

mGlu1 α -dependent recruitment of excitatory GABAergic input to neocortical Cajal-Retzius cells

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ARTICLE INFO

Article history:

Received 3 April 2012
Received in revised form
20 April 2012
Accepted 23 April 2012

Keywords:

GABA
Interneuron
Development
IPSC
Network
Excitability

ABSTRACT

Cajal-Retzius cells are thought to play an important role for cortical development, and receive primarily spontaneous GABAergic input mediated by GABA_A receptors. However, neither the effects of synaptically-released GABA on their excitability nor the cellular source(s) of spontaneous GABAergic currents have been yet determined. By directly recording electrophysiological responses from identified Cajal-Retzius cells of the CXCR4-EGFP mouse, we show that GABAergic input can trigger supra-threshold responses, and that the pharmacological activation of mGlu1 α receptors with the group I agonist DHPG powerfully increases the frequency of spontaneous GABAergic currents. These effects appeared mediated by a network mechanism, because responses to DHPG were completely prevented both by surgical disconnection of layer I from lower layers and by exposure of slices to TTX.

We propose that the cellular source underlying the observed effect of DHPG are layer I-targeting Martinotti-like interneurons, which we show express functional group I mGluRs and respond to DHPG with supra-threshold depolarization already at early developmental stages.

In conclusion, our work suggests that conditions of enhanced glutamate release may be critical at early developmental stages for the recruitment of an mGlu1 α -dependent micro-circuit, which then leads to the activation of Cajal-Retzius cells.

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1. Introduction

The embryonic marginal zone/postnatal layer I has been suggested to play a critical role in orchestrating the development of the neocortex (Marín-Padilla, 1998). In fact, after completing their radial migration, developing pyramidal neurons first make synaptic contacts with the marginal zone/layer I (Ramón y Cajal, 1904; Marín-Padilla and Marín-Padilla, 1982), and then are displaced toward deeper layers by newly arrived migrating cells, thus generating an inside-out pattern of cortical development (Angevine and Sidman, 1961). Therefore, all pyramidal cells seem to receive progressively, as they mature, the same kind of information from layer I. However, the detailed functions performed by the micro-circuitry operating within the marginal zone/layer I are not completely understood.

The principal neuron of the marginal zone/layer I is the Cajal-Retzius cell (reviewed by Soriano and Del Río, 2005), which has

been the subject of numerous studies as a cellular source of the glycoprotein reelin (Tissir and Goffinet, 2003), which is essential for several functions ranging from the correct organization of cortical layers (D'Arcangelo et al., 1995), to the maturation of dendritic arbors (via different signaling pathways, see Niu et al., 2004, 2008; and Chameau et al., 2009) and their synaptic channels (Qiu and Weeber, 2007; Campo et al., 2009). Cajal-Retzius cells appear spontaneously active both at embryonic and post-natal stages. In fact, spontaneous calcium transients in Cajal-Retzius cells have been found to be synchronous in correlated networks including other Cajal-Retzius cells and/or different types of local neurons (Schwartz et al., 1998; Aguiló et al., 1999). This pattern of activity has been suggested to play computational roles, which have been postulated to be important for the development of the cortex. Intriguingly, several studies have indicated that Cajal-Retzius cells receive predominant, if not exclusive, spontaneous excitatory synaptic input mediated by GABA_A receptors (Kilb and Luhmann, 2001; Soda et al., 2003). Synchronized calcium oscillations are sensitive both to tetrodotoxin, which blocks axonal conduction (Narahashi et al., 1964), and to bicuculline, which blocks GABA_A receptors (Curtis et al., 1970). Thus, GABAergic input to Cajal-Retzius cells of the

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marginal zone/layer I could play a critical role for the recruitment of assemblies of Cajal-Retzius cells involved in this type of spontaneous activity.

Various types of GABAergic fibers have been shown to target the developing layer I: axons of local interneurons (Hestrin and Armstrong, 1996; Zhou and Hablitz, 1996; Marín-Padilla, 2011a, 2011b; Wozny and Williams, 2011), subplate cells (Friauf et al., 1990; Myakhar et al., 2011; Marín-Padilla, 2011a, 2011b) and thalamic zona incerta neurons (Lin et al., 1990). Paired recording between layer I interneurons and Cajal-Retzius cells have revealed a very low degree of connectivity, suggesting that GABAergic pathways originating from lower layers may provide a more significant input (Soda et al., 2003).

Here, we unravel a massive source of GABAergic input to Cajal-Retzius cells, which is powerfully activated by pharmacological agonists of group I metabotropic glutamate receptors (mGluRs) via mGlu1 α . We propose that activation of GABAergic interneurons expressing mGlu1 α , possibly Martinotti cells, plays an important role in generating synchronous network activity in Cajal-Retzius cells of the developing layer I, and hence, in contributing to their computational functions.

2. Materials and methods

2.1. Slice preparation

All animal experiments were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals and approved by Northwestern University Animal Care and Use Committee. Slices were prepared from newborn (P5–P10) CXCR4-EGFP mouse pups (www.gensat.org) as described previously (Marchionni et al., 2010), or, in a few cases, from GIN mice, which identify somatostatin-expressing interneurons (strain: FVB-Tg(GadGFP)45704Swn/J, Jackson Labs, see Oliva et al., 2000). Mice were deeply anaesthetized using isoflurane, quickly decapitated and the brain dissected out in ice-cold modified ACSF (in mM: 130 NaCl, 24 NaHCO₃, 3.5 KCl, 1.25 NaH₂PO₄, 1 CaCl₂, 2 MgCl₂, 10 glucose oxygenated with 95% O₂/5% CO₂ at pH 7.4). Horizontal slices were cut at 250 μ m on a vibratome (Leica, VT 1000 S), and then transferred to a warm (30 °C) holding chamber filled with ACSF (in mM: 130 NaCl, 24 NaHCO₃, 3.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, 10 glucose oxygenated with 95% O₂/5% CO₂ at pH 7.4) for 30 min, and then at room temperature until use. For slices that had layer I surgically dissected (Fig. 5), before recording, a small incision was made between layer I and layer II/III with the aid of a dissection microscope.

2.2. Electrophysiological methods

During recording, slices were superfused with preheated ACSF maintained at a constant temperature of 33 ± 1 °C. Cajal-Retzius cells were identified by their location in layer I, stereotypical “tadpole” shape visualized with a 60 X IR immersion DIC objective, and EGFP fluorescence excited by X-Cite Series 120 light source visualized with a VE1000 camera (DAGE-MIT, Michigan City, IN, USA). Interneurons were identified by their location in layers I and II/III, shape, firing patterns, and, when using the GIN mouse, fluorescence in layer II/III.

Conventional whole-cell and cell-attached patch-clamp recordings were performed. Patch pipettes (~ 3 – 5 M Ω resistance) were pulled from thin-walled borosilicate glass capillaries and filled with (in mM) 125 KCl, 10 NaCl, 16 KHCO₃, 4 MgATP, 0.3 NaGTP, 10 QX-314 equilibrated with 95% O₂/5% CO₂ to a pH = 7.3 for voltage-clamp recordings of postsynaptic currents in Cajal-Retzius cells. Interneurons were recorded with a modified version of the above intracellular solution (KCl reduced to 20 mM, and inclusion of 115 mM KMeSO₄). For cell-attached recordings, either ACSF or a modified internal solution with KCl reduced to 40 mM, and inclusion of 85 mM KMeSO₄ was used.

Recordings were made using a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA). Holding potentials were -60 mV for experiments involving whole-cell voltage-clamp recordings of Cajal-Retzius cells. When cell-attached configuration was used, the pipette was set at 0 mV when filled with ACSF and at -45 mV when the modified internal solution was used. Data were filtered at 3 kHz, and digitized at 10 – 20 kHz using a Digital A/D board and pClamp 9 software suite (Molecular Devices). Series resistance was monitored throughout recordings via a -5 mV step in voltage-clamp, or a -5 pA current pulse in current-clamp configurations, and balanced via a bridge circuit in current-clamp mode. Drugs were applied through bath perfusion. Field stimulation was achieved through use of monopolar electrodes (FHC Inc., Bowdoin, ME, USA) connected to a constant current isolation unit (A360, World Precision Instruments, Sarasota, FL, USA) and positioned at the border of layer I/layer II/III.

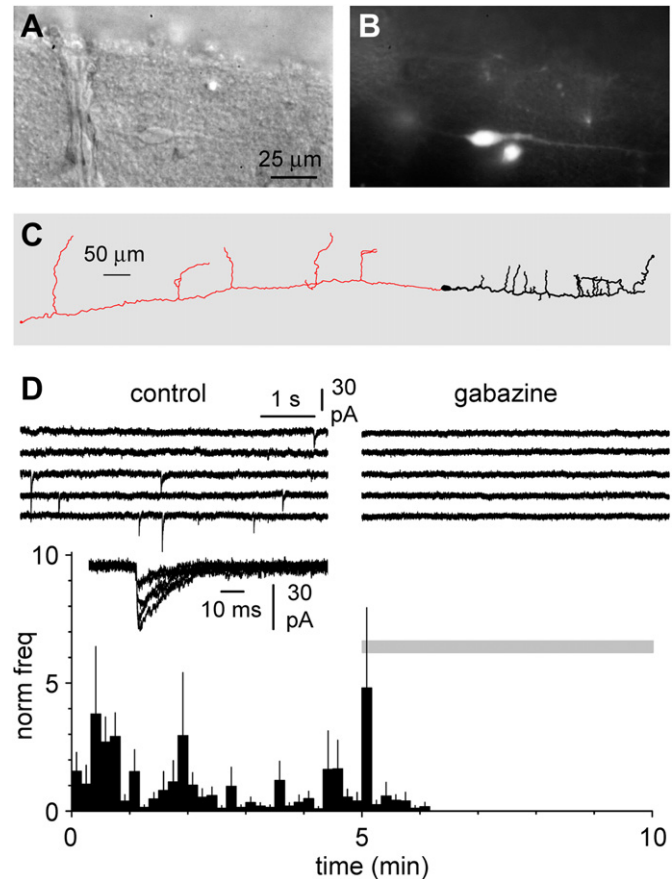


Fig. 1. Anatomically identified Cajal-Retzius cells of the CXCR4-EGFP mouse receive exclusively spontaneous GABAergic currents. A: DIC image of a Cajal-Retzius cell from a slice prepared from a P6 animal. Notice the typical “tadpole” shape with a single dendrite. B: same as in A, observed under epifluorescence. C: reconstruction of a biocytin-filled Cajal-Retzius cell from a P8 CXCR4-EGFP mouse. Soma and dendrite are shown in black, the axon in red. Notice the long span of the overall structure. D: summary plot of the frequency of spontaneous synaptic currents recorded in control ACSF and during application of the GABA_A receptor antagonist gabazine (12.5 μ M, gray bar). Upper insets show traces in control (left) and in the presence of gabazine (right). A few spontaneous events recorded in control conditions are superimposed and shown at a faster time scale.

2.3. Statistical methods

Data are presented as mean \pm SEM, and statistical significance was determined using Student's paired *t*-test, and repeated measures ANOVA with multiple comparisons posthoc Bonferroni test. Significance level was set at 0.05 .

2.4. Drugs

D-AP5, NBQX, gabazine, DHPG and LY367385 were obtained from Ascent Scientific. Tetrodotoxin was obtained from Sigma Aldrich, and QX-314 was obtained from Alomone Labs.

2.5. Visualization and reconstruction of biocytin – filled cells

Biocytin-filled cells were visualized using a slightly modified version of the protocol described by Lübke et al. (2000). Slices were fixed overnight in 4% para-formaldehyde in 0.1 M PB. Endogenous peroxidase activity was quenched using a 3% H₂O₂ solution for 15 min. Slices were incubated overnight at 4 °C in avidin–biotinylated-HRP complex (Vectastain ABC Elite kit) with 0.1% Triton X-100 in PB, followed by a peroxidase reaction with 3′3-diaminobenzidine tetrahydrochloride (DAB) as a chromogen, and intensified with 1% NiNH₄SO₄ and 1% CoCl₂. Cells were then checked for contrast under light microscopy, and briefly postfixed with 0.1% OsO₄ in PB (1 – 3 min). Slices were then mounted on slides with Moviol (Hoescht AG, Frankfurt, Germany) and coverslipped. After drying, cells were reconstructed using manual neuron tracing in Neurolucida and Neuroexplorer 9 software suite.

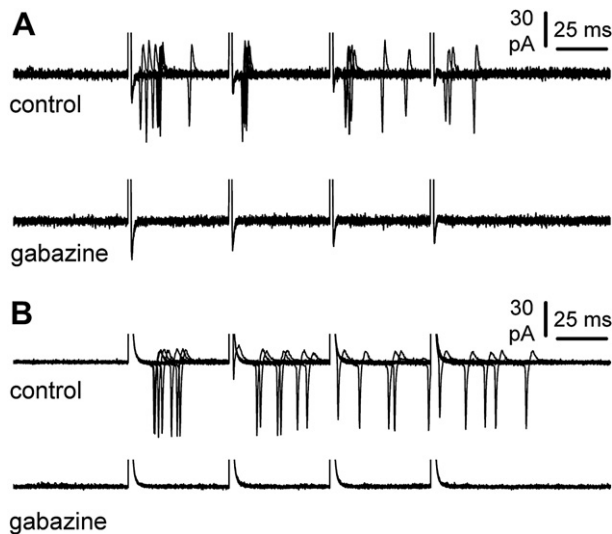


Fig. 2. Synaptically-released GABA can trigger action potentials in Cajal-Retzius cells. **A:** non-invasive cell-attached recording from a Cajal-Retzius cell during a four-pulse 20 Hz stimulation. Notice the presence of action currents, which are abolished in the presence of gabazine (12.5 μ M). **B:** similar result as in **A** for an experiment obtained in the presence of ionotropic glutamate receptor antagonists (NBQX: 20 μ M and D-AP5: 50 μ M).

3. Results

3.1. GABAergic input to Cajal-Retzius cells

When recorded in whole-cell voltage-clamp configuration, identified neocortical Cajal-Retzius cells of the developing layer I (**Fig. 1A, B and C**) show rare and irregularly-occurring spontaneous synaptic events. These synaptic events were completely abolished by gabazine (12.5 μ M, **Fig. 1D**), indicating that they were mediated by GABA_A receptors. Compared to a control value of 66 ± 26 mHz, bath perfusion of gabazine decreased the spontaneous frequency to 0 ± 0 mHz, ($n = 8$, $p < 0.05$, paired t -test). This indicates that, although Cajal-Retzius cells express glutamate receptors (Lu et al., 2001; Chan and Yeh, 2003; Marchionni et al., 2010), under our experimental conditions spontaneous synaptic currents are exclusively GABAergic. This finding is in good agreement with previous work (Kilb and Luhmann, 2001; Soda et al., 2003).

The relevance of a predominant, if not exclusive, GABAergic input to Cajal-Retzius cells has been highlighted by several studies showing that neocortical and hippocampal Cajal-Retzius cells maintain a high level of intracellular chloride, which generates a depolarized E_{GABA} (Mienville, 1998; Achilles et al., 2007; Marchionni et al., 2010). This ionic condition suggests that synaptic input mediated by GABA_A receptors may generate supra-threshold responses and thus control the level of activity and synchronization of networks of Cajal-Retzius cells.

To our knowledge, despite several studies providing evidence for a depolarizing role of exogenously applied GABA, the issue of whether synaptically-evoked GABAergic input is able to trigger firing in Cajal-Retzius cells remains undetermined. We decided to take advantage of a non-invasive configuration such as cell-attached recording to observe the effect of synaptic GABAergic input on Cajal-Retzius cell excitability. Extracellular stimulation at the border of layer I/layer II/III with a four-pulse train at 20 Hz could effectively trigger action currents in Cajal-Retzius cells (**Fig. 2A and B**). The average probability of firing was 0.45 ± 0.09 , 0.25 ± 0.07 , 0.25 ± 0.08 , and 0.20 ± 0.04 for the first, second, third, and fourth stimulus, respectively ($n = 9$). Subsequent application of gabazine ($n = 8$) resulted in the complete suppression of the observed firing.

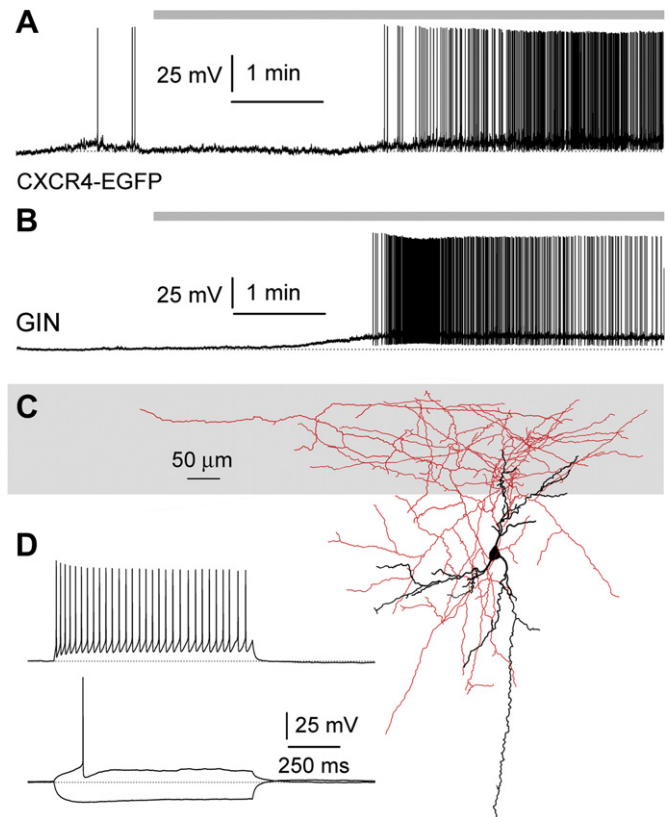


Fig. 3. Activation of group I metabotropic glutamate receptors generate supra-threshold depolarizations in somatostatin-expressing interneurons targeting layer I. **A:** example of an interneuron responding with firing to DHPG (10 μ M) application recorded in a slice prepared from a CXCR4-EGFP mouse. **B:** similar result obtained from a DHPG responding EGFP-expressing cell in a slice obtained from a P8 GIN mouse. **C:** anatomical properties of the cell shown in **B** reveal it for a young Martinotti interneuron. Notice the localization of its soma in the lower layers and the dense axonal arborization (red) and extensive targeting of layer I (gray). **D:** firing pattern of the cell shown in **B** and **C**. Current steps were +100 pA (upper trace) and +40 pA and -100 pA (lower traces).

3.2. Activation of group I mGluRs promotes firing in Martinotti cells of layer II/III

A potential candidate originating GABAergic input to Cajal-Retzius cells is the Martinotti cell, which heavily targets layer I (Martinotti, 1889; Ramón y Cajal, 1891; Fairen et al., 1984; Marín-Padilla, 2011a, 2011b). Martinotti cells are identified by specific molecular markers such as the peptide somatostatin (Wahle, 1993; Kawaguchi and Kubota, 1996), which is associated with high levels of expression of the group I metabotropic glutamate receptor 1 α (mGlu1 α , see Baude et al., 1993; Cauli et al., 2000; Stinehelfer et al., 2000; Fanselow et al., 2008). Furthermore, Martinotti cells can be found in different layers and areas of the mammalian neocortex at various developmental stages. For example, early Martinotti-like cells can be recognized as a critical component of the subplate (Friauf et al., 1990), and, at later stages, new Martinotti cells are progressively integrated in the maturing cortical plate (Marín-Padilla, 2011a, 2011b).

When we recorded responses to the application of the group I agonist DHPG in non-pyramidal neurons of layers II/III (of either CXCR4-EGFP or GIN mice, **Fig. 3A and B**, see Methods for details), we found depolarizing responses leading to repetitive firing in $\sim 40\%$ of the recorded neurons (8/21 neurons, combining 3/10 recordings from CXCR4-EGFP mice and 5/11 recordings from GIN mice). When the anatomy of the responding interneurons was examined, seven

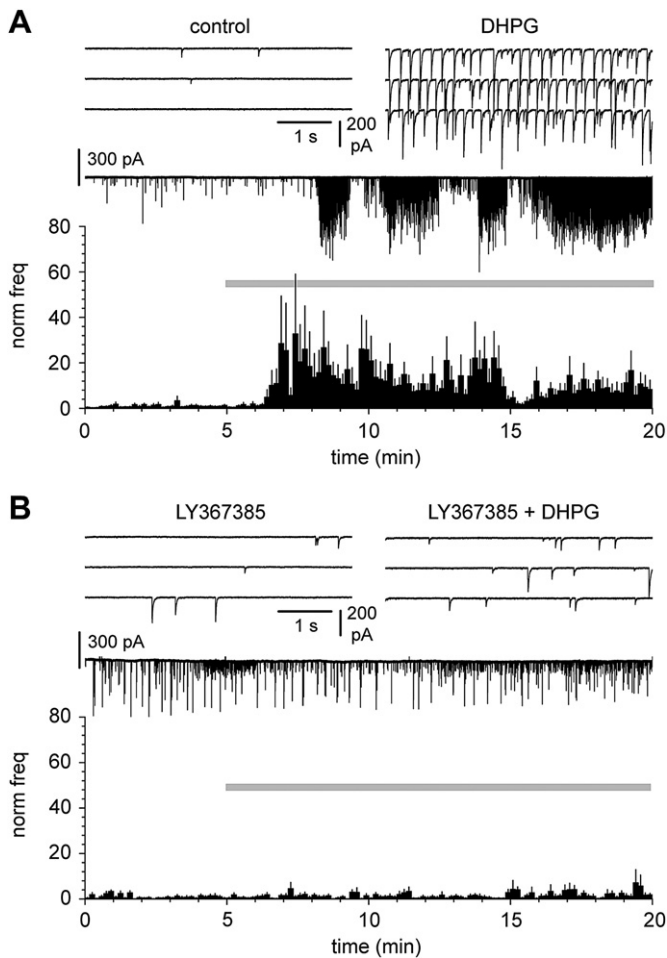


Fig. 4. Massive increase of the frequency of spontaneous GABAergic currents caused by DHPG is mediated by mGlu1 receptors. A: summary graph of the effect of DHPG (10 μ M, gray bar) application on the frequency of spontaneous synaptic events. Notice the massive increase in the presence of the drug. The upper insets show traces in control (left) and in the presence of DHPG (right). Ionotropic glutamatergic synaptic transmission was blocked throughout the experiment (NBQX: 20 μ M, D-AP5: 50 μ M). B: as in A, but in slices continuously exposed to the mGlu1 antagonist LY367385 (50 μ M). Notice the complete loss of DHPG effect.

of the eight responding cells had axonal projections to layer I (2/3 recordings from CXCR4-EGFP mice and 5/5 recordings from GIN mice). In contrast, when responses to DHPG were monitored from neurons with the cell body and main dendritic tree located in layer I, only sub-threshold depolarization or no effect were observed (9/9 cells, combining 8/8 from CXCR4 mice and 1/1 from a GIN mouse).

Thus, a prediction of these experiments is that if Martinotti cells provided a significant GABAergic input to Cajal-Retzius cells, then application of DHPG should cause a large increase in the frequency of the synaptic currents occurring in Cajal-Retzius cells.

3.3. DHPG increases the frequency of spontaneous postsynaptic currents in Cajal-Retzius cells via activation of mGlu1 α

We directly tested this hypothesis by recording spontaneous postsynaptic currents (sPCs) from Cajal-Retzius cells before and after application of DHPG. As shown in Fig. 4A, application of DHPG massively increased the frequency of sPCs from 122 ± 33 mHz in control to 746 ± 235 mHz in the presence of the drug ($p < 0.05$, $n = 15$, paired t -test). Thus, GABAergic input to Cajal-Retzius cells can be powerfully recruited by the activation of group I mGluRs.

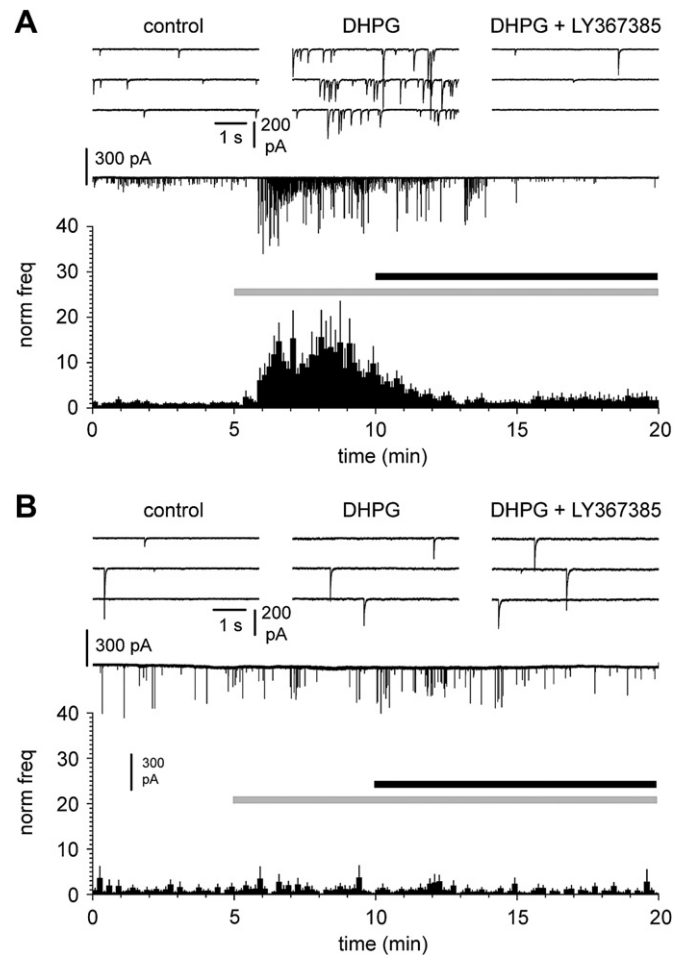


Fig. 5. DHPG-induced increase of the frequency of spontaneous GABAergic currents is mediated by metabotropic glutamate receptors expressed by neurons located deeper than layer I. A: summary graph of the effect of DHPG (50 μ M, gray bar) and subsequent application of LY367385 (100 μ M) in control slices. Notice the similarity of the effects to the ones shown in Fig. 4. The upper insets show traces in control (left), in the presence of DHPG (middle), and in the presence of both DHPG and LY367385 (right). Glutamatergic ionotropic synaptic transmission was blocked by NBQX (20 μ M) and D-AP5 (50 μ M). B: as in A, but in surgically-treated slices to cut ascending GABAergic axon targeting layer I. Notice the complete abolishment of DHPG effects.

This is consistent with synaptic events being provided by Martinotti cells and with a general role of group I mGluRs in regulating GABAergic input in cortical (Chu and Hablitz, 1998) and hippocampal networks (Mannaioni et al., 2001).

Because, specifically, Martinotti cells express mGlu1 α , we investigated whether the strong increase of sPCs triggered by DHPG was mediated by this receptor subtype. We applied DHPG in slices continuously exposed to the mGlu1 α antagonist LY367385 (Fig. 4B). Under these experimental conditions, the frequency of sPCs did not change. From a value of 192 ± 147 mHz in the presence of the antagonist, the frequency of sPCs non-significantly decreased to 137 ± 82 mHz after the further addition of DHPG ($p > 0.05$, $n = 6$ paired t -test). This result indicates that the massive increase of frequency of sPCs is mediated specifically by the activation of mGlu1 α receptors.

However, Martinotti cells are not the exclusive neuronal type endowed with mGlu1 α receptors. In addition to Martinotti cells, mGlu1 α is strongly expressed by Cajal-Retzius cells themselves (Martínez-Galán et al., 2001; López-Bendito et al., 2002). Therefore, an alternative interpretation could be that the increased frequency of spontaneous events may reflect the unmasking of “silent”

GABAergic synapses, which would be triggered by a cascade of events initiated by the activation of mGlu1 α receptors on the membrane of Cajal-Retzius cells.

We thought that this possibility was unlikely for two reasons. First, Cajal-Retzius cells are particularly sensitive to whole-cell recording conditions (Mienville and Pesold, 1999), which have been shown to depress responses triggered by other G-protein coupled receptors such as the chemokine metabotropic receptor CXCR4 (Marchionni et al., 2010, in press). Second, the massive increase of spontaneous GABAergic events would indicate that most synapses are silent under baseline conditions, which seems unlikely.

3.4. mGlu1 α -mediated effect is generated by interneurons located below layer I

Nevertheless, we decided to test this hypothesis by comparing the effect of DHPG-mediated activation of mGlu1 α in naïve slices vs. preparations in which a surgical cut had been done at the lower border of layer I. We reasoned that this manipulation would completely sever the axonal projections made by GABAergic neurons of deeper locations, but would not affect much the axons and dendrites of Cajal-Retzius cells, which are mostly confined to layer I (Radnikow et al., 2002). Hence, if DHPG-mediated increase in synaptic events was originated by mGlu1 α expressed in the somatodendritic membrane of Martinotti cells, no effect of DHPG should be observed. Alternatively, if the critical mGlu1 α receptors were expressed by Cajal-Retzius cells themselves, then responses to DHPG should remain mostly unaffected.

In naïve slices (Fig. 5A), as expected, application of DHPG strongly potentiated the frequency of sPCs from 334 ± 167 mHz before the addition of the agonist to 1646 ± 371 mHz in its presence ($p < 0.05$, $n = 14$, repeated measures ANOVA with Bonferroni posthoc test). Frequency was then reduced by the subsequent addition of LY367385 to 295 ± 71 mHz ($p < 0.05$, $n = 14$, repeated measures ANOVA with Bonferroni posthoc test).

In contrast, when the same experimental protocol was applied to surgically-treated slices (Fig. 5B), no changes were measured in the frequencies of spontaneous events (77 ± 24 mHz in control, vs. 86 ± 36 mHz in the presence of DHPG, and 64 ± 23 mHz in DHPG plus LY367385, $n = 11$, $p > 0.05$, repeated measures ANOVA with Bonferroni posthoc test). Therefore, we concluded that, under our experimental conditions, mGlu1 α expressed by Cajal-Retzius cells did not play a significant role in the observed DHPG-induced modulation of spontaneous events.

Lastly, we corroborated this result by showing that exposure to TTX, which blocks voltage-dependent sodium channels and action potentials, both reduced spontaneous synaptic activity and prevented the effect of DHPG. Application of TTX (1 μ M, Fig. 6) reduced the frequency of spontaneous PSCs from 155 ± 48 mHz to 41 ± 12 mHz ($n = 11$, $p < 0.05$, repeated measures ANOVA with Bonferroni posthoc test). However, when DHPG (50 μ M) was added in the presence of TTX, no significant effect was observed (frequency in TTX + DHPG was 38 ± 9 mHz, $n = 11$, $p > 0.05$, repeated measures ANOVA with Bonferroni posthoc test).

Thus, DHPG-induced GABAergic input observed on Cajal-Retzius cells depends on firing activity of cells located below layer I, which express mGlu1 α , all characteristics that fit very well with Martinotti cells.

4. Discussion

To our knowledge, this work establishes for the first time two important novel results. First, we show that *synaptically*-released GABA is able to trigger action potentials in Cajal-Retzius cells via

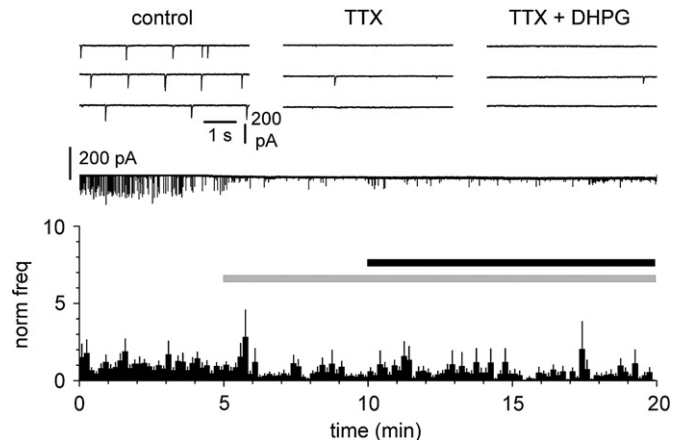


Fig. 6. A network mechanism underlies DHPG-induced increase of the frequency of spontaneous GABAergic currents in Cajal-Retzius cells. A: summary graph of the effect of DHPG (50 μ M, black bar) following pre-exposure to TTX (1 μ M, gray bar). Notice that TTX completely prevents the effect of DHPG. The upper insets show traces in control (left), in the presence of TTX (middle), and in the presence of both TTX and DHPG (right). Glutamatergic ionotropic synaptic transmission was blocked by NBQX (20 μ M) and D-AP5 (50 μ M).

GABA_A receptors. Second, we demonstrate that an important source of GABAergic input to Cajal-Retzius cells is massively controlled by the activation of mGlu1 α receptors. Therefore, our data predict that conditions associated with sustained glutamate release, which may occur in vivo and lead to the activation of metabotropic glutamate receptors, would be especially effective in activating Cajal-Retzius cells via a disynaptic pathway.

4.1. Cajal-Retzius cells: GABAergic input and computational roles

Although Cajal-Retzius cells have been long studied as a major cellular source of the glycoprotein reelin, which is critically involved in cortical development (D'Arcangelo et al., 1995), their computational roles have received less attention and are also less understood. Yet, Cajal-Retzius cells are endowed with membrane excitability and can fire action potentials (Hestrin and Armstrong, 1996; Zhou and Hablitz, 1996; Radnikow et al., 2002), they show network- and mGluR- modulated intracellular calcium oscillations (Schwartz et al., 1998; Aguiló et al., 1999; Martínez-Galán et al., 2001), and are integrated within neocortical networks (Radnikow et al., 2002; Soda et al., 2003).

However, both the identification of the presynaptic cells originating synaptic inputs converging onto Cajal-Retzius cells and the characterization of their postsynaptic effects on their cellular targets are still unclear, despite the importance of this type of information to understand their role(s) in the circuit. Our work, in agreement with several other studies in the neocortex (Kilb and Luhmann, 2001; Soda et al., 2003) and hippocampus (Marchionni et al., 2010), confirms that Cajal-Retzius cells receive predominantly, if not exclusively, spontaneous GABAergic input. Furthermore, using non-invasive recording techniques that maintain unperturbed chloride levels, we show that field electrical stimulation is able to trigger synaptically-evoked action potentials, which are prevented by the addition of the GABA_A receptor blocker gabazine. This result fits very well with electrophysiological evidence obtained in perforated-patch (Mienville, 1998; Achilles et al., 2007) or cell-attached configuration (Marchionni et al., 2010) showing that the electrochemical gradient for GABA_A receptor-induced currents is depolarized compared to resting potential, and that Cajal-Retzius cells respond with firing when exposed to exogenously applied GABA. Our finding is also

consistent with the reported expression of the NKCC1 (Achilles et al., 2007), but not KCC2 transporter (Pozas et al., 2008), which would favor the maintenance of high intracellular chloride levels, as NKCC1 (Yamada et al., 2004) and KCC2 (Rivera et al., 1999) play opposite roles in regulating intracellular chloride homeostasis.

Thus, if the major synaptic input that can generate action potentials in Cajal-Retzius cells is mediated by GABAergic fibers, a critical question becomes defining the subtype of presynaptic interneurons that originate this input. Here we add an important piece of the answer to this question by showing that activation of mGlu1 α massively recruits a population of interneurons that target Cajal-Retzius cells.

In the juvenile/adult mouse neocortex a strong enrichment in the expression of mGlu1 α has been repeatedly demonstrated in somatostatin-expressing interneurons such as Martinotti cells. Although Martinotti cells show variable anatomical and electrophysiological properties (Wahle, 1993; Kawaguchi and Kubota, 1997; Wang et al., 2004; Fanselow et al., 2008), they reliably express somatostatin and send axonal projections to layer I. In contrast, mGlu1 α expression has been shown to be low in fast-spiking interneurons (Beierlein et al., 2000; Cauli et al., 2000) such as basket cells and chandelier interneurons (Kawaguchi, 1995; Kawaguchi and Kubota, 1997). Therefore, we propose that Martinotti cells are the source of the mGlu1 α -dependent GABAergic input to layer I Cajal-Retzius cells observed in our experiments. Consistently, we have shown that in our slice preparation, a high proportion of neurons activated to supra-threshold levels by DHPG sent axonal projection to layer I. It is also important to note that Martinotti-like cells are among the earliest recognized local-circuit interneuron in the developing mammalian neocortex and are essential components of the subplate (Friauf et al., 1990; Marín-Padilla, 2011b). Although the GABAergic input described by Myakhar et al. (2011) is likely to originate from these Martinotti-like cells, immunoreactivity for mGlu1 α has been reported to be virtually absent in subplate neurons, at least at embryonic stages (López-Bendito et al., 2002).

Thus, the most parsimonious explanation is that the effect we observed was either mediated by Martinotti cells of upper layers or that some subplate neurons may increase their level of expression of mGlu1 α postnatally.

It needs also to be explicitly acknowledged that Martinotti cells are not the only interneuronal type expressing group I mGluRs in the neocortex. In fact, we occasionally observed depolarizing responses to DHPG in local layer I interneurons, although they never reached supra-threshold levels. Whether this is the result of our preparation, which may have disproportionately affected the axonal arborization of one class of cell vs. the other or may reflect the early developmental stage examined, remains to be determined. However, it nevertheless corroborates our suggestion that the observed increase in spontaneous synaptic events did not originate from local layer I interneurons, but from Martinotti cells of deeper layers.

Another important point that needs to be explicitly recognized is that our suggestion that the massive synaptic input unveiled by mGlu1 α activation originates from Martinotti cells does not imply that, overall, Martinotti cells are the major source of GABAergic input to Cajal-Retzius cells. Similarly powerful connections may be established by other classes of GABAergic cells located in layer I (Hestrin and Armstrong, 1996; Zhou and Hablitz, 1996; Chu et al., 2003; Marín-Padilla, 2011a, 2011b; Wozny and Williams, 2011) or in other areas (Friauf et al., 1990; Lin et al., 1990; Myakhar et al., 2011; Marín-Padilla, 2011a, 2011b). All these neurons may play an equally important role in the regulation of Cajal-Retzius cell activity. Future work based on paired recordings will hopefully provide direct information on this issue.

4.2. Functions of Martinotti → Cajal-Retzius cell connections

The axon of Martinotti cells is considered an integral part of the layer I micro-circuitry and has been shown to be present, in humans, at very early stage of development and most likely persist until adulthood (Marín-Padilla, 2011a). Although recent work in adult animals has highlighted the powerful inhibitory role of Martinotti cells on the apical dendrites of pyramidal cells by modulating local calcium electrogenesis (Murayama et al., 2009), their role at early developmental stages may be different because of the immature stage of pyramidal cell dendritic excitability (Zhu, 2000).

Direct calcium imaging of layer I neurons at embryonic and early postnatal stages has revealed the presence of an active network showing correlated activity (Schwartz et al., 1998; Aguiló et al., 1999). Intriguingly, de-coupling of spontaneous calcium activity could be obtained by the pharmacological blockade of GABA_A receptors and voltage-dependent sodium channels. We suggest that GABA release from Martinotti cells onto postsynaptic Cajal-Retzius cells and local neurons may be critical for the selection of neuronal assemblies involved in this correlated activity. Interesting, the activation of metabotropic glutamate receptors has been shown to trigger rhythmic firing activity in a network of electrically coupled low threshold spiking neurons (Beierlein et al., 2000), which are the electrophysiological phenotype of somatostatin-expressing interneurons and of a subtype of Martinotti cells (Kawaguchi, 1995; Kawaguchi and Kubota, 1997; Goldberg et al., 2004; Wang et al., 2004).

According to our hypothesis, Martinotti-cell driven oscillatory calcium activity could be important for several functions. First, it could regulate reelin secretion/release and hence affect a large number of pathways critical for pyramidal cell dendritic and synaptic development. Although in other neuronal types reelin secretion has been shown to follow a constitutive pathway (Lacor et al., 2000), indirect evidence for activity-dependent secretion of reelin in Cajal-Retzius cells has also been put forward. For example, Derer et al. (2001) have proposed axonal localization of reelin in specialized membrane-delimited structures they termed “axonal reelin reservoirs”. In addition, reelin levels during early development appear diminished following blockade of 5-HT₃ serotonergic excitatory input to Cajal-Retzius cells (Chameau et al., 2009), and exposure of hippocampal cultured slices to kainate (to activate glutamate receptors) can modulate reelin levels (Tinnes et al., 2011). However, these last results are complex to interpret because additional factors such as kainate-dependent depolarization block of Cajal-Retzius cells, cellular toxicity, and impact on the cleavage of the molecule need to be considered (Duveau et al., 2011; Tinnes et al., 2011).

Second, although a direct determination of the major conventional neurotransmitter released by Cajal-Retzius cells is still lacking, several studies have suggested that they are likely to be glutamatergic (del Río et al., 1995; Radnikow et al., 2002; Hevner et al., 2003; Ina et al., 2007). Assuming that this was the case, glutamate release could play a trophic role (Balazs, 2006) during the maturation of dendrites (Lüthi et al., 2001) and synapses (Durand et al., 1996) on the apical tufts of pyramidal neurons. By directly controlling these functions, GABAergic input to Cajal-Retzius cells could be critically involved in essential developmental functions. However, the conclusive determination of the neurotransmitter released by Cajal-Retzius cells and of the receptors mediating its postsynaptic effects on target cell will be required to validate or disprove this hypothesis.

4.3. Conclusions

Our work has revealed that mGlu1 α expressed by a subpopulation of neocortical interneurons, likely to be Martinotti cells, has

the ability to powerfully control spontaneous GABAergic input to Cajal-Retzius cells. Although in the mature cortex Martinotti cells have been proposed to play a critical role as gatekeepers of dendritic excitability of pyramidal neurons, we propose that, at early developmental stages, they may play a different role, by synchronizing populations of Cajal-Retzius cells and potentially other layer I neurons. These functions may finally depend on reelin, glutamate or a yet unidentified output molecule as final effector(s).

Conflict of interest

None.

Acknowledgments

This work was supported by the National Institute of Neurological Disorders and Stroke (grant number NS064135 to GM).

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