Muscarinic presynaptic modulation in GABAergic pallidal synapses of the rat

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ABSTRACT

The external globus pallidus (GPe) is central for basal ganglia processing. It expresses muscarinic cholinergic receptors and receives cholinergic afferents from the pedunculopontine nuclei (PPN) and other regions. The role of these receptors and afferents is unknown. Muscarinic M₁-type receptors are expressed by synapses from striatal projection neurons (SPNs). Because axons from SPNs project to the GPe, one hypothesis is that striatopallidal GABAergic terminals may be modulated by M₁ receptors. Alternatively, some M₁ receptors may be postsynaptic in some pallidal neurons. Evidence of muscarinic modulation in any of these elements would suggest that cholinergic afferents from the PPN, or other sources, could modulate the function of the GPe. Here, we show this evidence using striatopallidal slice preparations: after field stimulation in the striatum, the cholinergic muscarinic receptor agonist, muscarine, significantly reduced the amplitude of inhibitory postsynaptic currents (IPSCs) from synapses that exhibited short-term synaptic facilitation. This inhibition was associated with significant increases in paired pulse facilitation and quantal content (CV²) was proportional to IPSC amplitude. These actions were blocked by atropine, pirenzepine and mamba toxin-7; suggesting that receptors involved were M₁. In addition, we found that some pallidal neurons have functional post-synaptic M₁-receptors. Moreover, some evoked IPSCs exhibited short-term depression and exhibited a different kind of modulation: they were indirectly modulated by muscarine via the activation of presynaptic cannabinoid-1 receptors (CB₁). Thus, pallidal synapses presenting distinct forms of short-term plasticity were modulated differently.

**Key words:** external globus pallidus, pallidal synapses, presynaptic modulation, muscarinic receptors, endocannabinoids.
Most projection neurons of the external globus pallidus (GPe) are GABAergic and inhibitory in the adult rodent. These neurons have multiple targets: the striatum (Str), the subthalamic nucleus (STN), the reticular nucleus of the thalamus, the internal globus pallidus (GPI; rodent entopeduncularis) (Kita 2007), the substantia nigra pars reticulata (SNr) (Aceves et al. 2011), the auditory cortex, the pedunculopontine nucleus (PPN) (Mena-Segovia et al. 2004; Moriizumi et al. 1992; Takakusaki et al. 2013), the periaqueductal gray matter (Shammah-Lagnado et al. 1996) and the inferior colliculus (Kita 2007). A single GPe neuron may project to several targets.

On the other hand, the GPe receives afferents from the Str (mainly from the “indirect” basal ganglia pathway), the parafascicular nucleus of the thalamus, the STN and the brain stem including the PPN (Bolam et al. 2000; Charara and Parent, 1994; Kita 2007; Mallet et al. 2012; Mena-Segovia et al. 2004; Parent and Hazrati 1995). Str afferents are abundant (Schwab et al. 2013). All these connections suggest that the GPe is an important integrative center, not just a relay nucleus (Chan et al. 2005; Goldberg and Bergman 2011; Kita 2007; Mena-Segovia et al. 2004; Schwab et al. 2013).

Synaptic studies in the GPe have focused on glutamatergic, GABAergic and dopaminergic inputs (Abedi et al. 2013; Anaya-Martínez et al. 2006; Jaeger and Kita 2011). Cholinergic inputs have received less attention. The present work explores whether cholinergic modulation can be detected in the GPe, either pre- or post-synaptically. A positive answer to this question may provoke more detailed studies.

One basis to posit this question was: inside the striatum, a presynaptic activation of muscarinic M₁-type receptors has been described. It reduces GABA release from the terminals of SPNs local axon collaterals (Perez-Rosello et al. 2005). The acetylcholine (ACh) that induces striatal presynaptic modulation is in part released from striatal cholinergic interneurons (Mesulam et al. 1992; Wilson et al. 1990), and in part from cholinergic afferents coming from the brain stem (Dautan et al. 2014).

But synaptic axons from striatal projection neurons of the indirect pathway (iSPNs) and some from the direct pathway (dSPNs) leave the Str and innervate the GPe (Gerfen and Surmeier 2011; Kawaguchi et al. 1990).
That is, many GABAergic synapses in the GPe come from the same axons whose terminals are modulated by ACh inside the Str (Schwab et al. 2013) and striatal projection neurons express muscarinic M₁-type receptors (Yan et al. 2001). Therefore, a first hypothesis would be that many GABAergic synapses in the GPe will also be modulated by presynaptic muscarinic M₁-type receptors (Cortés et al. 1987; Goldberg et al. 2012; Piggott et al. 2002). A related question is whether M₁-type receptors modulate GABA release in the same way: reducing inhibitory postsynaptic currents (IPSCs). In case these hypotheses were proven, a function for cholinergic innervation to the GPe would have been disclosed (Gorbachevskaya and Chivileva 2006; Woolf and Butcher 1986). Cholinergic fibers from the PPN innervate the telencephalon and diverse basal ganglia nuclei including the GPe (Chan et al. 2005; Charara and Parent, 1994; Mena-Segovia et al. 2004). In addition, the GPe may have local cholinergic neurons (Rodrigo et al. 1998) and perhaps receive cholinergic innervation coming from the ventral pallidum (Bengtson and Osborne 2000).

Therefore, another question was whether some pallidal neurons exhibit postsynaptic responses to muscarinic cholinergic agonists. Finally, because some GABAergic synapses in the GPe arise from pallidopallidal axon collaterals (Jaeger and Kita 2011; Miguelez et al. 2012; Sims et al. 2008) it was important to observe if they are also modulated. Importantly, electrophysiological and optogenetic techniques have already demonstrated that a main difference between striatopallidal and pallidopallidal synapses is their short-term synaptic plasticity: striatopallidal connections display short-term facilitation (STF) while terminals from pallidal neurons exhibit short-term depression (STD) (Miguelez et al. 2012; Sims et al. 2008). Striatopallidal synapses have a lower release probability than pallidopallidal synapses. In agreement with these previous studies we divided the GABAergic synapses studied here by the type of short-term plasticity that they exhibit. Then, we asked whether both of them could be modulated by muscarinic receptors and if that is true, whether they are modulated in the same way.
MATERIALS AND METHODS

Animals. All procedures were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (National Institutes of Health, 1996 revision) and followed the guidelines from the Institutional Committee for the Production, Care and Use of Laboratory Animals (NOM-062-Z00-1999) of the Instituto de Fisiología Celular from the Universidad Nacional Autónoma de México. Because the scientific questions of the present investigation were to disclose cholinergic muscarinic actions in the GPe, a precise identification of the origin of synaptic inputs or neurons was not required. Therefore, it was decided that there is no need to use transgenic animals at this stage. In addition, every effort was made to minimize the number of animals needed to attain statistical significance. To our knowledge, our procedures do not induce animal suffering of any type.

Slice preparation. The experiments were performed on brain slices obtained from Wistar rats. Briefly, the rats (postnatal day 15-30) were anesthetized and perfused intracardiacally with a choline-Cl solution (in mM): 124 (CH₃)₃ N (Cl) CH₂CH₂OH, 2.5 KCl, 1.3 MgCl₂, 2.4 CaCl₂, 1.2 NaH₂PO₄, 26 NaHCO₃ and 10 glucose saturated with 95% O₂ and 5% CO₂. Thereafter, animals were decapitated and their brains obtained. Each hemisphere was cut using a vibratome (1000 Classic, Warner Instruments, Hamden, USA). Sagittal slices (350 μm thick) were cut at an angle of 10 degrees to the midline using the vibratome (Beurrier et al. 2006). Slices contained both the Str and the GPe. Slices were then transferred to saline containing (in mM): 124 NaCl, 2.5 KCl, 1.3 MgCl₂, 2.0 CaCl₂, 26 NaHCO₃, 1.2 NaH₂PO₄, 10 glucose, 0.2 ascorbic acid and 0.2 thiourea (pH = 7.4, 25°C-27°C saturated with 95% O₂ and 5% CO₂, 298 mOsm/L) and left for equilibration in this saline solution for at least 1 h. Single slices were transferred to a recording chamber and superfused continuously with oxygenated saline solution (4–5 ml/min).

Whole cell recordings. Recordings were made at room temperature (~25°C). Neurons were visualized using infrared differential interference microscopy (Nikon Instruments Inc, Melville, N.Y. U.S.A). Micropipettes for whole cell
recordings were pulled (Sutter Instrument, Novato, CA) from borosilicate glass tubes (1.5 mm OD, WPI, Sarasota, FL) for a final DC resistance of 4–6 MΩ when filled with internal saline containing high Cl⁻ and the following composition (in mM): 72 KH₂PO₄, 36 KCl, 10 NaCl, 10 EGTA, 10 HEPES, 1 CaCl₂, 2.5 MgCl₂, 2.4 Mg²⁺-ATP, 0.4 Na⁺-GTP, 5 lidocaine N-ethyl bromide –QX-314– and 1% byocitin (pH=7.2, 282 mOsM/L). In these conditions IPSCs are inward currents when recorded at -80 mV holding potential; where intrinsic currents are either small or not available (Perez-Rosello et al. 2005; Tecuapetla et al. 2007).

IPSCs were evoked by field stimulation in the Str (≥ 500 µm outside the GPe border) and recorded in GPe neurons. We used a concentric bipolar tungsten electrodes with12.5 µm diameter at the tip (FHC, Bowdoinham, ME).

Recordings were obtained with an Axopatch 200B electrometer (Axon Instruments, Foster City, CA, USA) and monitored with an oscilloscope (Tektronix Inc, Beaverton, OR, USA). Series resistance ranged from 5 to 20 MΩ and was commonly compensated up to 80%. Input and access resistance were continuously monitored during the experiment with a voltage command and experiments were discarded if changes > 20 % in the evoked transmembrane current were observed. Recordings were done in the presence of CNQX (10 µM) and APV (50 µM), antagonists of glutamate receptors. IPSCs could be totally blocked by 10 µM bicuculline or gabazine (Perez-Rosello et al. 2005; Tecuapetla et al. 2007). Stimulation consisted in either stimulus trains of ten shocks at 20 Hz every 20 s to assess short-term synaptic plasticity (STSP) and alternatively in two shocks (paired pulse) with a 50 ms interpulse interval delivered at 0.1 Hz. Stimulation parameters were adjusted to obtain synaptic currents of 100–500 pA during the control (about half the maximal amplitude). These responses were achieved with stimulus strengths from 1 to 20 V. IPSC amplitudes were measured from basal line to peak for the first response in a train (IPSC₁). For the subsequent responses (IPSCₙ), the basal line remaining from the previous response was subtracted. We measured the STSP ratio as IPSC₇–10 / IPSC₁.

Amplitudes of IPSCs after trains of stimuli that evoked short-term depression (STD) were normalized and fitted ad hoc with a single exponential decay in order to approximate a global time constant for depression:
\[ IPSC(t) = y_0 + A_1 e^{-t/\tau} \]

Where \( y_0 \) is the asymptotic baseline, \( A_1 \) is the exponential coefficient and \( \tau \) the time constant in ms.

For IPSCs trains that exhibited short-term facilitation (STF) a good fit was also obtained with a single inverted exponential function with one time constant:

\[ IPSC(t) = y_0 - A_1 e^{-x/\tau} \]

Where \( y_0 \) is the asymptotic maximal value, \( A_1 \) is the exponential coefficient and \( \tau \) is the time constant. Ten pulses separated by 50 ms intervals were given to induce the trains of IPSCs to see if short-term synaptic plasticity (STSP) was depressing (STD) or facilitating (STF), so that time constants ± estimation errors are reported in milliseconds. The paired pulse ratio (PPR) was the amplitude ratio between the second IPSC and the first IPSC (IPSC\textsubscript{2} / IPSC\textsubscript{1}).

Immunoctytochemical procedures. After recordings, neurons were injected with biocytin 1% to identify the cells and were processed for immunocytochemistry as previously described (Ibañez-Sandoval et al. 2006). The slices were incubated in streptavidin conjugated with Cy3 (1:200 dissolved in PBS, Zymed Laboratories, San Francisco CA). This allowed visualize the recorded neuron. Thereafter slices were incubated 30 min with 1% bovine albumin to block nonspecific binding sites. Then, slices were incubated 36 h with a rabbit antibody against parvalbumin (anti-PV; 1:2000, Abcam, dissolved in PBS containing 0.25% Triton-X). The slices were then rinsed thrice with PBS and incubated with a goat versus rabbit secondary antibody during 1 h. This antibody was conjugated with FITC (Vector Laboratories, Burlingame, CA). Samples were mounted with vectashield (Vector Laboratories, Burlingame, CA) and observed in a confocal microscope Olympus FV-1000. Most neurons recorded were PV-positive.

Dissociated neurons. In the present work the GPe was dissected from 300 μm thick brain slices incubated 20 min with 1 mg/ml papain (Calbiochem, Billerica, MA) at 34 °C. Slices were transferred to a low Ca\textsuperscript{2+} (0.4 mM CaCl\textsubscript{2}) saline solution to obtain isolated GPe neurons by mechanical dissociation. The
suspension was plated into a Petri dish mounted on the stage of an inverted microscope (Nikon, Melville, NY, USA) with saline containing (in mM): 0.001 tetrodotoxin, 130 NaCl, 3 KCl, 5 BaCl₂, 2 MgCl₂, 10 HEPES, and 10 glucose (pH=7.4 with NaOH; 300 ± 5 mOsm/L with glucose). Voltage-clamp recordings were performed on GPe neurons of 13-30 µm main diameter (capacitance = 12-13 pF). Internal saline contained (in mM): 180 N-methyl-D-glucamine, 40 HEPES, 10 EGTA, 4 MgCl₂, 2 ATP, 0.4 GTP and 0.1 leupeptin (pH = 7.2 with H₂SO₄; 280 ± 5 mOsm/L). Whole-cell recordings were obtained with an Axopatch 200B electrometer (Axon Instruments) and monitored with pClamp (v8) and a 125 kHz DMA interface (Axon Instruments). Series resistance (<10 MΩ) was compensated (70–80%). Ba²⁺ (5 mM) currents were recorded while blocking Na⁺-channels (1 µM tetrodotoxin). Current–voltage relationships (I–V plots) of I₉ were built before and after drug applications with current responses to either voltage step commands (20 ms) from -80 to 50 mV (in 10 mV steps) or with 200 ms voltage ramp commands (0.7 mV/ms) from -80 to 50 mV. Both methods yielded equal results (Perez-Burgos et al. 2008). The present paper shows representative responses to ramp commands where peak evoked currents correspond to the minimum of the I-V plot.

**Drugs.** Drugs were prepared prior to each experiment and added to the superfusion saline in the final concentration indicated. AMPA / KA selective antagonist 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline disodium salt (10 µM CNQX), NMDA antagonist DL-2-Amino-5-phosphonopentanoic acid (50 µM AP5), tetrahydro-4-hydroxy-N,N,N,5-tetramethyl-2-furanmethanammonium chloride ((±)-muscarine chloride 1 µM), tropine tropate, α-(hydroxymethyl) benzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester (atropine sulfate salt monohydrate); 5,11-dihydro-11-[(4-methyl-1-piperazinyl)acetetyl]-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one dihydrochloride (pirenzepine dihydrochloride); (5S)-5-[(6R)-6,8-dihydro-8-oxofuro[3,4-e]-1,3-benzodioxol-6-yl]-5,6,7,8-tetrahydro-6,6-dimethyl-1,3-dioxolo[4,5-g]isoquinolinium iodide (1 (S), 9 (R)-(−)-bicuculline methiodide); and N-Ethyl-2-phenyl-N-(4-pyridylmethyl)hydracrylamide (tropicamide) were obtained from Sigma-RBI (St. Louis, MO, USA). CB₁-receptor antagonist, N-(piperidin-1-yl)-5-(4-iodophenyl)-
1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM 251), the cannabinoid agonist, R-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylat (WIN 55,212-2 mesylat), were obtained from Tocris (Ellisville, Missouri, USA). Mamba toxins 7 and 3 (MT-7 and 3) were obtained from Alomone (Israel).

**Statistics.** In many cases distributions of measurements of small samples were not normal. Therefore, distribution-free statistics were used. Unless stated otherwise, cases comparing recordings from the same neurons before and after a treatment used Wilcoxon T tests. Unpaired samples were compared with Mann-Whitney U test. In the same way, multiple treatments were compared with the either Friedman-ANOVA or Kruskal-Wallis ANOVA and post hoc Tukey or Dunn statistics. P<0.05 was used as significance level. Statistical analysis of synaptic variability was assessed with the coefficient of variation (CV = standard deviation of IPSC peak amplitude / mean IPSC peak amplitude). The mean quantal content was approximated as customary: (mean IPSC amplitude)² / (IPSC amplitude variance)² ≈ CV⁻² (Bekkers and Stevens, 1990; Lupica et al. 1992; Rodriguez-Moreno et al. 1997; Sims et al. 2008). A relation with positive slope between CV⁻² and IPSC amplitude is expected only when the cuase of change is presynaptic.

**Abbreviations.** CB₁R: cannabinoid receptor-1; IPSCs: evoked inhibitory postsynaptic currents; GPe: external globus pallidus or rodent pallidum; GPI: internal globus pallidus; SPNs: striatal projection neurons. iSPNs: striatal projection neurons of the indirect basal ganglia pathway which form the main striatopallidal connection; dSPNs: striatal projection neurons of the direct basal ganglia pathway which have a minor contribution to the striatopallidal connection. Str: striatum; PPN: pedunculopontine nucleus; PPR: paired pulse ratio; SNr: substantia nigra pars reticulata; STN: subthalamic nucleus; STSP: short-term synaptic plasticity; STD: short-term depression; STF: short-term facilitation.
Results

There are different types of short-term synaptic plasticity in GABAergic synapses of the GPe.

A field stimulus delivered within the Str will activate axons whose terminals may make synapses with postsynaptic GPe neurons (Figs. 1A-C) (Copper and Stanford 2001). This finding has been corroborated in many instances and was done using mainly electrophysiological methods in vitro and in vivo (rev. in Jaeger and Kita 2011). But electrical stimulation of the Str might activate both striatopallidal and pallidostriatal axons (Goldberg and Bergman 2011; Kita 2007; Mallet et al. 2012; Schwab et al. 2013). The terminals they activate onto GPe neurons are distinguished by the type of short-term synaptic plasticity that they exhibit: striatopallidal terminals exhibit facilitation (short-term facilitation STF) while pallidopallidal synapses show depression (short-term depression: STD; Miguelez et al. 2012; Sims et al. 2008). When strong enough stimuli are applied, the latency cannot (Ogura and Kita 2000) distinguish between them. Therefore, because we used electrophysiological methods for this investigation, and did not need to precisely isolate the origin of the synapses studied to reach our research goals, we functionally designed the evoked inputs by the class of short-term synaptic plasticity that they exhibited.

Thus, in one of our samples, a thin field electrode (see Materials and Methods) located in the Str will evoke IPSCs exhibiting short-term facilitation (STF-synapses; Fig. 1A) in \( n = 16 \) out of 22 trials. In the rest, \( n = 6 \) out of 22 trials (1/3) we evoked IPSCs that exhibited short-term depression (STD-synapses; Fig. 1B; Beurrier et al. 2006). Here, however, these synapses will be referred as STF- or STD-synapses due to possible contamination when using field stimulus. In addition, perhaps, not all sources of GABA that target GPe neurons have been identified (rev. in Mena-Segovia et al. 2004). The STF ratio = mean amplitude of last 7th to 10th IPSCs divided over mean amplitude of first IPSC was (mean ± SEM) 1.72 ± 0.023 (\( n = 10 \); median 1.59; Fig. 1A). An ad hoc fitting of a single inverted exponential function (see Materials and Methods)
to facilitating synapses yielded a time constant ($\tau$; fitted value ± estimation error) of 150 ± 30 ms ($r^2 = 0.97$; Fig. 1D).

In contrast, STD-synapses (Figs. 1B, C) had a STD ratio = 0.60 ± 0.01 (n = 6; median 0.6) and a simple exponential decay yielded $\tau = 83 ± 11$ ms ($r^2 = 0.98$; Fig. 1E). When IPSCs from STD-synapses were activated by intrapallidal stimuli in coronal slices (Fig. 1C), the STD ratio was 0.45 ± 0.01 (n = 4; median 0.46) and a simple exponential decay yielded a $\tau = 67 ± 12$ ms ($r^2 = 0.97$; Fig. 1F; not significantly different from that obtained in sagittal slices). These results corroborated that striatal field stimulation can evoke IPSCs in postsynaptic pallidal neurons with two different types of short-term plasticity: STF and STD. The larger proportion corresponded to STF-synapses when field stimulus was delivered inside the Str. When field stimulus was delivered within the GPe only STD-synapses could be recorded.

In the next section we will show the muscarinic modulation of STF- and STD-synapses.

**Activation of cholinergic muscarinic receptors presynaptically inhibits STF- and STD-synapses.**

The actions of muscarinic receptor activation were examined in STF-synapses while evoking IPSCs in postsynaptic GPe neurons (only paired responses are illustrated; Cooper and Stanford 2001). Figure 2A shows a representative time course of IPSCs amplitude (each symbol represents the average of 12 individual events ± SEM, in absolute amplitude) in two different conditions: control (1), and during the addition of 1 $\mu$M muscarine (2) into the bath saline (horizontal bar). Muscarine reduced IPSCs amplitude (first IPSC of the pair) in all cases. Insets at right show traces taken at different times during the time course (indicated by numbers). This connection exhibited paired pulse facilitation in control (1) and after IPSC reduction by muscarine (2) paired pulse facilitation was enhanced as seen with normalized and superimposed traces.

Figure 2B shows a similar experiment in a STD-synapse. There is paired pulse depression in the control (paired IPSCs are illustrated). In this synapse muscarine also decreased IPSCs amplitude in all experiments. IPSC reduction
by muscarine converted paired pulse depression into paired pulse facilitation as seen in the insets at the right.

Muscarinic actions looked similar in both STF- and STD-synapses. A sample from both synapses showed that the paired pulse ratio (PPR) increased in all connections tested (Fig. 2C; n = 9; **P<0.01), suggesting a presynaptic site of action. Muscarine also induced IPSCs decrease in all synapses tested either with STD or STF in control conditions (Fig. 2D; in percentage a 56 ± 5 % decrease; n = 22; ***P<0.001). The change in IPSC amplitude was accompanied by an increase in the coefficient of variation (CV; Fig. 2E: n = 19; ***P<0.001), again suggesting a presynaptic origin.

A comparison of mean quantal content (CV^2; see Material and methods) as a function of IPSC amplitude before and during muscarine disclosed a relation between CV^2 and IPSC amplitudes in both facilitating and depressing synapses (Fig. 2F; Bekkers and Stevens, 1990; Rodríguez-Moreno et al. 1997; Salgado et al. 2005; Sims et al. 2008; Tecuapetla et al. 2007), suggesting that muscarine preserved this relation. That is, reduction in amplitude signified less quanta of released transmitter in both STF- and STD-synapses.

Taken these results together, the following partial conclusions can be reached: muscarine decreases IPSC amplitude in inhibitory pallidal synapses. These synapses may exhibit either facilitation or depression suggesting that one role of cholinergic inputs in the GPe is to regulate GABA release at the presynapsis, as previously shown for the terminals of local axon collaterals interconnecting striatal projection neurons (SPNs) (Perez-Rosello et al. 2005). This conclusion is supported by an increase in the PPR and CV as well as a dependency of CV^2 on IPSC amplitude (Rodríguez-Moreno et al. 1997; Salgado et al. 2005).

Muscarinic modulation of STF-synapses is mediated by M1-class receptors. Figure 3A illustrates that muscarinic actions are specific and reversible in IPSCs evoked from STF-synapses. A time course similar as that shown in Figure 2A (each symbol in the time course is the average of 12 individual events ± SEM) shows that muscarine (1 μM) decreased IPSC amplitude in all evaluated neurons, and that addition of 10 μM atropine to the bath saline induced a recovery of IPSC amplitude, suggesting that muscarine actions were blocked at
its receptor. Atropine blocked muscarinic actions in all synapses tested. Insets at right are representative traces taken from the time course as indicated by numbers. Paired line graph in Figure 3B shows a summary of results for the corresponding sample, in absolute IPSC amplitudes (before and during muscarine: \( n = 6; **P < 0.01 \)), and after addition of atropine in the presence of muscarine (\( n = 6; **P < 0.03 \)).

Figure 3C illustrates an alternative protocol showing the time course of the actions of 1 μM pirenzepine, a preferring antagonist for \( M_1 \)-class receptors, in a STF-synapse. Pirenzepine (bar) had no significant actions by itself. However, the actions of 1 μM muscarine added in the presence of pirenzepine were blocked completely (the same result as in Fig. 3A is obtained when pirenzepine was administered after muscarine; not illustrated). Inset at right shows paired responses taken at different times during the time course as denoted by numbers.

Paired lines graph in figure 3D shows no significant changes in IPSC amplitude during addition of muscarine in the presence of pirenzepine. The same results were obtained when mamba toxin-7 (500 nM MT-7) was used instead of pirenzepine (\( n = 3; \) not illustrated). In contrast, 1 μM tropicamide, a preferring \( M_4 \)-type receptor antagonist (Betz et al. 2007), could not block the actions of muscarine (\( n = 3; \) not illustrated) and mamba toxin-3 (500 nM MT-3), a specific \( M_4 \)-type receptor antagonist was also used with negative results (\( n = 3; \) not illustrated).

A conclusion from these results suggests that STF inhibitory synapses in the GPe are regulated presynaptically by \( M_1 \)-type receptors (Perez-Rosello et al. 2005). Pirenzepine also blocked muscarinic actions on STD-synapses (\( n = 4; \) not illustrated, but see below).

Mediation of muscarinic actions differs in STF- as compared to STD-synapses.

To see whether muscarinic actions were mediated directly or indirectly by presynaptic cannabinoids in STF- or STD-synapses, a selective antagonist of \( CB_1 \)-receptors was employed (Fukudome et al. 2004; Lau and Vaughan 2008). It is known that striatopallidal terminals possess presynaptic cannabinoid \( CB_1 \)-
type receptors that decrease GABA release (Chen et al. 2011; Engler et al. 2006; Ergetová and Elphick 2000; Szabo et al. 1998).

First, we examined whether CB1-type receptors antagonists could block muscarinic modulation in STF-synapses. Figure 4A illustrates the time course of IPSC amplitude before and during the addition of 1 μM AM 251, a CB1-class receptor antagonist. The cannabinoid antagonist had no actions by itself (bars denote time of drugs application), suggesting that endogenous cannabinoids are at very low concentrations in the extracellular fluid of this preparation. In the continuous presence of AM 251, a subsequent addition of 1 μM muscarine had its usual action: it decreased IPSC amplitude. Inset at right shows IPSC traces acquired at numbered moments of the time course. There is paired pulse facilitation. This result suggests that the action of muscarine on STF-synapses was independent of and not mediated via cannabinoids. In this particular experiment, the GABA_A-receptor antagonist, gabazine (10 μM), blocked all the remaining current, suggesting that all IPSC was mediated by GABA and therefore these inhibitory pallidal STF-synapses activated in the Str are GABAergic. Paired line graph in Figure 4B shows the individual behavior of STF-synapses: muscarine acted in the presence of AM 251 in 12 out of 18 GPe neurons recorded during intra-striatal field stimulation for a 49 ± 7 % decrease in IPSC amplitude (***P < 0.001).

A similar experiment was performed in STD-synapses (Fig. 4C). As in STF-synapses, the amplitude of IPSCs recorded on a GPe cell had no significant changes during the time of addition of 1 μM AM 251 (bars denote time of drugs application). But, in contrast to STF-synapses, the actions of 1 μM muscarine were blocked by AM 251 in STD-synapses. The inset shows IPSC traces acquired at the moments signaled by numbers in the time course (see paired pulse depression). Paired line graph in Figure 4D shows the individual behavior of a sample of STD-synapses: muscarine actions were antagonized in the presence of AM 251 (n = 6). Again, gabazine blocked all synaptic current showing that STD-synapses in the GPe are also GABAergic. When the action of muscarine in the presence of AM 251 was compared in STF vs. STD connections, differences were significant (***P < 0.001). The results then suggest that cannabinoids released to the extracellular space and acting as
retrograde transmitter (Engler et al. 2006) mediate the actions of muscarine in
STD-synapses. Retrograde actions of cannabinoids have been described in
many brain synapses and the synthesis of them is commonly associated with
metabotropic receptors which are present in the striatopallidal complex (e.g.,
Chen et al. 2011; Poisik et al. 2003).

However, if the above conclusion is true, then, STD-synapses should express
presynaptic cannabinoid receptors. Moreover, muscarinic signaling may also
induce the synthesis of cannabinoids, that is, M₁-type receptor signaling may
occur through a G_{q/11} protein that produces changes in intracellular calcium
(e.g., Perez-Burgos et al. 2008), therefore, we should observe postsynaptic
actions in some GPe neurons and presynaptic modulation of STD-synapses
should be blocked by calcium chelation (e.g., 20 mM intracellular BAPTA).

None of these hypotheses had been tested in the GPe.

A representative experiment of a sample designed to explore these inferences
is shown in the time course of Figure 5A: Ca²⁺ chelation in a postsynaptic
pallidal neuron, before addition of muscarine, blocked all muscarinic actions in a
STD-synapse. This suggests that muscarinic action requires a postsynaptic
signaling cascade. Next, we added a selective cannabinoid CB₁-type receptor
agonist, 10 μM WIN 55212-2, to the bath saline. The CB₁-agonist depressed
IPSCs amplitude by an average of 54 ± 14 %, suggesting that modulation by
CB₁-type receptors is present in STD-synapses. The inset shows representative
traces taken at numbered moments during the time course. Figure 5B shows a
paired line graph showing that similar actions were obtained from a sample of
STD-synapses (n = 5; *P < 0.05). These results support an indirect action of
muscarine in these synapses.

The above results imply that some GPe neurons posses functional
postsynaptic muscarinic receptors. To test this hypothesis we recorded the
postsynaptic actions of muscarine in acutely dissociated GPe neurons. Figure
6A shows the time course of the action of 1 μM muscarine in whole-cell Ca²⁺
current amplitudes evoked in a GPe neuron with ramp-like voltage commands
(Perez-Burgos et al. 2008; Perez-Rosello et al. 2005). Clearly and reversibly,
muscarine reduced the Ca²⁺ current (shaded areas) in 6 out of 16 neurons from
the GPe (37% in this sample of recorded neurons dissociated from the GPe
center; Fig. 6C). Figure 6D shows that MT-7 blocked the actions of muscarine
when administered together (bars indicate time of drug application) and I-V plots in Figure 6E show that I-V plot with muscarine plus MT-7 do not differ from that in the control conditions. It was upon washing MT-7 when the actions of muscarine were revealed. This is clearly seen in a sample of neurons (Fig. 6F; n = 9; ***P < 0.001 with Friedman ANOVA and *P < 0.05 for the post hoc test between muscarine plus MT-7 vs. muscarine alone). This result suggests that post-synaptic receptors in GPe neurons that express muscarinic receptors are M₁; reason why both STF and STD synapses could be blocked by both pirenzepine and MT-7. This action could also be blocked by atropine (n = 3; not shown).

Nevertheless, the calcium current of most GPe neurons did not respond to muscarine (n = 10/16 or 63%; Figs. 6G-I). Probably, the cell population in the GPe is heterogeneous (Goldberg and Bergman 2011; Hoover and Marshall 2002; Mallet et al. 2012; Poisik et al. 2003). These results imply that although all pallidal inhibitory synapses may be modulated, only a subset of postsynaptic neurons expresses muscarinic receptors and may produce cannabinergic mediators upon their activation.

DISCUSSION

Facilitating synapses are modulated by muscarinic receptors.

Field stimulation inside the Str in sagittal slices (Beurrier et al. 2006) evokes IPSCs in postsynaptic GPe neurons (Cooper and Stanford 2001) in the presence of CNQX plus APV (see Materials and Methods). These IPSCs are GABAergic since they can be completely blocked by the actions of gabazine or bicuculline (Goldberg and Bergman 2011; Jaeger and Kita 2011). These IPSCs were significantly reduced in amplitude after activation of muscarinic receptors in a way similar to that observed for the IPSCs recorded between SPNs inside the Str (Perez-Rosello et al. 2005). Muscarinic action is most probably presynaptic since it was accompanied by increases in both PPR, CV and a relation between CV² and IPSC amplitude (Bekkers and Stevens, 1990; Cooper and Stanford 2001; Rodríguez-Moreno et al. 1997; Salgado et al. 2005; Sims et al. 2008; Tecuapetla et al. 2007). These responses could be recorded
in both STF-synapses and STD-synapses. Thus, a main purpose of the present work, to demonstrate the functionality of cholinergic muscarinic receptors within the GPe was fulfilled.

A correlation between the type of short-term synaptic plasticity and the origin of the synapses has been previously shown (Miguelez et al. 2012): STF-synapses are mostly striatopallidal synapses whereas STD-synapses are mostly pallidopallidal synapses, and in fact, we could only record from STF-synapses when field stimulation was given inside the Str and not inside the GPe. Moreover, most evoked IPSCs after field stimulation inside the Str followed STF plasticity. When field stimulation was given inside the GPe we could only record STD-synapses. However, because a field stimulus cannot precisely isolate the origin of the IPSCs, and because other sources of GABA may be present (Mena-Segovia et al. 2004), here, we referred to the synapses recorded as facilitating (STF-synapses) or depressing (STD-synapses) during intrastriatal stimulation, as most previous electrophysiological studies (Jaeger and Kita 2011; Sims et al. 2008).

Previous studies show that about two thirds of GPe neurons are immunoreactive to parvalbumin (PV+). These neurons mainly project to STN, GPi and SNr. On the other hand, about one third of GPe neurons are PV- (Kita, 2007). The latter may express preproenkephalin (Mallet et al. 2012) and project to the striatum. However, other types of neurons are not discarded (Cooper and Stanford, 2000; Nambu and Llinas, 1994; Rodrigo et al. 1998). Available knowledge points towards a predominant type of neuron receiving STF-synapses (rev. in Jaeger and Kita 2011). Here, we show that in a small sample of PV+ neurons that were recorded and labeled the synapses recorded were STF-synapses. Because terminals from the same axons have been shown to express and are responsive to M₁-receptor activation inside the Str (Perez-Rosello et al. 2005), we conclude that these synapses may be modulated directly by ACh. However, all possible retrograde messengers have not been discarded.

Muscarine actions were specific because they were blocked by muscarinic receptor antagonists of the M₁-class receptors such as M₁-antagonists pirenzepine and MT-7, but not by the M₄-class antagonists such as tropicamide or MT-3.
While M₁ and M₄ receptors are the functional muscarinic receptors in SPNs, only M₁ receptors are expressed in both classes of SPNs. M₄-receptors are preferentially expressed in dSPNs. Therefore, M₁ receptors are virtually the only functional muscarinic receptors of iSPNs (Yan et al. 2001) and these neurons configure a most important projection to the GPe (Schwab et al. 2013). Thus, the present results demonstrate that M₁ receptors presynaptically regulate STF-synapses (most probably striatopallidal synapses). These results may have been expected given the previous knowledge mentioned above, but they had not been observed experimentally. Thus, STF-synapses constitute a first target for cholinergic modulation in the GPe. A possible contamination of other inputs due to field stimulation runs against obtaining this robust result. Blockade by selective CB₁-receptor antagonist, AM 251, did not affect muscarinic modulation of STF-synapses (Engler et al. 2006; Fukudome et al. 2004; Lau and Vaughan 2008), therefore, we discarded that muscarinic presynaptic modulation of STF-synapses was mediated indirectly via cannabinoid synthesis and CB₁-presynaptic receptors known to be present in striatopallidal terminals (Chen et al. 2011; Engler et al. 2006; Ergetová and Elphick 2000; Szabo et al. 1998). The combined modulation of M₁ and CB₁ receptors is out of the scope of the present work.

Sources of ACh to exert this modulation are: the PPN (Charara and Parent, 1994; Mena-Segovia et al. 2004; Woolf and Butcher 1986) and some cholinergic neurons within or in the vicinity of the GPe (Bengtson and Osborne 2000; Rodrigo et al. 1998). PPN afferents also reach the Str (Dautan et al. 2014; Mena-Segovia et al. 2004; Woolf and Butcher 1986) although a main cholinergic source in this nucleus comes from interneurons (Wilson et al. 1990).

Depressing synapses are modulated by muscarinic receptors in an indirect way.

IPSCs from STD-synapses decreased their amplitude and increased both their PPR and CV during activation of muscarinic receptors, again supporting a presynaptic mechanism. However, the behavior of STD-synapses was different: a previous application of AM 251 blocked all muscarinic modulation. This result is consistent with an indirect modulation mediated by cannabinoids. In support of this inference, application of the selective CB₁-receptor agonist, WIN 55212-
2, reduced IPSCs from STD-synapses. The interpretation of these results is:
first, CB₁-receptors are present and functional in the synaptic terminals of STD-
synapses, and second, muscarinic actions are mediated indirectly by CB₁-
receptors. These results on STD-synapses were observed despite possible
contamination due to field electrode stimulation, suggesting that they are robust.
Consistent with these results, we show that chelation of intracellular calcium in
recorded postsynaptic GPe neurons abolished muscarinic modulation of STD-
synapses. This result shows that mediation of a postsynaptic component was a
necessary step (Engler et al. 2006) for presynaptic inhibition to occur.
Moreover, during recordings of whole-cell Ca²⁺-currents in a sample of
dissociated GPe neurons, we found that about one third of them were
responsive to the muscarinic agonist. This result demonstrates the existence of
postsynaptic muscarinic receptors in some GPe neurons. The action of
muscarine on the Ca²⁺-currents of these GPe neurons was robust, reversible
and could be blocked by atropine and MT-7.
Taken all together the interpretation of these results is as follows: another
target for muscarinic actions in the GPe is the postsynaptic receptors in some
type of GPe neurons. Secondly, perhaps GPe with postsynaptic muscarinic
receptors release endocannabinoids which retrogradely affect CB₁-receptors
present in STD-synapses.
Muscarinic modulation of synaptic transmission mediated by cannabinoids
has been found in other systems (Fukudome et al. 2004; Lau and Vaughan
2008; Ohno-Shosaku and Kano, 2014). What is of interest in the GPe is that
two functional classes of GABAergic terminals are modulated differently by
ACh. Modulation of STF-synapses is very probably mediated by M₁-receptors
while modulation of STD-synapses is probably indirectly mediated by
cannabinoids. Action of group 1 metabotropic glutamate receptors on GPe
neurons involves the PLC-IP₃-PKC pathway (Poisik et al. 2003). Therefore, one
possible explanation for the synthesis of endocannabinoids via the activation of
M₁-receptors could be the activation of a similar pathway and the consequent
increase in intracellular Ca²⁺ released from internal stores (Ohno-Shosaku and
Kano 2014; Perez-Burgos et al. 2008; Poisik et al. 2003). Nevertheless, future
research is needed to disclose the complete signaling pathway.
In summary the findings of the present work show that cholinergic inputs to the GPe (Charara and Parent, 1994; Mena-Segovia et al. 2004) have presynaptic and postsynaptic targets. However, the GPe projects to the PPN (Mena-Segovia et al. 2004; Morizumi et al. 1992; Takakusaki et al. 2013). Therefore a loop between these nuclei may exist and explain why 6-hydroxy-dopamine administered in the GPe reproduces major disorders of PD (Abedi et al. 2013).

A recapitulation of the above described findings follows: 1) field stimulation within the Str activates at least two types of GABAergic synapses as recorded in GPe neurons: STF- and STD-synapses (Jaeger and Kita, 2011; Miguelez et al. 2012; Sims et al. 2008), 2) IPSCs from both connection types are presynaptically modulated by muscarinic receptors, mainly M₁-receptors, 3) Muscarinic modulation of STF-synapses cannot be blocked by CB₁-receptor antagonists and is very probable mediated by M₁-receptors. 4) Muscarinic modulation of STD-synapses is indirect and mediated by cannabinoids, 5) Indirect muscarinic modulation of STD-synapses involves the activation of postsynaptic muscarinic receptors, and indeed, some GPe neurons were found to be postsynaptically responsive to muscarine. The receptor involved in these cases is the M₁-type.

Probable physiological relevance.

The aim of the present work was to demonstrate a muscarinic modulation in the GPe. However, this demonstration supports some wider questions. Not only the cholinergic system innervates most basal ganglia nuclei (Mena-Segovia et al. 2004), but the dopaminergic system accomplishes a similar role (Benazzouz et al. 2014). Therefore, when it is said that there is a balance between these two systems in the basal ganglia, how the balance is reached in each of these nuclei simultaneously? Is the unbalance in any of them a source of disorder?

One partial answer was to show the role of ACh in the GPe. Interestingly, it had diverse roles: pre- and post-synaptic. In vivo, GPe neurons commonly exhibit tonic firing with pauses (Bugaysen et al. 2010; Goldberg and Bergman 2011). Excitatory inputs from the STN or inhibitory inputs from the striatum or GPe axon collaterals increase or decrease their basal firing, respectively, perhaps generating a temporal code that carries striatal population coded activity based on neuronal ensembles (Carrillo-Reid et al. 2008; Goldberg and...
Bergman 2011). When GABAergic transmission is blocked, firing rate is increased and becomes more regular (Goldberg and Bergman 2011; Jaeger and Kita 2011; Schwab et al. 2013). Here, we show that one physiological way to decrease inhibitory inputs to the GPe is the activation of muscarinic receptors: a suppression of inhibition will make GPe neurons less sensitive to incoming inputs. Some GPe neurons express muscarinic M₁-receptors postsynaptically, and the balance between pre- and post-synaptic actions is at the moment unknown.

Consistent with the above results, there is a heterogenous population of GPe neurons (Cooper and Stanford 2000; Mallet et al. 2012; Nambu and Llinas 1994) although most are immunoreactive to parvalbumin (Kita 2007; Mallet et al. 2012). And a single class of GPe neuron may display a whole variety of firing patterns (Deister et al. 2013; Goldberg and Bergmann 2011; Jaeger and Kita 2011). However, PV-expressing cells fire antiphase to STN neurons and preferentially target downstream basal ganglia nuclei. Neurons expressing preproenkephalin fire in-phase with STN neurons and preferentially project to the Str (Hoover and Marshall 2002; Mallet et al. 2012).

The present findings add to the evidence that supports heterogeneity in GPe neurons and synapses and posit a number of questions, as for example, whether STF- or STD-synapses preferentially innervate one or the other neuron type and whether metabotropic glutamate receptors co-exist in the same neurons with muscarinic receptors, or if they are located in different neuron classes. Finally, how dopaminergic, cholinergic and cannabimimetic modulations interact in normal and diseased subjects has not been investigated. Clearly, more research is needed to answer these questions.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
RH-M conducted whole cell patch recording experiments and primary data analysis. He developed the experimental design together with JJA, PER-O and JB. TH-F and OH-G conducted patch-clamp recordings in dissociated neurons. All authors took part in the evaluation of the experimental data. RH-M, JB and EG wrote the manuscript in interaction with all authors. All authors approved the final version.

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**Figure Captions**

Fig. 1. Short-term synaptic plasticity in GPe inhibitory synapses. A 20 Hz stimulus train with a field electrode located in the Str in slightly inclined parasagittal slices that include the Str and the GPe evoked IPSCs in postsynaptic recorded GPe neurons in all cases. A: Most IPSCs recorded exhibited short-term facilitation (STF-synapses) when filed stimulus was
delivered inside the Str. Average of \( n = 15 \) traces is denoted by a thick black trace and variation of responses in different trials is denoted by the thin grey traces in all panels. B: In one third of cases evoked IPSCs exhibited short-term depression (STD-synapses). C: When the stimulus was given inside the GPe while recording a GPe neuron in coronal slices, STD-synapses were also recorded. D: A single exponential function was reasonably well fitted to STF. E, F: A single exponential function was reasonably well fitted to STD. Time constants obtained with exponential fits in STD-synapses recorded in sagittal or coronal slices were not significantly different. STF-synapses were never recorded when the stimulating electrode was in the GPe.

Fig. 2. Presynaptic muscarinic modulation of STF- and STD-synapses.
A: Time course of muscarinic action on IPSCs evoked by stimulating a STF-synapse (in the presence of 10 \( \mu \)M CNQX and 50 \( \mu \)M APV). IPSCs are reduced in amplitude after 1 \( \mu \)M muscarine. Averaged amplitude ± SEM of first IPSC of the pair is plotted in the time course. Bar indicates time of muscarine application. Representative traces at right are pairs of responses evoked at different moments as indicated by numbers in the time course. Superimposed traces normalized to the first IPSC amplitude are also shown for comparison. B: Time course of muscarinic action on IPSCs evoked in an STD-synapse: IPSCs are also reduced in absolute amplitude by 1 \( \mu \)M muscarine. Traces at the right illustrate representative IPSCs taken at different moments during the time course as indicated by numbers. Bar indicates time of muscarine application. Superimposed traces normalized to the first IPSC show that synaptic depression turned to facilitation. C: Paired lines plot of paired pulse ratio (PPR =
second IPSC / first IPSC). PPR was increased significantly (**P < 0.01). D:
IPSCs absolute amplitudes were decreased in all cases of a sample of
inhibitory synapses (**P < 0.001). E: The coefficient of variation (CV) was
increased in most cases (**P < 0.001). F: Quantal content (CV^2) as a function
of IPSCs amplitude was similar before and after muscarine (NS).

Fig. 3. Muscarinic actions are mediated by M_1-class receptors in STF-
synapses. A: Time course of an IPSC from a STF-synapse before and during
muscarine (1 μM) into the bath saline. IPSC amplitude was reduced. Posterior
addition of 10 μM atropine blocked muscarine action and recovered IPSC
amplitude. Bars indicate time of drugs application. Each symbol represents the
mean ± SEM of 12 individual synaptic events. Inset at right illustrates
representative paired pulse responses obtained from the time course as
indicated by the numbers. B: Time course of an IPSC from a STF-synapse
when 1 μM pirenzepine is administered before muscarine. Pirenzepine had not
significant action by itself and addition of 1 μM muscarine had no action when
pirenzepine, a preferring M_1-class receptor antagonist, was present. Inset at
right illustrates representative responses to paired pulse stimulation taken from
the time course in B as indicated by the numbers. C: Paired line graph shows
percentage change of IPSC amplitude during muscarine (n = 6; ***P < 0.01).
Subsequent addition of atropine produce a significant recovery of IPSC
amplitude (n = 6; **P < 0.03). D: Paired line graph shows that muscarine had no
significant action in the presence of pirenzepine.
Fig. 4. Muscarinic presynaptic modulation differs in STF- and STD-synapses. 

A: Time course of IPSC amplitude before (1) and during (2) the addition of the CB₁-class receptor antagonist: 1 μM AM 251. The antagonist had no action by itself. Subsequent addition of 1 μM muscarine added in the presence of AM 251 had its usual action: reduction in IPSC amplitude. Thus, blockade of CB₁-receptors did not reduce muscarinic actions in STF-synapses. Inset at right shows representative paired responses taken from the time course as denoted by numbers. B: Summary of sample statistics (n = 10; ***P < 0.001 comparing AM 251 vs. AM 251 plus muscarine). C: Time course of IPSC amplitude from a STD-synapse before (1) and during (2) the addition of the CB₁-class receptor antagonist: 1 μM AM 251. The antagonist had no action by itself. However, the action of 1 μM muscarine was blocked in the presence of AM 251 (cf., Fig. 2B). Right: representative recordings from the time course. D: Sample statistics of STD-synapses show no significant muscarinic actions in the presence of AM 251.

Fig. 5. Muscarinic actions in STD-synapses are blocked by chelation of intracelullar calcium. A: Time course of IPSC absolute amplitude from a STD-synapse in the presence of 20 mM of intracellular BAPTA (1). Bars indicate the times of drugs applications. Action of muscarine was blocked after Ca²⁺ chelation (2). A subsequent addition of 10 μM WIN 55212-2 (3) decreased IPSC amplitude, suggesting that STD-synapses express functional CB₁-type receptors. The inset shows representative traces taken from the time course as denoted by numbers. B: A sample of STD-synapses tested in this way (n = 5; *P < 0.05) had the same behavior.
Fig. 6. Postsynaptic action of muscarine in some GPe neurons. A: Time course of Ca\(^{2+}\)-current modulation by 1 µM muscarine in an acutely dissociated GPe neuron. Modulation was reversible. B: Current-voltage relationships before and during muscarine (obtained with ramp commands, see: Perez-Burgos et al. 2008). C: Paired lines graph illustrate a sample of responsive cells (n = 6; *P < 0.05). D: 50 nM MT-7 blocked muscarine action (1 µM), bars indicate time of drugs application. However, when MT-7 was washed off the action of muscarine is readily revealed. E: Current-voltage relationships before and during muscarine in the presence or absence of MT-7. F: A sample of neurons treated with MT-7 and muscarine illustrate muscarinic actions only after MT-7 is washed (n = 9; ***P < 0.001 for Friedman ANOVA and *P < 0.05; for the pair MT-7 plus muscarine vs. muscarine alone after post hoc test). G: Most GPe neurons (about 2/3) did not respond to muscarine. H: I-V plots of non-responsive cells before and after muscarine application. I: Sample of non-responsive cells.
A. Short-term facilitation (STF)

B. Short-term depression (STD)

C. Short-term depression (STD)

D. $t = 150 \pm 30 \text{ ms}$

$\text{IPSC}_n / \text{IPSC}_1$

$T^2 = 0.97$

$n = 16$

E. $t = 83 \pm 11 \text{ ms}$

$\text{IPSC}_n / \text{IPSC}_1$

$T^2 = 0.98$

$n = 6$

F. $t = 67 \pm 12 \text{ ms}$

$\text{IPSC}_n / \text{IPSC}_1$

$T^2 = 0.97$

$n = 4$
A) STF-synapse

- **1 μM muscarine**

- Abs. IPSC amplitude (pA)

  - Time (min): 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55

  - Abs. IPSC amplitude (pA): 0, 50, 100, 150, 200, 250

B) STD-synapse

- **1 μM muscarine**

- Abs. IPSC amplitude (pA)

  - Time (min): 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65

  - Abs. IPSC amplitude (pA): 0, 20, 40, 60, 80, 100

C) PPR

- **control**, **muscarine**

- CV

- IPSC amplitude (pA)

D) Abs. IPSC amplitude (pA)

- **control**, **muscarine**

E) CV

- **control**, **muscarine**

F) CV^2

- IPSC amplitude (pA)

- **control**, **muscarine**
Hernandez-Martinez et al. Figure 3

A: 
STF-synapse

1 μM muscarine

+10 μM atropine

1 μM muscarine

abs. IPSC amplitude (pA)

B:

abs. IPSC amplitude (nA)

***

**

control + atropine

+ muscarine

+ pirenzepine

abs. IPSC amplitude (pA)

C:

STF-synapse

1 μM pirenzepine

+1 μM muscarine

1 μM pirenzepine

D:

abs. IPSC amplitude (nA)

0.6

0.4

0.2

0.1

0

control + muscarine

pirenzepine

+ muscarine

abs. IPSC amplitude (nA)

0.5

0.3

0.1
Figure 3

A) STF-synapse

- Control
- 1 μM AM251
- + 1 μM muscarine
- + 10 μM gabazine

B) Abs. IPSC amplitude (nA)

***

C) STD-synapse

- Control
- 1 μM AM251
- + 1 μM muscarine
- + 10 μM gabazine

D) Abs. IPSC amplitude (pA)

50 ms

100 pA

400 pA
Hernandez-Martinez et al. Figure 5.

A

STD-synapse

+10 μM gabazine

+10 μM WIN 55212-2

1 μM muscarine

w/ 20 mM intracel. BAPTA

abs. IPSC amplitude (pA)

time (min)

B

abs. IPSC amplitude (pA)

control

muscarine

+WIN55212.2

100 pA

50 ms

*