Different Inhibitory Inputs Onto Neostriatal Projection Neurons as Revealed by Field Stimulation

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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 from axon collaterals interconnecting these cells (Czubayko and Plenz 2002; Koos et al. 2004; 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 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” (I-A) experiments obtained from inhibitory postsynaptic currents (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 Ca2+ 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 this study, acute neostriatal sagittal slices (300 μm thick) were prepared from postnatal day (PD) 12–14 Wistar rats (27–28 g) from either sex, as previously described (see Guzman et al. 2003). In principle, we cannot discard that brain 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 and National Institutes of Health 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, Melville, NY) with an adapted CCD camera (CCD-100, Dage-MTI, 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 Instruments, 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 Instruments). Standard patch pipettes (3–6 MΩ) were.

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RESULTS

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

I-A experiments were performed on bicuculline-sensitive IPSCs recorded on striatal spiny projection neurons (Fig. 1) and evoked with a predominant stimulation of spiny neurons axons (stroiofugal) from the GP (Fig. 1A; GP→NS) (Guzman et al. 2003) or by stimulation in the NS (Fig. 1B; NS→NS). Glutamatergic transmission was blocked by 10 μM CNQX and 50 μM AP5 (Fig. 1, C and D). All experiments were performed on the bicuculline-sensitive component obtained by stimulating at each location (Fig. 1, C and D) (Guzman et al. 2003). Each I-A experiment was fitted by a three parameter sigmoidal function (see METHODS), where $A_{\text{max}}$ denoted IPSC maximal amplitude, $I_h$ was the stimulus to obtain half $A_{\text{max}}$, and $k$ was the slope factor proportional to the recruitment of active synaptic sites as a function of stimulus strength (Fig. 1, E and F). Thick gray lines (Fig. 1, E and 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 strio-fugal 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, intra-striatal 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 probably belong to different types of interneurons and some axon collaterals. Function averages for each sample are superimposed in Fig. 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, whereas 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 one to three 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 virtually reached with threshold stimulus (Fig. 2, C and 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 (Fig. 3, C or D) 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. tu, threshold units.

### TABLE 1. I-A parameters for field-evoked inhibition recorded on spiny cells

<table>
<thead>
<tr>
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<th>GP→NS (Axon Collaterals)</th>
<th>NS→NS (Interneurons)</th>
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<tr>
<td>( A_{\text{max}} )</td>
<td>( 151 \pm 11 ) (0.171)</td>
<td>( 298 \pm 42 ) (0.419)</td>
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<td></td>
<td>157 (105–174) (pA)</td>
<td>287 (145–494) (pA)</td>
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<tr>
<td>( k^* )</td>
<td>10.3 ± 3 (7.7–4.4)</td>
<td>3.7 ± 0.7 (3.8–1.1)</td>
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<tr>
<td></td>
<td>(pA/threshold units)</td>
<td>(pA/threshold units)</td>
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<tr>
<td>( I_0 )</td>
<td>1.3 ± 0.07 (1.15)</td>
<td>2.1 ± 0.23 (1.34)</td>
</tr>
<tr>
<td></td>
<td>1.3 (1.1–1.5) (threshold units)</td>
<td>1.8 (1.3–3.4) (threshold units)</td>
</tr>
<tr>
<td>( A_{\text{h}} )</td>
<td>72 ± 4.2 (58–86) (pA)</td>
<td>149 ± 21 (74–250) (pA)</td>
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Cells, from top to bottom, have mean ± SE and median followed by range in parenthesis *\( P < 0.01 \); †\( P < 0.025 \); ‡\( P < 0.004 \). I-A plots were fitted by the equation: \( A(I) = A_{\text{max}} / (1 + e^{-k(I - I_0)}) \), where \( A_{\text{max}} \) denotes maximal amplitude of evoked IPSC in pA, \( k \) denotes a slope factor (pA/stimulus), \( I_0 \) denotes stimulus intensity, in threshold units, necessary to obtain \( A_{\text{h}} \) 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. Coefficient of variation, in parenthesis, is included in \( A_{\text{max}} \) and \( I_0 \). I-A, intensity–amplitude; GP, globus pallidus; NS, neostriatum.
Sensitivity to \(\text{Ca}^{2+}\) channel blockers

The next set of experiments attempted to investigate two properties that may differ in synaptic terminals: the \(\text{Ca}^{2+}\) channels that they use for transmitter release and their short-term dynamics. Thus the sensitivity of GABAergic IPSCs recorded on spiny neurons to \(\text{N-type}\) and \(\text{P/Q-type}\) 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 reduction of 64 ± 7\% (SE; \(n = 8\)). 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 blockade of \(\text{N-type}\) \(\text{Ca}^{2+}\) channels acted presynaptically to decrease release probability in a synapse that could reverse their short-term release dynamics (Dunwiddie and Hass 1985; Guzman et al. 2003).

In contrast, Fig. 3B depicts a representative case for one-half the experiments evoking IPSCs on spiny neurons while stimulating into the NS (NS→NS; see I-A profile in Fig. 1F). \(\omega\)-CgTx produced a significant smaller reduction with an average of 5\% (\(n = 6\); \(P < 0.005\); Mann-Whitney \(U\) test between both samples). In these cases, with reduced \(\omega\)-CgTx sensitivity, a strong PPD was always found in control conditions (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). \(\omega\)-CgTx did not reverse the release dynamics of these synapses (Fig. 3B, inset 2). However, \(\omega\)-AgaTK (400 nM) completely blocked these synapses. However, even when these responses were at the brink of total blockage, PPD (reduced) remained as the characteristic short-term dynamics (insets 1 and 2). \(\omega\)-Aga toxin TK (\(\omega\)-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 \(\omega\)-Agatx (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 one-half of cases, \(\omega\)-CgTx also blocked intrastriatally evoked IPSCs. Most cases could not be distinguished from IPSCs evoked from the GP, except for I-A profile (Fig. 1H). F: however, when \(\omega\)-CgTx sensitivity was high, it coincided with a large PPF in control conditions (inset 1).
delivered in either the GP or the NS while recording in spiny cells (cf. Fig. 1, A and B).

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 (Fig. 3E and 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 nonsignificant 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 Fig. 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 so that properties such as I-A profile, Ca2+ 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 (Amax) 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 (Ik) 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 ω-CgTx and ω-AgaTK, indicating that both P/Q and N types Ca2+ channels participate in transmitter release in this type of synapse.

Second, NS stimulation evoked two types of IPSCs. Both exhibited larger mean Amax 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 \(\Pi\)Q-type \(\mathrm{Ca}^{2+}\) 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 and could exhibit PPD or PPF; 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: \(A_{\text{max}}\), \(k\), and \(l_h\).

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. 1) 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 \(A_{\text{h}}\), 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). 2) GP stimulation may evoke antidromic action potentials in spiny neurons when QX-314 is not used in the recording pipette (Guzman et al. 2003). 3) 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). 4) These synapses may use both N and \(\Pi\)Q type \(\mathrm{Ca}^{2+}\) channels for transmitter release, and medium spiny neurons possess both types of \(\mathrm{Ca}^{2+}\) channels (Bargas et al. 1994). Other projection neurons also express N and \(\Pi\)Q type \(\mathrm{Ca}^{2+}\) channels for transmitter release (Iwasaky et al. 2000; Takahashi and Moriyama 1993; Wu and Saggau 1997). 5) 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, they 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, these 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 as follows. 1) In the cortex and the neostriatum, contacts between principal cells decrease down to zero when distance between pair recordings is \(>250\, \mu\text{m}\) (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 this work, distance between recording and stimulating electrodes was 0.5–1.0 mm during both GP and NS stimulation. 2) 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 compared with interneuron-spiny pairs (Koos et al. 2004; 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 \(k\), suggesting that recruitment dynamics of terminals was different in each experiment when stimulation was made at the NS. 3) The variety of interneurons in the telencephalon sometimes is divided into two genres: FS and low threshold spiking (LTS; or nonfast 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). 4) 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. 5) One-half of IPSCs evoked on spiny neurons during NS stimulation exhibited little sensitivity to the N-type \(\mathrm{Ca}^{2+}\) 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 one-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 (e.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, these 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 \(>50\%\) 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, a complete characterization of these synaptic properties may help to perform pharmacological analysis on single boutons.

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

Finally, these experiments showed that ω-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 one-half of the synapses activated within the NS, ω-CgTx and ω-AgaTK blockade present the phenomenon known as “supaperadditivity” (see Wu and Saggau 1997), which is present in many other CNS synapses.

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