P/Q-type, but not N-type, calcium channels mediate GABA release from fast-spiking interneurons to pyramidal cells in rat prefrontal cortex

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\textbf{Running title:} P/Q-type channels mediate GABA release in fast-spiking cell

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Abstract

The Cav2.1 (P/Q-) and Cav2.2 (N-type) voltage-gated calcium channels (VGCCs) play a predominant role in neurotransmitter release at central synapses, but their distribution is not uniform across different types of synapses. Although the functional significance of the differential distribution of N-type and P/Q-type VGCCs is poorly understood, distinct types of VGCCs appear to differentially affect synaptic properties. For example, P/Q-type VGCCs are located closer to release sites and are less affected by G-protein-mediated inhibition than are N-type VGCCs. Thus, P/Q-type VGCCs might be beneficial at synapses with high probability of release and precise timing of neurotransmission, such as the inhibitory inputs from parvalbumin-containing fast-spiking (FS) interneurons to pyramidal cells (PCs) in the neocortex. To determine whether VGCCs types predominate at synapses from FS interneurons to PCs in rat prefrontal cortex, whole-cell paired recordings (n=14) combined with intracellular labeling and fluorescence immunohistochemistry for parvalbumin were performed in acute slices. Bath application of the specific N-type VGCC blocker ω-conotoxin-GVIA (1 μM) did not alter IPSP amplitude, failure rate or synaptic dynamics; in contrast, application of P/Q-type VGCC blocker ω-agatoxin-IVa (0.5 μM) completely and irreversibly blocked neurotransmission. These results indicate that P/Q-type VGCCs mediate the GABA release from parvalbumin-positive FS interneurons to PCs in the rat neocortex.
Introduction

The arrival of an action potential at a presynaptic axon terminal activates voltage-gated calcium channels (VGCCs) and Ca\(^{2+}\) influx through the channels, which in turn, triggers neurotransmitter release. The Cav2.1 (P/Q-type) and Cav2.2 (N-type) VGCCs play a predominant role in neurotransmitter release at both excitatory and inhibitory synapses in the cortex (Reid et al. 2003; Evans and Zamponi 2006).

Previous studies suggest that some GABAergic synapses use mostly one channel subtype or the other (Poncer et al. 1997; Wilson et al. 2001; Hefft and Jonas 2005; Ali and Nelson 2006). Although the advantage of a differential distribution of N-type and P/Q-type Ca\(^{2+}\) channels (Reid et al. 2003) remains unclear, distinct types of VGCCs appear to differentially affect synaptic properties. For example, Ca\(^{2+}\) influx through P/Q-type channels triggers release more efficiently than Ca\(^{2+}\) influx through N-type channels due to the closer location of P/Q-type VGCCs to release sites in central synapses (Wu et al. 1999; Hefft and Jonas 2005). This difference might explain the reported differences in short-term modification; P/Q-type calcium channels are associated with transmitter release at connections displaying synaptic depression, whereas N-type channels are predominantly associated with connections displaying facilitation (Ali and Nelson 2006). In addition, there are differences in G-protein-mediated inhibition of N-type and P/Q-type VGCCs (Currie and Fox 1997; Colecraft et al. 2000). This inhibition at P/Q-type synapses affects synaptic efficacy, but not the timing or precision of neurotransmission. In contrast, G-protein-mediated inhibition at N-type VGCCs synapses not only decreases synaptic efficacy to a greater extent, but might impact the timing and coincidence detection properties of such synapses (Sabatini and Regehr 1999).

Thus, P/Q-type VGCCs might be beneficial at synapses with high probability of release and precise timing of neurotransmission, such as the inhibitory inputs from parvalbumin-containing fast-spiking (FS) interneurons to pyramidal cells (PCs) in the neocortex. Previous studies indirectly support this hypothesis in hippocampus (Poncer et al. 1997; Wilson et al. 2001; Hefft and Jonas 2005), but contradictory observations have been found in young rat neocortex (Ali and Nelson 2006).

Consequently, in order to determine whether VGCCs types predominate at FS interneurons to PCs synapses in rat neocortex, we applied specific calcium channel blockers ω-conotoxin-GVIA and ω-agatoxin-IVA during whole cell paired recordings, combined with
intracellular labeling and fluorescence immunohistochemistry for parvalbumin, in acute slices from rat prefrontal cortex.

**Materials and Methods**

*Slice preparation*

Male Wistar albino rats (19-22 postnatal days) were deeply anesthetized with halothane and decapitated in accordance with University of Pittsburgh Animal Care and Use Committee and in agreement with National Institutes of Health (USA) guidelines. The brain was rapidly removed and immersed in ice-cold pre-oxygenated (95% O₂/5% CO₂) artificial cerebrospinal fluid (ACSF) and 350-µm-thick coronal slices were cut with a vibratome (Leica VT1000S, Leica, Germany). The slices were maintained in the ACSF at 37°C for 1 h and, later, at room temperature. Slices were transferred to a recording chamber perfused with the ACSF at 31-32°C. Through all steps of these experiments, the ACSF of the following composition was used (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1 MgSO₄, 2 CaCl₂, 24 NaHCO₃ and 20 dextrose.

*Single and paired recordings*

Neurons in layers 2-3 of medial prefrontal cortex were identified visually using infrared transmitted illumination from a Axioscop 2 microscope (Carl Zeiss, Inc., Germany) equipped with differential interference contrast optics and Dage MTI NC-70 video camera (Dage-MTI of Michigan City, Inc., USA) for contrast enhancement. Patch electrodes (4-7 MΩ) were pulled from borosilicate capillary glass. The internal solution for presynaptic interneurons contained (in mM): 114 K-gluconate, 6 KCl, 10 HEPES, 4 ATP-Mg and 0.3 GTP (pH was adjusted to 7.25 with KOH). To reverse and increase the amplitude of GABAₐ receptor-mediated IPSPs in postsynaptic pyramidal cells at resting membrane potential, an internal solution of high chloride concentration was used (in mM): 120 KCl, 10 HEPES, 4 ATP-Mg and 0.3 GTP (pH was adjusted to 7.25 with KOH). The chloride equilibrium potential was estimated to be -2.6 mV, using the Nernst equation calculated for a temperature of 32°C. In both internal solutions, biocytin (0.5%) was added for later morphological identification of the recorded neurons.

Whole cell voltage recordings were made with MultiClamp 700A amplifiers (Axon Instruments, Union City, CA) operating in a bridge-balance mode and employing capacitance
neutralization. Signals were filtered at 4 kHz and acquired at a sampling rate of 20 kHz using a 16 bit-resolution Power 1401 interface and Signal software (CED, Cambridge, UK). Access resistance and capacitance were compensated on-line. Access resistance (15–25 MΩ) was constantly monitored and remained relatively stable during the experiments (≤ 30% increase).

Intrinsic membrane properties were assessed from the voltage responses to the series of 500-ms hyper- and depolarizing current steps with 5-10 pA increments at 0.5 Hz. Synaptically connected pairs were identified during simultaneous dual whole cell voltage recordings as follows: single spikes were evoked in the presynaptic neurons by injection of short-duration (2 ms) suprathreshold (1–1.5 nA) current steps; 7-10 consecutive postsynaptic voltage responses were averaged on-line and the start of fast voltage deflection within 1-2 ms delay after presynaptic spike was interpreted as monosynaptic unitary IPSP. Once the presence of a synaptic connection was established, single stimuli or trains of 5 presynaptic APs at 20 Hz were applied every 10-20 s. The level of stimulation current, injected into presynaptic neurons, was adjusted to elicit APs with little trial-to-trial variability in spike latency.

To block the presynaptic VGCCs, peptide toxins ω-conotoxin-GVIA (1 µM, Bachem, Torrance, CA) and ω-agatoxin-IVA (0.5 µM, Bachem) were administrated by bath application.

Data analysis and statistics

Data were analyzed using Signal software. Input resistance was determined as the slope from a linear regression fit to the plot of injected current/voltage response. Linear current/voltage relationship was usually preserved between –50 and –10 pA and voltage responses were measured at the end of the 500-ms step. Membrane time constant was determined by fitting a single exponential to the on phase of the averaged voltage responses to –10 … –30 pA current steps. Properties of action potential were measured using the first spike in a sweep evoked by a near-threshold current step. Spike frequency adaptation (adaptation ratio) was determined as the ratio between the 1st interspike interval to the last interspike interval, measured at twice the threshold level of the depolarizing current steps.

Synaptic latency (peak of presynaptic action potential to onset of IPSP), IPSP amplitude (baseline to the peak of the IPSP), rise time (10-90% of IPSP peak amplitude), and decay time (time constant of a monoexponential decay function) were determined on traces obtained by averaging 30–50 consecutive responses, including failures. Standard deviation of the synaptic latency distribution was calculated based on measurements of synaptic latency at the
individual traces. To characterize IPSPs dynamics during repetitive stimulation of presynaptic neurons, 30–50 consecutive traces including failures were averaged and the amplitude of each IPSP in the train was measured from the baseline directly preceding the rising phase.

IPSPs failures were considered when membrane potential was <1.5 x the noise within 1-2-ms time window of the expected IPSP peak. Value of noise was measured in a 1-2-ms time window prior to the IPSP onset (Gonzalez-Burgos et al. 2005). The detected IPSPs failures were also confirmed by visual inspection of the individual traces.

To analyze the mechanisms of short-term synaptic dynamics, the coefficient of variation (CV) method was used (Faber and Korn 1991). According to this method, quantal parameters are assumed to be invariant, and a simple binomimial description is adopted. The proportional change in the inverse square of the coefficient of variation (CV$^{-2}$) was compared with the proportional change in the mean postsynaptic potential amplitude ($M$) to determine whether the quantal amplitude ($q$), the release probability ($p$) or the number of release sites ($n$) had changed. In a binomial distribution, $CV^{-2} = [np/(1 – p)]$, and is therefore independent of $q$, while $M = npq$. When mean postsynaptic potential amplitude changes, therefore, no change in $CV^{-2}$ indicates that only $q$ has changed and a postsynaptic site is involved. A larger proportional change in $CV^{-2}$ than in $M$ indicates that $p$ has changed, while a similar proportional change in the two parameters indicates that $n$ has changed. Both presynaptic and postsynaptic sites are affected when a smaller proportional change in $CV^{-2}$ than in $M$ is revealed.

Values are given as mean ± s.e.m. and error bars in figures also indicate s.e.m. unless otherwise indicated.

**Histological Processing**

After recordings, slices were immersed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) and then were kept in storing solution (33% glycerol, 33% ethylene glycol, in 0.1 M PBS) at -80°C. Some recorded interneurons were processed for parvalbumin. For this purpose the fixed slices were transferred to 0.1 M PBS, serially re-sectioned at 60 μm, and processed using double immunofluorescent labeling. First, sections were incubated for 12-24 hours at 4°C in blocking serum (10% normal goat serum; 2% bovine serum albumin; 0.4% Triton X-100 in PBS). Then, to visualize biocytin and PV, the sections were incubated with a mixture of streptavidin-Alexa Fluor 633 conjugate (Invitrogen, USA, dilution 1:500) and
mouse monoclonal anti-parvalbumin antibodies (Swant, Bellinzona, Switzerland, dilution 1:2000) in 0.1 M PBS with 10% normal goat serum for 48-72 hours at 4°C. After thorough rinsing in 0.1 M PBS, the sections were incubated with a goat anti-mouse IgG conjugated with Alexa Fluor 488 (Invitrogen, USA, dilution 1:500) in 0.1 M PBS with 10% normal goat serum for 2 hours at room temperature. Rinsed sections were kept in storing solution until they were analyzed using Olympus Fluoview™ 500 confocal laser scanning microscope (Olympus Corp.). After confocal reconstruction, the sections were treated with 1% H2O2 for 2-3 hours at room temperature, rinsed and incubated with the avidin-biotin-peroxidase complex (1:100; Vector Laboratories, Burlingame, CA) in PBS for 4 hours. Sections were rinsed, stained with 3,3'-diaminobenzidine (DAB), mounted on gelatin coated glass slides, dehydrated and coverslipped. Neurons were reconstructed using the Neurolucida tracing system (MicroBrightField, Williston, VT).

**Results**

*Electrophysiological and morphological properties of FS interneurons and PCs*

Whole cell recordings were performed in 14 connected pairs of electrophysiologically identified FS interneurons and PCs in layer 2-3 of rat medial prefrontal cortex. FS interneurons were recognized according to previously specified criteria (Kawaguchi 1995). The interneurons displayed fast action potentials (≤0.50 ms at half amplitude), followed by fast and deep monophasic afterhyperpolarization (22.5±0.8 mV). These cells had a low input resistance ($R_i = 212±10 \, \text{M} \Omega$) and fast membrane time constant (7.8±0.9 ms), and they did not typically show significant time-dependent changes of voltage responses during application of hyperpolarizing or subthreshold depolarizing current steps. At supra-threshold current level FS interneurons displayed a high-frequency firing pattern without a significant spike frequency adaptation. All PCs exhibited regular-spiking firing pattern (Fig. 1A).

8 connected pairs were morphologically reconstructed. FS interneurons had multipolar aspiny dendrites and relatively uniformly- or predominantly horizontally-spreaded axonal arbors (Fig. 1B). These anatomical features of the FS interneurons are consistent with previous description of interneurons with local and long horizontal axonal arbors in rat neocortex (Kawaguchi 1995). PV immunoreactivity in a sample of FS interneurons (n=4) was detected by indirect immunofluorescent technique with primary antiserum against PV (Swant) and secondary antibody conjugated with Alexa 488. All examined FS interneurons contained...
PV (Fig. 1C), and their membrane properties were similar to those of the other FS interneurons (Fig. 1D). Pyramidal cells were recognized by their apical and basal dendrites containing numerous spines.

**Connections between FS interneurons and PCs have high synaptic efficacy and precise timing of neurotransmission**

Recordings from PCs were done with the electrodes containing the high chloride concentration in the internal solution to reverse and amplify the IPSP amplitudes. Figure 2 illustrates representative examples of unitary IPSPs recorded in PCs after eliciting action potentials in presynaptic FS interneurons by somatic injection of suprathreshold current steps. Quantitative measures of the unitary IPSPs are provided in Figure 2C. The IPSP latency was short (0.85 ± 0.05 ms) and had small variation across trials. The average standard deviation (SD) of the latency distribution, a quantitative measure for the synchrony of release, was 0.13 ± 0.02 ms. Neurotransmission between FS interneurons and PCs was highly reliable. For action potentials evoked in the presynaptic FS interneurons at frequencies of 0.1 Hz, the mean failure rate of neurotransmission was only 8.0 ± 3.8%. Furthermore, 7 out of the 14 connections did not exhibit failures.

Rise time of the IPSP was rapid (10–90% rise time 2.01 ± 0.13 ms). The IPSP decay phase could be accurately described with a mono-exponential decay function, which yielded a value of 39 ± 3 ms at resting membrane potential of pyramidal cells. Averaged amplitude of IPSPs varied significantly among recorded pairs from 0.22 to 9.4 mV.

**FS interneurons to PCs inhibitory connections exhibit presynaptic short-term depression**

To determine the effects of repetitive presynaptic firing on the functional properties of IPSPs, trains of 5 suprathreshold current steps were applied to the presynaptic FS interneurons at 20 Hz. As illustrated in Figure 2D-E, repetitive presynaptic stimulation resulted in depression of amplitude during trains to 70-80% of initial values.

Depression of IPSP amplitudes during the trains was most likely generated by presynaptic mechanisms. First, depression in amplitude was associated with an increase in the event failure rate (Fig. 2E). Failures of transmission were more commonly observed following the fifth as compared to the first presynaptic action potential (18.1±6.7% vs. 9.9±3.5%, respectively; n=10). Further evidence for a presynaptic contribution to the depression was
obtained from the coefficient of variation (CV) analysis of the IPSPs amplitudes (Faber and Korn 1991). In the majority of the pairs tested, comparison of 1\textsuperscript{st} and 5\textsuperscript{th} responses revealed a greater or equal proportional decrease in CV\(^2\) than in mean values of postsynaptic potential amplitudes (Fig. 2F), suggesting a significant contribution of the decreasing release probability (\(p\)) and/or number of release sites (\(n\)) to IPSP amplitude depression.

To distinguish these two possibilities, we determined if the number of the release sites (\(n\)) changed during the trains. For this purpose, a coefficient of correlation between the amplitudes of first and second IPSPs was calculated. It was shown that if \(n\) decreases during trains due to depletion of the readily and immediately available pools of vesicles, an inverse correlation between the amplitude of the first and the amplitude of a second postsynaptic potentials is often apparent (Thomson 2000). Calculated coefficients of correlation varied within the range from -0.26 to 0.24 and did not reach the level of significance (\(p>0.05\)). These results indicate that depression of IPSP is due to a decrease in release probability.

\textit{N-type channels are not required for GABA release from FS interneurons to PCs}

Finally, we pharmacologically identified the VGCCs mediating GABA release in the FSI to PC connections. In all tested pairs (\(n=5\)), bath application of \(\omega\)-conotoxin-GVIIa (1 \(\mu\)M), \(N\)-type specific calcium channel antagonist during 10-15 min had no significant effect (paired t-test, \(p>0.05\)) on the IPSP amplitude, failure rate or synaptic dynamics (Fig. 3). In contrast, bath application of \(\omega\)-agatoxin-IVa (0.5 \(\mu\)M), a P/Q-type specific calcium channel antagonist totally blocked unitary the IPSPs in all tested pairs (\(n=11\), in 5 pairs effect of \(\omega\)-agatoxin-IVa was tested after application of \(\omega\)-conotoxin-GVIIa and in 6 pairs the effect of \(\omega\)-agatoxin-IVa was tested alone).

After application of \(\omega\)-agatoxin-IVa, the IPSP amplitude exponentially declined to 0 mV at a rate with a time constant of 38 \(\pm\) 2.8 s (\(n=5\)). This effect was not reversed within 10-20 minutes of toxin wash out.

To determine if \(\omega\)-conotoxin-GVIIa (1 \(\mu\)M) and \(\omega\)-agatoxin-IVa (0.5 \(\mu\)M) are actually effective in another system, 4 reciprocal connections were studied as a control (Fig. 3). Bath application of \(\omega\)-agatoxin-IVa (3 pairs) resulted in a considerable reduction in EPSPs amplitude (11%; 13%; and 31% of the control). Effect of \(\omega\)-conotoxin-GVIIa (1 pair) was smaller than of \(\omega\)-agatoxin-IVa but still substantial: amplitudes of single EPSPs were reduced to 47% of the control.
Because of a non-linear relation between presynaptic calcium currents and neurotransmitter release (Bollmann and Sakmann 2005), small calcium currents through VGCCs other than P/Q-type might possibly contribute to release; however, when acting alone, these calcium currents are insufficient to trigger release. Their impact in mediating release probably increases during high-frequency train of spikes when accumulation of residual calcium might be significant. To examine this possibility we injected prolonged (500 ms) depolarizing suprathreshold currents steps to FS interneurons (n=2) before and after application of ω-agatoxin-IVa. In control conditions, high-frequency firing of FS interneurons produced strong depolarization in postsynaptic regular spiking PCs and increased frequency of spontaneous events after the train; after application of ω-agatoxin-IVa, firing of presynaptic FS interneurons did not affect the postsynaptic PCs.

These results indicate that N-type channels are not required for GABA release and neurotransmission from FS interneurons to PCs is mediated by P/Q-type calcium channels.

**Discussion**

This study provides direct pharmacological evidence that P/Q-type, but not N-type, VGCCs mediate GABA release from presynaptic terminals at the inhibitory synapses from parvalbumin-positive FS interneurons to PCs in rat neocortex. In agreement with these findings, in the rat hippocampus GABA release at synapses on distal apical dendrites of pyramidal cells was completely blocked by ω-conotoxin MVIIA (N-type channel specific antagonist), whereas neurotransmission from interneurons establishing mostly perisomatic and proximal dendritic contacts, were blocked by ω-agatoxin-IVA (Poncer et al. 1997). However, proximal inhibitory inputs are not all the same; for instance, excitatory neurons in many cortical areas receive proximal inputs from both PV-containing FS interneurons and CCK-containing non-fast-spiking cells (Freund 2003). Consistent with these findings, Wilson et al (2001) reported that somatic GABAergic boutons contain either N-type or P/Q-type VGCCs. Neurons with smaller adaptation and higher firing rates, which can be considered as FS interneurons, have only P/Q channels, while other interneurons with larger adaptation and lower firing rate have only N-type channels. More recently, it was reported that GABA release from PV-positive interneurons in hippocampus is exclusively mediated by P/Q-type and from CCK-containing interneurons is mediated by N-type VGCCs (Hefft and Jonas 2005).
In contrast, in the only report from rat neocortex unitary IPSPs from FS interneurons to PCs were not affected by ω-agatoxin-IVA, but totally blocked by N-type Ca\(^{2+}\) channel blocker ω-conotoxin-GVIA (Ali and Nelson 2006). A possible explanation of the discrepancy between our results and the results of Ali&Nelson may reflect differences in the laminar location of the neurons examined and indicate a large heterogeneity of central interneurons. In agreement with our results, a subunit of P/Q-type VGCCs was shown to be co-expressed with parvalbumin in rat neocortex (Toledo-Rodriguez et al. 2004).

**Possible functional implications of P/Q-type VGCCs predominance at synaptic contacts between FS interneurons and PCs**

G-protein-mediated inhibition of N-type VGCCs is stronger than that of P/Q-type VGCCs (Currie and Fox 1997). Moreover, voltage-dependent relief of G-protein-mediated inhibition, which occurs during trains of action potentials, is stronger for P/Q-type channels than for N-type channels (Currie and Fox 2002). Consequently, synapses in which P/Q-type VGCCs dominate would be inhibited less and so would operate over a smaller range (between uninhibited and maximally inhibited states) than synapses in which N-type channels dominate, thus providing fine tuning of the regulation of release (Currie and Fox 1997).

In addition, at the single channel level inhibited P/Q channels are electrically silent, while N-type VGCCs manifest relatively infrequent openings (reluctant openings) (Colecraft et al. 2001). These striking contrasts in single-channel properties produce fundamental differences in the effect of G-protein inhibition on the waveform of Ca\(^{2+}\) entry elicited by action potentials. Inhibition of N-type currents produced both decreased Ca\(^{2+}\) current amplitude and temporally advanced waveform, effects that would not only reduce synaptic efficacy, but also influence the timing of synaptic transmission. On the other hand, inhibition of P/Q-type currents showed diminished amplitude without shape alteration. Consequently, synaptic efficacy would be reduced, but the timing and precision of neurotransmission would be preserved (Sabatini and Regehr 1999; Colecraft et al. 2001).

Thus, these specific properties of P/Q-type VGCCs create conditions permitting nearly linear information processing in the postsynaptic neuron as a function of presynaptic action potential. Predominance of P/Q-type VGCCs at synaptic terminals of FS interneurons appears especially beneficial for neurotransmission, since these PV-containing interneurons are always able to fire repetitively at a high constant rate (Kawaguchi 1995). As a slow Ca\(^{2+}\)-buffer,
parvalbumin could quickly bring down the calcium concentration at presynaptic terminals, preventing the cumulative facilitation (Caillard et al. 2000) or potential desensitization of Ca\textsuperscript{2+} sensors. Parvalbumin should also effectively reduce the number of events with long latency. Thus, the presence of parvalbumin, a large pool of readily and immediately available vesicles at presynaptic terminals, and P/Q-type VGCCs permits highly reliable neurotransmission during trains of spikes in terms of timing and efficacy in connections of FS interneurons to PCs.

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2. **Bollmann JH and Sakmann B.** Control of synaptic strength and timing by the release-site Ca\(^{2+}\) signal. *Nat Neurosci* 8: 426-434, 2005.


**Figure legends**

*Figure 1. Physiological and morphological properties of FS interneurons and PCs in layers 2-3 of rat prefrontal cortex.* A. Representative example of the firing pattern of neocortical FS interneuron and PC. FS interneuron exhibits high-frequency firing rate and a pattern without a significant spike frequency adaptation. PC displays membrane properties of a typical regular spiking cell with a significant spike frequency adaptation. B. Neurolucida reconstruction of the FS interneuron – PC connected pair. Interneuron soma and dendrites are drawn in green and the axon in black. Pyramidal soma and dendrites are drawn in red, axon of PC is not shown. C. Double immunostaining for biocytin (red) and PV (green) in the same connected pair. Biocytin and PV are co-localized in the FS interneuron. Arrows indicate PV-positive FS interneuron. D. Membrane properties of recorded FS interneurons (circles) and PCs (triangles). Boxes represent s.e.m.; whiskers represent standard deviation; horizontal lines are medians.

*Figure 2. Physiological properties of the inhibitory connections from FS interneurons to PCs in layer 2-3 of prefrontal cortex.* A. Example of 47 superimposed subsequent unitary IPSPs recorded from a PC (top trace) in response to the presynaptic action potentials evoked in a FS cell (bottom trace). This connection exhibited no failures. Baselines in all traces were aligned. Note that the IPSP latency distribution is narrow (inset). B. Example of the inhibitory connection with failures (8 failures out of 63 subsequent trials, bottom trace). The top trace represents the averaged IPSP waveform. Arrow indicates action potentials evoked in the presynaptic FS interneuron. C. Statistical description of inhibitory connections. Each circle represents data from one connected pair, bar – mean value, and error bar – standard error measurement. D. Representative examples of IPSPs trains. Arrows indicate action potentials elicited in the presynaptic FS interneuron. The top trace represents the averaged waveforms of the IPSPs. E. Failure rate increases in the subsequent responses within the trains. Average amplitudes decrease in the subsequent IPSPs within the trains; the amplitudes are normalized to the amplitude of the 1st IPSPs. F. A plot of CV^2 ratio vs. amplitude ratio of 5th to 1st IPSPs in the trains. Each circle represents the connected pair.

*Figure 3. P/Q-type calcium channels exclusively mediate GABA release in synapses from FS interneurons to PCs.* A. Plot of the IPSP amplitudes recorded in the postsynaptic PC
before and during subsequent bath application of ω-conotoxin-GVIA (1 µM), and, subsequently, of ω-agatoxin-IVa (0.5 µM). Application of ω-conotoxin-GVIA, N-type specific calcium channel antagonist, had no significant effect on the IPSP amplitude. In contrast, ω-agatoxin-IVa, a specific P/Q-type calcium channel antagonist, completely blocked the synaptic responses. B. Average IPSPs from the same experiment. C. Plot of normalized IPSP amplitudes against recording time (5 pairs) after application of ω-agatoxin-IVa. IPSP amplitude exponentially declined to 0 mV at a rate with a time constant of 38 ± 2.8 s. D. Plot of normalized IPSP amplitudes during trains in control condition and after application of ω-conotoxin-GVIA. N-type specific calcium channel antagonist had no significant effect on the IPSP synaptic dynamics. E. Average amplitude of IPSP did not change after application of ω-conotoxin-GVIA (Ctx, n=5 pairs), whereas application of ω-agatoxin-IVa alone (Aga, n=6) or after bath application of ω-conotoxin-GVIA (Ctx+Aga, n=5) completely blocked synaptic transmission. F-G. Both ω-agatoxin-IVa (0.5 µM) (F) and ω-conotoxin-GVIA (1 µM) (G) reduced EPSP amplitudes in the reciprocal excitatory connections from PC to FS cell. H. Blocking P/Q-type VGCCs by ω-agatoxin-IVa fully prevented the neurotransmission from FS cell to PC even during the high-frequency firing of the presynaptic FS interneuron.
Figure 1. Physiological and morphological properties of FS interneurons and PCs in layers 2-3 of rat prefrontal cortex. A. Representative example of the firing pattern of neocortical FS interneuron and PC. FS interneuron exhibits high-frequency firing rate and a pattern without a significant spike frequency adaptation. PC displays membrane properties of a typical regular spiking cell with a significant spike frequency adaptation. B. Neurolucida reconstruction of the FS interneuron - PC connected pair. Interneuron soma and dendrites are drawn in green and the axon in black. Pyramidal soma and dendrites are drawn in red, axon of PC is not shown. C. Double immunostaining for biocytin (red) and PV (green) in the same connected pair. Biocytin and PV are co-localized in the FS interneuron. Arrows indicate PV-positive FS interneuron. D. Membrane properties of recorded FS interneurons (circles) and PCs (triangles). Boxes represent s.e.m.; whiskers represent standard deviation; horizontal lines are medians.
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F. A plot of CV\(^{-2}\) ratio vs. amplitude ratio of 5\(^{th}\) to 1\(^{st}\) IPSPs in the trains. Each circle represents the connected pair.
Figure 3. P/Q-type calcium channels exclusively mediate GABA release in synapses from FS interneurons to PCs. 

A. Plot of the IPSP amplitudes recorded in the postsynaptic PC before and during subsequent bath application of \( \theta \)-conotoxin-GVIIa (1 \( \mu \)M), and, subsequently, of \( \theta \)-agatoxin-IVA (0.5 \( \mu \)M). Application of \( \theta \)-conotoxin-GVIIa, N-type specific calcium channel antagonist, had no significant effect on the IPSP amplitude. In contrast, \( \theta \)-agatoxin-IVA, a specific P/Q-type calcium channel antagonist, completely blocked the synaptic responses. 

B. Average IPSPs from the same experiment. 

C. Plot of normalized IPSP amplitudes against recording time (5 pairs) after application of \( \theta \)-agatoxin-IVA. IPSP amplitude exponentially declined to 0 mV at a rate with a time constant of \( 38 \pm 2.8 \) s. 

D. Plot of normalized IPSP amplitudes during trains in control condition and after application of \( \theta \)-conotoxin-GVIIa. N-type specific calcium channel antagonist had no significant effect on the IPSP synaptic dynamics. E. Average amplitude of IPSP did not change after application of \( \theta \)-conotoxin-GVIIa (Ctx, n=5 pairs), whereas application of \( \theta \)-agatoxin-IVA alone (Aga, n=6) or after bath application of \( \theta \)-conotoxin-GVIIa (Ctx+Aga, n=5) completely blocked synaptic transmission. 

F-G. Both \( \theta \)-agatoxin-IVA (0.5 \( \mu \)M) (F) and \( \theta \)-conotoxin-GVIIa (1 \( \mu \)M) (G) reduced EPSP amplitudes.
in the reciprocal excitatory connections from PC to FS cell. H. Blocking P/Q-type VGCCs by 
(1)-agatoxin-IVa fully prevented the neurotransmission from FS cell to PC even during the 
high-frequency firing of the presynaptic FS interneuron.