Different inhibitory inputs onto neostriatal projection neurons as revealed by field stimulation

Fatuel Tecuapetla, Luis Carrillo-Reid, Jaime N Guzmán, Elvira Galarraga and José Bargas

Depto. de Biofísica. Instituto de Fisiología Celular, UNAM PO Box, 70-253. México City, D.F. México. 04510.

Abbreviated Title: Neostriatal inhibition

Number of Figures = 4
Tables 1
Abstract = 222 words
Main text: 4045 words

Correspondence: José Bargas, Instituto de Fisiología Celular, Circuito exterior s/n (PO Box: 70-253 for ordinary mail) UNAM, Mexico City DF 04510, Mexico. Tel.: 525 55 5622 5670; FAX: 525 55 5622 5747 or 5607. (e-mail: jbargas@ifc.unam.mx).
Abstract

This work investigated if diverse properties could be ascribed to evoked inhibitory postsynaptic currents (IPSCs) recorded on rat neostriatal neurons when field stimulation was delivered at two different locations: the globus pallidus (GP) and the neostriatum (NS). Previous work stated that stimulation in the GP could antidromically excite projection axons from medium spiny neurons. This maneuver would predominantly activate the inhibitory synapses that interconnect spiny cells. In contrast, intrastriatal stimulation would preferentially activate inhibitory synapses provided by interneurons. The present work shows that, in fact, Intensity-Amplitude experiments are able to reveal different properties for IPSCs evoked from these two locations (GP and NS). In addition, while all IPSCs evoked from the GP were always sensitive to \( \omega \)-conotoxin GVIA (Ca\(^{2+} \)\textsubscript{V2.2} or N-channel blocker), half of the inhibition evoked from the NS exhibited little sensitivity to \( \omega \)-conotoxin GVIA. Characteristically, all \( \omega \)-conotoxin GVIA insensitive IPSCs exhibited strong paired pulse depression, whereas, \( \omega \)-conotoxin GVIA sensitive IPSCs evoked from either the GP or the NS could exhibit short-time depression or facilitation. \( \omega \)-agatoxin TK (Ca\(^{2+} \)\textsubscript{V2.1} or P/Q-channel blocker) blocked IPSCs evoked from both locations. Therefore: a) distinct inhibitory inputs onto projection neostriatal cells can be differentially stimulated with field electrodes. b) N-type Ca\(^{2+} \) channels are not equally expressed in inhibitory terminals activated in the NS. c) Synapses that interconnect spiny neurons use both N- and P/Q-type Ca\(^{2+} \) channels.
INTRODUCTION

The laminar architecture of cortical structures allows field stimulation of defined afferents or inhibitory interneurons (e.g., Miles et al. 1996; Shlosberg et al. 2003). This greatly facilitates pharmacological studies on afferent inputs. However, basal ganglia nuclei do not have a laminar structure, and there are problems to identify the origin of synaptic inputs during field stimulation studies.

Inhibition onto neostriatal GABAergic projection neurons comes from axon collaterals interconnecting these cells (Czubayko and Plenz 2002; Koos et al. 2002; Tunstall et al. 2002), and from several types of GABAergic interneurons (Kawaguchi et al. 1995; Kita 1993; Koos and Tepper 1999). It has recently been proposed that antidromic stimulation of spiny cells axons, from the globus pallidus (GP), can isolate the inhibitory component conveyed by the recurrent axon collaterals that interconnect spiny neurons (Guzman et al. 2003). In addition, several studies have posited that GABAergic interneurons are preferentially activated with field stimulation within the neostriatum (NS) (Jaeger et al. 1994; Kita 1993,1996; Koos and Tepper 1999; Tepper et al. 1998). Here we compared “Intensity-Amplitude” experiments obtained from IPSCs evoked from these two locations and show that, in fact, significant functional differences can be found for IPSCs evoked from either the GP or the NS.

Among the diverse functional properties that synaptic terminals may exhibit, some very important ones are the Ca\(^{2+}\) channels involved in transmitter release (e.g., Poncer et al. 1997, 2000; Takahashi and Momiyama 1993), and their short-term release dynamics (Gibson and Connors 2003; Macaferri and Lacaille, 2003; Poncer et al. 2000). In addition, interneurons may express
different pre and postsynaptic receptors (Freund 2003; Jonas et al. 2004; Koos and Tepper 2002; Poncer et al. 2000; Wu and Saggau, 1997). Thus, we questioned if these functional differences between afferent inputs can be made evident during field stimulation experiments. A preliminary report of this work has been published in abstract form (Tecuapetla et al. 2003).

METHODS

Preparation of slices and electrophysiological recordings

In the present study, acute neostriatal sagittal slices (300 µm thick) were prepared from postnatal days 12 to 14 (PD12-14), Wistar rats (27-28 g) from either sex, as previously described (see Guzmán et al. 2003). In principle we cannot discard that brains slices cut in other ways (e.g., horizontally) may also yield successful connections with the present protocols. The protocols followed the National University of Mexico (UNAM) and National Institutes of Health (NIH) guidelines for the use of animals in biomedical experiments.

Slices were continuously superfused (3-6 ml/min) with saline containing (in mM): 123 NaCl, 3 KCl, 1 MgCl₂, 2.0 CaCl₂, 25 NaHCO₃, and 11 glucose; aerated with 95% CO₂ - 5% O₂ to a final pH of 7.4 at room temperature (23-25°C). Neostriatal neurons were visually located with the help of a water immersion objective and an upright microscope (Diaphot, Nikon Inc. Melville NY) with an adapted CCD camera (CCD-100, Dage-MTI Inc. Michigan City IN) working with infrared illumination. Neurons were recorded using the whole-cell patch clamp technique in the voltage-clamp configuration. IPSCs recordings were carried out with Axoclamp 2A/2B (Axon Ins., Foster City CA) amplifiers and data were filtered at 1-3 KHz and digitized with an AT-MIO-6040E, a DAQ
(NI-DAQ) board (National Instruments, Austin TX) and a PC clone. On-line data acquisition used custom programs made in the LabVIEW environment (National Ins.). Standard patch pipettes (3-6 MΩ) were pulled on a Flaming/Brown puller (Sutter Instrument Corp. Novato CA). Pipettes were filled with saline containing high Cl (in mM): 72 KH$_2$PO$_4$, 36 KCl, 2 MgCl$_2$, 10 HEPES, 1.1 EGTA, 0.2 Na$_2$ATP, 0.2 Na$_3$GTP, 5 mM QX-314 and 0.1 % biocytin (pH=7.2, 275 mOsM/l). Experiments were abandoned if changes > 10% were encountered in access resistance.

Field stimulation was done via sharp (pencil shape) concentric bipolar tungsten electrodes (12 µm at the tip; 50 ± 8 kΩ d.c. resistance) (FHC Inc., Bowdoinham ME) attached to an isolation unit (Digitimer LTD, Hertfordshire UK) connected to the AT-MIO card. The electrode was positioned either in the globus pallidus (GP) (Fig. 1A) or in the neostriatum (NS) (Fig. 1B). Paired stimulus were delivered with 45-50 ms interstimuli interval, 0.2-0.4 ms duration, 1 - 4 V and 0.1 Hz. Distance between recording and stimulating electrode in all configurations was 0.5-1 mm. Experiments were done in the presence of the L-α-amino-3-hydroxy-5-methyl-isoxazolepropionate and 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine/kainate (AMPA/KA) antagonist 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline disodium salt (CNQX) (10 µM) and the N-methyl-D-aspartate (NMDA) antagonist D-(-)-2-amino-5 phosphonovaleric acid (AP5) (50 µM). Most traces shown are the average of 4 min recordings (24 traces) taken when the amplitude had been stabilized for a given condition.

Off-line analysis of digitized saved data used commercial graphing software (Origin v. 6. Microcal, Northampton MA). Peak IPSC amplitude was measured from basal line. Intensity-Amplitude (I-A) experiments were fitted to:
\[ A(I) = \frac{A_{\text{max}}}{1 + \exp(-k(I - I_h))} \quad (1) \]

where \( A_{\text{max}} \) denotes maximal amplitude of evoked IPSC, \( I \) denotes stimulus intensity normalized to threshold units, \( I_h \) denotes the stimulus intensity that evokes IPSCs of half maximal amplitude or \( A_h = 0.5A_{\text{max}} \), and \( k \) denotes a slope factor (proportional to the number of terminals recruited as a function of stimulus intensity). Statistics used distribution-free statistical procedures: Mann-Whitney’s U test.

Drugs were dissolved in the bath saline before applying them, using a gravity-driven superfusion system. All chemicals used in these experiments were obtained from Sigma (St. Louis) except for synthetic \( \omega \)-conotoxin GVIA (\( \omega \)-CgTx) (Alomone Labs., Jerusalem, Israel) and \( \omega \)-agatoxin TK (\( \omega \)-AgaTK) (Peptides International, Louisville KY).

**Histology**

Slices with a single biocytin filled neuron were fixed overnight in 4% paraformaldehyde and 1% picric acid in 0.1 M phosphate buffer saline (PBS; pH = 7.4). The slices were then infiltrated with 30% sucrose and cut on a vibratome into 40 \( \mu \)m sections. The sections were incubated 4 - 6 h in PBS solution containing 0.2 Triton-X-100 and avidin conjugated to Texas Red (12.5 \( \mu \)g/ml, Vector Laboratories, Burlingame, CA) (Horikawa and Amstrong 1988). Most neurons reported in this work were medium spiny neostriatal neurons. Of the three putative interneurons whose I-A plots were separated from those obtained from medium spiny cells, one was positively identified as an aspiny cell and other was defined as such due to its firing pattern (Fig. 2).
RESULTS

I-A experiments revealed distinct populations of GABAergic terminals depending on stimulus location

Intensity-Amplitude experiments (I-A plots) were performed on bicuculline sensitive IPSCs recorded on striatal spiny projection neurons (Fig. 1) and evoked with a predominant stimulation of spiny neurons axons (striofugal) from the GP (Fig. 1A; GP→NS) (Guzman et al. 2003) or by stimulation in the neostriatum (NS) (Fig. 1B; NS→NS). Glutamatergic transmission was blocked by 10 μM CNQX and 50 μM AP5 (Figs. 1C, D). All experiments were performed on the bicuculline sensitive component obtained by stimulating at each location (Figs. 1C, D)(Guzman et al. 2003). Each I-A experiment was fitted by a three parameter sigmoidal function (see METHODS): where $A_{max}$ denoted IPSC maximal amplitude, $I_h$ was the stimulus to obtain half $A_{max}$, and $k$ was the slope factor proportional to the recruitment of active synaptic sites as a function of stimulus strength (Figs. 1E, F). Thick grey lines (Figs. 1E, F) are the means of all functions from each sample. Note compactness, around their mean, of I-A plots obtained by stimulating in the GP while recording in spiny neurons (Fig. 1E); suggesting that most stimulated afferents shared the same properties, probably those of striofugal axons since these same characteristics were found in a previous study after injuring the intrinsic neurons of the GP and thus, the pallido-striatal fibers (Guzman et al. 2003). In contrast, intrastriatal stimulation in the NS while recording spiny neurons disclosed a larger diversity (Fig. 1F); likely reflecting a greater variability on the types of afferents stimulated in each
experiment: which probable belong to different types of interneurons and some axon collaterals. Function averages for each sample are superimposed in Figure 1H (GP→NS and NS→NS). All parameters of these I-A plots were significantly different (Table 1); suggesting that experiments stimulated different populations of inhibitory afferents, since both the stimulating electrode and its distance to the recording electrode were the same. Noticeably, besides exhibiting more dispersion, average \( A_{\text{max}} \) was significantly larger for IPSCs evoked from the NS (\( p < 0.01 \); Mann-Whitney’s U test) (Table 1). Also, while IPSCs evoked from the GP while recording spiny cells needed less than one threshold unit to reach amplitude saturation, IPSCs evoked from the NS while recording spiny cells needed from 1 - 5 threshold units to saturate; suggesting that diverse types of afferents could be recruited in each experiment (Erlanger and Gasser 1937). There was a significantly different average slope factor (\( p < 0.025 \); Table 1).

Although in most recordings the intracellular saline had QX-314 to avoid firing (see METHODS), this blocker was avoided in selected cases. I-A characteristics at each stimulation site were maintained in those conditions (data not shown). In one case without QX-314, a striatal interneuron was identified by its characteristic firing pattern (Fig. 2A). IPSCs recorded in this interneuron while stimulating in the GP (Fig. 2B) revealed an I-A profile (Fig. 2C; GP→NS(in)) strikingly different to those revealed by recording in spiny cells after either NS or GP stimulation; suggesting that we were isolating a third class of afferents; probably those of pallido-striatal afferents that preferentially target striatal interneurons (Kita and Kita 2001). In this and two other similar cases, \( A_{\text{max}} \) was as large as that obtained with stimulation in the NS (compared in Fig.
1H) but the slope factor was larger than that measured after GP stimulation while recording in spiny cells. $A_{\text{max}}$ was reached virtually with threshold stimulus (Fig. 2C, D), and increasing stimulus strength did not further increase IPSC amplitude (Fig. 2D), as though release were highly synchronized (Jonas et al. 2004). This proposal was supported by observing strong paired pulse depression (Fig. 2B); confirming a high release probability during the first shock. This type of depression was never observed on IPSCs evoked on spiny neurons when stimulating in the GP (Figs. 3A, B); probably indicating that different afferents predominate when recording spiny neurons or interneurons after GP stimulation (v.g., recurrent collaterals or pallido-striatal afferents, respectively). Two other cells had IPSCs with these characteristics.

Sensitivity to Ca\(^{2+}\) channel blockers

The next set of experiments attempted to investigate two properties that may differ in synaptic terminals: the Ca\(^{2+}\) channels that they use for transmitter release and their short-term dynamics. Thus, the sensitivity of GABAergic IPSCs recorded on spiny neurons to N-type and P/Q-type peptidic channel blockers, $\omega$-CgTx and $\omega$-AgaTK, respectively, was investigated with the paired pulse protocol (see METHODS). Figure 3A, insets 1-2, shows a representative case of IPSCs evoked with GP stimulation while recording in spiny neurons (GP→NS; see I-A profile in Fig. 1E): Time course of $\omega$-CgTx (1 $\mu$M) blockade showed that $\omega$-CgTx always decreased the amplitude of these IPSCs with a mean percent (mean ± s.e.m.) reduction of 64 ± 7 % (n = 8). Figure 3A shows responses to the paired pulse protocol, exhibiting paired pulse depression (PPD) in control conditions (Fig. 3A inset 1) that became paired pulse facilitation (PPF) during $\omega$-CgTx (Fig. 3A inset 2). Thus, blockage of N-type Ca\(^{2+}\) channels
acted presynaptically to decrease release probability in a synapse that could reverse their short-term release dynamics (Dunwindde and Hass 1985; Guzman et al. 2003).

In contrast, Figure 3B depicts a representative case for half the experiments evoking IPSCs on spiny neurons while stimulating into the NS (NS→NS; see I-A profile in Fig. 1F): ω-CgTx produced a significant smaller reduction with an average of 22 ± 5 % (n = 6; p < 0.005; Mann-Whitney U test between both samples). In these cases, with reduced ω-CgTx sensitivity, a strong PPD was always found in control conditions (n = 6; Fig. 3B inset 1). Slope factor $k$ was smaller than that obtained for IPSCs evoked with GP stimulation (GP→NS; compared in Table 1 and Fig. 1H). ω-CgTx did not reverse the release dynamics of these synapses (Fig. 3B inset 2). However, ω-AgaTK (400 nM) completely blocked these synapses. But even when these responses were at the brink of total blockage by ω-AgaTK, PPD was still their short-term dynamics (Fig. 3B, inset 3); suggesting a high release probability. However, besides the type of postsynaptic neuron recorded, differences between these responses (NS→NS recording on spiny cells; Fig. 3B) and those from the putative pallidostristrial pathway described above (GP→NS (in) recording on interneurons; Fig. 2) remained: e.g., $k$ value (Fig. 1H), but not $A_{max}$. Taken together, these results strongly suggested that ω-CgTx was acting on different terminals when the stimulus was delivered in either the GP or the NS while recording in spiny cells (cf. Figs 1A and 1B).

ω-AgaTK blocked inhibitory currents evoked from both sites (GP and NS), with a similar potency, and in all cases, when recording in spiny cells: 86 ± 5 % (n = 6) for IPSCs evoked from the NS (Fig. 3D) and 89 ± 7 % (n = 8) for
IPSCs evoked from the GP (Fig. 3C). For IPSCs evoked on spiny neurons while stimulating in the NS (NS→NS), control conditions revealed either facilitation (Fig. 3D inset 1) or depression (Fig. 3B inset 1) and both types of synapse were blocked by ω-AgaTK. This suggests that P/Q-type channels are important for transmitter release at most classes of neostriatal GABAergic terminals impinging on spiny cells. However, only those synapses that showed depression displayed little sensitivity to ω-CgTx (Fig. 3B). All IPSCs recorded in spiny cells, after activating NS→NS synapses that showed PPF or that could be turned into PPF, were sensitive ω-CgTx (Figs. 3E, F). In fact, some of them could be completely blocked by ω-CgTx (Fig. 3F). Mean reduction for this last group was 65 ± 6 % (n = 6; p < 0.01 with respect to NS→NS sample exhibiting little sensitivity but non significant with respect to IPSCs evoked from the GP while recording on spiny neurons). However, other differences with respect to those responses evoked from the GP while recording on spiny cells remained: i.e., k and Amax parameters. Results of toxin blockage are graphically summarized in Figure 4. Note that ω-CgTx divides the IPSCs evoked from the NS in two halves.

DISCUSSION

We have investigated the possibility of activating different classes of inhibitory inputs making contact with spiny projection neurons of the neostriatum with field stimulation. We based this investigation on the fact that dual recordings have shown that synapses from different origins may express different functional properties (Gibson and Connors, 2003; Jonas et al. 2004; Whittington and Traub 2003), and that available evidence suggests that this is
also true in the neostriatum (Kawaguchi et al 1995; Koos and Tepper 1999; Kubota and Kawaguchi 2000). Therefore, our working hypothesis posited that synapses with different functional properties could be observed when IPSCs were evoked with field stimulation, while recording in spiny cells. To achieve this end we did not expect to completely isolate distinct separate afferents, but to reasonably activate a predominant input such that properties as I-A profile, Ca$^{2+}$ channels used for release, and short-term release dynamics, could be clearly different under different circumstances.

Accordingly, inhibitory inputs with diverse properties were observed when recording from medium spiny neurons while the stimulating electrode was placed at two different locations, the GP and the NS.

First, GP stimulation evoked IPSCs with mean maximal amplitude ($A_{\text{max}}$) of about 150 pA and a recruiting factor ($k$) of about 10 pA per log threshold unit. The stimulus necessary to get an IPSC of half maximal amplitude ($I_h$) was very near threshold: 1.3 threshold units; suggesting that fibers and/or terminals with similar properties were being stimulated (Table 1). These IPSCs could exhibit short-term depression (PPD) or facilitation (PPF) and were sensitive to both $\omega$-CgTx and $\omega$-AgaTK indicating that both P/Q and N types Ca$^{2+}$ channels participate in transmitter release in this type of synapse.

Secondly, NS stimulation evoked two types of IPSCs. Both exhibited larger mean $A_{\text{max}}$ values than those evoked after GP stimulation: about 300 pA; suggesting that synaptic force is stronger for these synapses. However, the recruiting factor was smaller: 4 pA per log threshold unit, indicating higher anatomical dispersion of afferents around the stimulating electrode, afferents and terminals with different thresholds, and/or afferents with more diverse
conduction velocities. These data suggest that intrastriatal stimulation may activate a different class of input each time. Heterogeneity is expected as the product of different afferents arising from a diverse set of interneurons (e.g., Jonas et al. 2004; Whittington and Traub 2003). About two threshold units were necessary to reach half maximal amplitude. In addition, one type (ca., 50%) of these synapses always exhibited PPD; suggesting high release probability.

Concomitantly, these depressing synapses displayed little \(\omega\)-CgTx sensitivity and high \(\omega\)-AgaTK sensitivity; suggesting that P/Q-type Ca\(^{2+}\) channels are preferentially used for transmitter release. On the other hand, the other type of synapses (ca., 50%) activated by NS stimulation were sensitive to both \(\omega\)-CgTx and \(\omega\)-AgaTK, could exhibit PPD or PPF and thus, the only functional differences detected in this work between these synapses and those activated after GP stimulation were the parameters of the I-A plot: \(A_{max}\), \(k\) and \(I_h\).

The synapses activated by GP stimulation while recording on spiny neurons most probably correspond to recurrent inhibition between spiny neurons based on the following facts: a) in a previous work, the intrinsic neurons of the GP were destroyed, thus cutting the other possible afferent that could be stimulated at the GP, the pallido-striatal fibers. In that case (Guzman et al. 2003), GP stimulation, while recording spiny neurons, yielded IPSCs with very similar properties (I-A profile, PPF or PPD, half maximal amplitude about 70 pA –see \(A_n\) in Table 1) than those recorded in the present work. Short-term facilitation or depression have also been described after dual recordings between spiny cells (Czubayko and Plenz, 2002), b) GP stimulation may evoke antidromic action potentials in spiny neurons when QX-314 is not used in the recording pipette (Guzman et al. 2003), c) local glutamate application in the GP
does not enhance asynchronous IPSCs on medium spiny neurons indicating that pallido-striatal fibers impinging on medium spiny neurons are scarce (Guzman et al 2003), d) these synapses may use both N and P/Q type Ca\textsuperscript{2+} channels for transmitter release and medium spiny neurons posses both types of Ca\textsuperscript{2+} channels (Bargas et al. 1994). Other projection neurons also express N and P/Q type Ca\textsuperscript{2+} channels for transmitter release (Iwasaky et al. 2000; Takahashi and Momiyama 1993; Wu and Saggau, 1997), and e) IPSCs recorded on striatal interneurons during GP stimulation have functional properties different to those recorded in spiny neurons, i.e., larger $A_{\text{max}}$ and recruiting factor $k$, a stronger PPD, and saturation with a threshold stimulus; suggesting a much higher release probability. These latter properties were never seen when recording from spiny cells during GP stimulation. Taken together, the data indicate that the IPSCs evoked on spiny neurons during GP stimulation most probably arise from axons belonging to other spiny neurons; axons that are stimulated antidromically at the GP. Contamination from pallido-striatal fibers cannot be completely ruled out. However, it should be scarce since pallido-striatal fibers mainly innervate interneurons (Kita and Kita 2001) and recordings from interneurons during GP stimulation yielded IPSCs with very different properties. In addition, the present data indicate that inhibition from medium spiny neurons onto fast spiking (FS) interneurons may also be scarce.

Next, the main reasons that support the view that stimulation in the NS while recording spiny neurons is biased toward the activation of inhibitory afferents arising from interneurons (Jaeger et al. 1994; Kita 1993, 1996; Koos and Tepper 1999; Tepper et al. 1998) are enlisted: a) in the cortex and the neostriatum, contacts between principal cells decrease down to zero when
distance between pair recordings is larger than 250 µm (Hellwig 2000; Holmgren et al. 2003; Koos and Tepper 1999; Tunstall et al. 2002). However, contacts between interneurons and principal cells do not decrease significantly with this distance (Holmgren et al. 2003) in agreement with their more extended and arborized axonal fields. In the present work, distance between recording and stimulating electrodes was 0.5 to 1.0 mm during both GP and NS stimulation, b) Although the distance between recording and stimulating electrodes was the same during GP and NS stimulation, maximal amplitude reached by IPSCs evoked from the GP was smaller than that reached by IPSCs evoked from the NS (Kita 1993; Koos and Tepper 1999). This result correlates with the fact that there is a weaker inhibition when recording from pairs of spiny neurons as compared to interneuron-spiny pairs (Koos et al. 2002; Plenz 2003). In fact, interneurons leave more terminals, than spiny neurons, on spiny cells (Kubota and Kawaguchi 2000). Moreover, other I-A parameters were significantly different as the slope factor ($k$) (see above); suggesting that recruitment dynamics of terminals was different in each experiment when stimulation was made at the NS, c) the variety of interneurons in the telencephalon sometimes is divided into two genres: fast spiking (FS) and low threshold spiking (LTS; or non-fast spiking) (e.g., Gibson and Connors, 2003; Jonas et al. 2004; Macaferri and Lacaille 2003). A more complex subdivision underlies these two genres so that a continuous spectrum of properties between these two extremes has been posited (Macaferri and Lacaille 2003); perhaps disclosing an array of 18 or more interneuron subtypes. Nevertheless, both genres (or functional extremes) have been well described in the neostriatum (Kawaguchi et al. 1995; Koos and Tepper 1999). Furthermore, they
have the same embryological origin as those in the cortex and the hippocampus (Marin et al. 2000), d) while some interneurons exhibit strong short-term depression others exhibit short-term facilitation (Gibson and Connors, 2003). Both types of responses were present after NS stimulation while recording in spiny cells, e) half of IPSCs evoked on spiny neurons during NS stimulation exhibited little sensitivity to the N-type Ca\(^{2+}\) channel blocker \(\omega\)-CgTx. These same IPSCs also exhibited strong short-term depression suggesting high release probability. Both these characteristics belong to synapses arising from hippocampal and cortical FS interneurons (Gibson and Connors, 2003; Jonas et al. 2004; Poncer et al. 1997, 2000). This same class of interneuron accounts for about half of cases of intrastriatally evoked inhibition (Kita 1993) and dual recordings between pairs of FS interneurons and spiny cells reveal that their synapses have a high release probability (Koos and Tepper 1999). In contrast, inhibition evoked from the GP was always sensitive to \(\omega\)-CgTx, could express synaptic facilitation and never displayed strong PPD (Guzman et al. 2003).

In the cases where NS stimulation evoked IPSCs that could present both short-term facilitation and \(\omega\)-CgTx sensitivity we could not rule out a contamination with synapses belonging to recurrent collaterals of spiny cells. However, a mean \(A_{\text{max}}\) that doubled that obtained after GP stimulation suggested that at least a fraction of these synapses belonged to the other interneuron genre (v.g., LTS interneurons) (Gibson and Connors, 2003; Jonas et al. 2004; Kawaguchi et al 1995; Koos and Tepper 1999; Kubota and Kawaguchi 2000; Poncer 2000). More importantly, the present experiments have shown that functional properties may differ between IPSCs evoked with
field stimulation, so that other properties could be defined in the near future to
differentiate between LTS and spiny recurrent synapses.

To conclude, the data suggest that, at least, in more than 50% of the
cases, stimulation in the NS is able to predominantly activate inhibitory inputs
from interneurons making synaptic contacts on spiny cells. Since field
stimulation can be used to functionally isolate single terminals (e.g., Hanse and
Gustafsson 2001; note that this cannot be done with dual recordings since a
single action potential in the presynaptic neuron will tend to activate all available
contacts), and since these terminals may reflect the release dynamics of their
companions, then, a complete characterization of these synaptic properties may
help, in future studies, to perform pharmacological analysis on single boutons.

On the other hand, the data also suggest that the pallidostriatatal pathway
may also be accessible for pharmacological studies using field stimulation from
the GP while recording neostriatal interneurons.

Finally, the present experiments demonstrated that \(\omega\)-Aga TK is the most
potent blocker for most inhibitory synapses in the neostriatum. Both in synapses
belonging to axon collaterals of spiny cells and in half of the synapses activated
within the NS, \(\omega\)-CgTx and \(\omega\)-AgaTK blockage present the phenomenon known
as “supperadditivity” (see Wu and Saggau, 1997); which is present in many
other CNS synapses.
Acknowledgments: The authors thank technical support to Dagoberto Tapia. This work was supported by the following grants: DGAPA-UNAM (Mexico) IN201603 IN219304 (JB) and IN200803 IN205804 (EG), CONACyT (Mexico) grants: 31839 (JB), 42636 (EG) and The Millenium Research Initiative: W-8072/35806 (JB & EG).

REFERENCES


Figure Legends

**Figure 1. Intensity-amplitude (I-A) plots.**

**A.** Illustrates the experimental protocol to evoke IPSCs from the globus pallidus (GP) by antidromic stimulation of striofugal axons from spiny cells. All graphs and records below this scheme (C, E, G), correspond to GP stimulation while recording in medium spiny cells.

**B.** Illustrates the experimental protocol to evoke IPSCs with stimulation into the neostriatum (NS). D and F show records and graphs after NS stimulation while recording in medium spiny cells. Protocol schemes depict a cortical pyramidal cell (light grey pyramid) that makes contacts with spiny neurons (3 black circles) and interneurons (dark grey star like). A pyramidal axon runs through the GP.

**C, D.** According to schemes in A, B, evoked IPSCs from both locations have glutamatergic (blocked with 10 μM CNQX and 50 μM AP5 in the present experiments) and GABAergic (blocked with 10 μM bicuculline) components. **E, F.** I-A plots obtained from IPSCs recorded in spiny cells and evoked stimulating their axons at the GP (E) or evoked within the neostriatum (F). Each experiment was fitted with equation 1 (H). Fig. 1E inset shows a representative experiment. Thick grey functions in E and F are sample averages I-A plots also graphed in Fig. H. Mean parameters from I-A plots are summarized in Table 1. **G.** Illustrates the I-V plot obtained from a representative IPSC evoked from the GP. Reversal potential = -29 mV (n = 13) was near $E_{Cl} = -30.5$ mV with the present intra and extracellular Cl concentrations. **H.** Depicts average I-A plots obtained from IPSCs evoked from the GP while recording a spiny cell (GP $\rightarrow$ NS), from IPSCs evoked from the NS while recording a spiny cell (NS $\rightarrow$ NS) and an I-A
plot obtained from IPSCs evoked from the GP while recording an interneuron (see Figure 2; GP→NS (in)) for comparison.

**Figure 2. IPSCs recorded in neostriatal interneurons during GP stimulation.** A. This neuron was recorded without QX-314 to observe its firing pattern before performing an I-A experiment on evoked IPSCs. Note that it corresponds to a fast spiking (FS) interneuron (responses to three different intracellular rectangular current steps). B. IPSCs evoked on this interneuron while stimulating in the GP. The paired pulse protocol was used to observe release dynamics. Two traces correspond to threshold stimulus and one to 1.5 X threshold intensity. Note large amplitude and strong paired pulse depression (cf., Fig. 3C or Fig. 3D) never observed when recording from spiny cells during GP stimulation. C. I-A plot for IPSCs shown in B. Note that saturating amplitude is reached with near threshold stimuli, suggesting highly synchronized terminals (high release probability). D. Time course of the experiment showing that strong inhibition can be maintained at low stimulating frequencies. Also note that suprathreshold stimuli does not increase mean IPSC amplitude. This was never seen after NS stimulation even on strong depressing synapses.

**Figure 3. Sensitivity to peptidic Ca²⁺ channel blockers and short-term dynamics.** A. Time course of ω-CgTx (1µM) blockage on the amplitude of IPSCs evoked from the GP while recording a spiny cell. Note change in short-term dynamics from paired pulse depression (PPD) in the control (inset 1) to paired pulse facilitation (PPF) after ω-CgTx (inset 2). Superimpositions of records is at the bottom in this and all cases B. Half of IPSCs evoked
intrastriatally exhibited a minor sensitivity to $\omega$-CgTx (Table 1). In all these cases, PPD was the characteristic short-term dynamics (insets 1 and 2). $\omega$-AgaTK blocked these depressing synapses. However, even at the brink of total blockage, PPD (reduced) remained as the characteristic release dynamics (inset 3). C. Time course showing the effects of $\omega$-AgaTK (400 nM) on the amplitude of IPSCs evoked from the GP while recording in a spiny cell. Note PPF (inset 2) when $\omega$-AgaTK had blocked part of the response. D. In all intrastriatally evoked IPSCs $\omega$-AgaTK was a powerful blocker. Note in this case, strong PPF since the control (inset 1). $\omega$-AgaTK had an effect no matter the short-term dynamics. E. In half of the cases $\omega$-CgTx also blocked intrastriatally evoked IPSCs. Most cases could not be distinguished from IPSCs evoked from the GP, except for the I-A profile (Fig. 1H). F. However, when $\omega$-CgTx sensitivity was high, it coincided with a large PPF in control conditions (inset 1).

Figure 4. Box plots illustrating the sensitivities to the peptidic Ca$^{2+}$ channel blockers. Note that $\omega$-CgTx divides the sample of intrastriatally evoked IPSC in two halves. The half with little $\omega$-CgTx sensitivity always exhibited PPD.
Figure 2

A

B

C

D

GP → NS (in)

\[ A_{max} = 333 \text{ pA} \]

\[ I_0 = 1.1 \text{ tu} \]

\[ k = 33 \text{ pA/tu} \]

1.5x 2x

0.6x 1x

Threshold units

IPSC amplitude (pA)

min
Figure 3

A. GP → NS

ω-CgTx

B. NS → NS

ω-Aga TK

C. GP → NS

ω-Aga TK

D. NS → NS

ω-Aga TK

E. NS → NS

ω-CgTx

F. NS → NS

ω-CgTx
Tecuapetla et al. Figure 4

A

ω-conotoxin GVIA

(% inhibition)

GP → NS
(axon collaterals)

NS → NS
(interneurons)

B

ω-agatoxin TK

(% inhibition)

GP → NS
(axon collaterals)

NS → NS
(interneurons)
<table>
<thead>
<tr>
<th></th>
<th>GP→NS (axon collaterals) n=6</th>
<th>NS→NS (interneurons) n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amax</strong></td>
<td>151 ± 11 (0.171) 157 (105 - 174) (pA)</td>
<td>298 ± 42 (0.419) 287 (145 - 494) (pA)</td>
</tr>
<tr>
<td><strong>k</strong></td>
<td>10.3 ± 3 7.7 (4.4 - 22) (pA / threshold units)</td>
<td>3.7 ± 0.7 3.8 (1.1 - 7) (pA / threshold units)</td>
</tr>
<tr>
<td><strong>I_h</strong></td>
<td>1.3 ± 0.07 (0.135) 1.3 (1.1 - 1.5) (threshold units)</td>
<td>2.1 ± 0.23 (0.334) 1.8 (1.3 - 3.4) (threshold units)</td>
</tr>
<tr>
<td><strong>A_h</strong></td>
<td>72 ± 4.2 70 (58 – 86) (pA)</td>
<td>149 ± 21 147 (74 – 250) (pA)</td>
</tr>
</tbody>
</table>

Table 1. Intensity-Amplitude parameters for field evoked inhibition recorded on spiny cells. I-A plots were fitted by the equation:

\[ A(I) = \frac{A_{max}}{1 + \exp(-k(I - I_{h}))} \]

\( A_{max} \) denotes maximal amplitude of evoked IPSC in pA, \( k \) denotes a slope factor (pA/stimulus), \( I_{h} \) denotes stimulus intensity, in threshold units, necessary to obtain \( A_{h} \) which denotes IPSCs with half the maximal amplitude. First column corresponds to parameters obtained with stimulation in the GP and second column corresponds to stimulation in the NS. Cells, from top to bottom, have mean ± s.e.m and median followed by range in parenthesis (*p < 0.01; **p < 0.025; ***p< 0.004). Coefficient of variation, in parenthesis, is included in \( A_{max} \) and \( I_{h} \).