoing oscillation. Less Wnt, or less activation of canonical Wnt pathway as with the antibody experiments, interferes with the oscillatory cycle. Instead, adding Wnt or the sustained activation of canonical Wnt pathway, as when we add Wnt3a or BIO-6, causes the de-fragmentation of the oscillatory cycle due to the excessive neurotransmitter release and/or due to disequilibrium in the excitatory/inhibitory balance.

On the other hand, our data indicates that the hippocampus behaves under different rules. In CA3, anti-Wnt3a/7a causes a significant reduction in firing activity but only during the first 30 min (Fig. S2 C2). This transient effect reveals the component of spike activity in CA3 that depends on the constitutive presence of a Wnt ligand and the consequent activation of the intracellular canonical signaling (Fig. S2 C2). In fact, the effect we observed in CA3 coincides with the peak in the accumulation rate of β-catenin after 30 min of Wnt3a stimulation (Hernandez et al., 2012) or after 15–30 min of tetanic stimulation (Chen et al., 2006), which returns back to basal levels after 1 h (Hernandez et al., 2012). In basal condition, β-catenin is continuously synthesized and degraded keeping a balanced intracellular concentration. The stimulation by Wnt can activate its accumulation reaching to a new high steady-state which is stabilized by maintaining simultaneously the accumulation and degradation processes (Hernandez et al., 2012). In our case, the antibody could block the autocrine Wnt activation causing the reduction in β-catenin and then the partial reduction in the firing activity in CA3. The recovery of the firing activity could obey to the restitution of the internal equilibrium toward more β-catenin synthesis. In the same CA3 area, the perfusion of anti-Wnt5a significantly reduces spiking activity by the end of 60 min, a later effect that reveals the component of the spiking activity in CA3 that depends on endogenous Wnt5a, which most likely exerts its effects to sustain the postsynaptic machinery to continue the synaptic activity as has been previously described (Cerpa et al., 2011; Varela-Nallar et al., 2010). The Wnt5a which is one of the non-canonical Wnt/Ca^{2+} ligands has been shown to be involved in the regulation of postsynaptic receptors such as NMDAR and components of postsynaptic density such as PSD-95 (Cerpa et al., 2011; Varela-Nallar et al., 2010).

We cannot rule out the other possibility, which is that the connectivity between different parts of the circuit commands the changes in the firing properties. Then, the initial reduction in the CA3 activity by the antibody may be due to the reduction in the oscillatory activity in mEC — that is, affected by the synaptic connectivity between mEC and CA3. However, in the presence of the antibody CA3 is able to recover the original activity despite that EC does not. Moreover, regardless of the well-described connectivity between EC-CA1 (Capogna, 2011), the hypothesis of the connectivity does not explain the effect in CA1, where the cells tend to fire more. We postulate an alternate hypothesis. Evidences in the literature suggest that the hippocampus works under a strict control of homeostatic rules. It has been shown that the way that synapses accommodate to changes in activity is extremely synapse specific and the homeostatic rules applied differentially for CA3 or CA1 (Kim and Tsien, 2008). In fact, during inactivity periods, CA1 is able to scale up but not CA3 (Kim and Tsien, 2008). Our experimental data supports this hypothesis. Pyrvinium pamoate, which blocks the intracellular activation of Wnt/β-catenin dependent pathway, causes the stronger effect we observed steadily decreasing the firing activity in CA3 (Figs. 5C–D), confirming that CA3 needs the basal activation of the intracellular canonical Wnt pathway to keep its circuits active. At the same time, during pamoate treatment the firing activity in CA1 increases significantly, showing that CA1 can increase its firing activity by mechanisms independently of canonical Wnt signaling, and instead uses homeostatic mechanisms to regulate its output. Moreover, it suggests that in our in vitro model the activity in CA1 is strongly driven by its presynaptic inputs coming from CA3. Therefore, these results suggest that in the hippocampus Wnt signaling could modulate the pre- and postsynaptic functional coordination. Furthermore, pamoate slowly reduces the oscillatory activity, oppositely to what BIO-6 does, and it shows a strong rebound of short-Up states after washout, confirming that endogenous canonical Wnt activity helps to keep the integrity of the oscillatory units.

Another support to this hypothesis comes from the experiments in magnesium-free recording solutions. In our experiments the activation of endogenous Wnt is able to control and even reverse the abnormal seizure activity induced by a magnesium-free environment in CA3 and CA1 area, respectively, strongly suggesting that CA1 is "the plastic output" of the system that scales-up or -down depending on the presynaptic driving force modulated by Wnt signaling. Altogether, these evidences show that an active Wnt pathway could work as a homeostatic adjustment of excitability of the system, controlling the firing rate and the strength of the synaptic connectivity to keep the status of the network activity differentially in the hippocampus than in the cortex.

Nevertheless, how can a molecule like Wnt modulate firing activity? Recently, it has been described that β-catenin regulates the expression of genes that encode for proteins involved in neuronal excitability in the thalamus (Wisniewska, 2013), and the hippocampus receives innervations from certain thalamic nucleus (Wouterlood et al., 1990). However, in our case the effects we observed are too fast to presume a regulated expression of particular conductances. On the other hand, some evidences have demonstrated that β-catenin and GSK3 interact with conductances such as Ca^{2+}-activated K^+ channels in hair cells (Lesage et al., 2004) and $KCNQ_2$ potassium channels in mouse prefrontal cortex (Kapfhamer et al., 2010), respectively, demonstrating that components of the intracellular Wnt cascade can physically interact, and perhaps modulate ion channel physiology. BDNF, a proposed target of Wnt signaling (Inestrosa and Varela-Nallar, 2014; Yi et al., 2012), also modulates intrinsic voltage-dependent conductance to adjust the excitability of cells in conditions of variable activity (Desai et al., 1999; Rutherford et al., 1998). Whether Wnt exerts its effects through BDNF is not possible to know from our study, but based on our results we can say the effect of Wnt exposure is acute enough to be produced through a direct activation of Wnt intracellular cascade. A very reasonable hypothesis has been recently proposed. GSK3 and β-catenin can regulate the neuronal excitability by controlling the voltage-gated sodium channels density at the axon initial segment (Tapia et al., 2013), region that concentrates the highest density of sodium channels to initiate the action potential and its propagation. Whether the activation of Wnt signaling exerts changes in cellular excitability by this way remains to be elucidated, but it could explain in part our results supporting the idea of a dynamic role of Wnt signaling modulating the firing properties of the cells. Our results represent a novel mechanism by which Wnt spontaneously released from active synapses could control non activity-dependent processes, but the state of the system, whereas different doses of Wnt3a or Wnt5a may emulate what happens during activity-dependent processes of different strengths.

Conclusions

Wnt is continuously released during postnatal life, Wnt signaling components are presents in areas actively participating in neurogenesis and its receptors are expressed in areas of the brain that undergo plasticity or involved in sensory processing and superior cognitive processes (Oliva et al., 2013a; Shimogori et al., 2004). Wnt is released spontaneously by cells but activity may also control its secreted levels. In this way, Wnt can actively modulate the synaptic transmission, improve the release of neurotransmitters and reliability of receptors, directly remodeling the structure of dendritic spines based on the

incoming activity, a property that can modify the connectivity, affect synaptic functions, and therefore, the entire output of the system (Caroni et al., 2012; Cerpa et al., 2011; Chen et al., 2006; Oliva et al., 2013a,b). But more, from our study we reveal that Wnt can directly change the spiking properties of the cells, an effect that can anticipate the structural changes constituting a positive feedback for the system that reinforces the release of Wnt ligands with the physiological effect. Especially in the entorhinal–hippocampal circuit, a molecule like Wnt could be fundamental consolidating, first functional and then structurally, the large amount of associational correlations that may able the storage of spatial information, to establish later the possibility of episodic memory with spatial components.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.expneurol.2015.03.027.

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