Direct Physiological Evidence for Synaptic Connectivity Between Medium-Sized Spiny Neurons in Rat Nucleus Accumbens In Situ

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Taverna, Stefano, Yvette C. van Dongen, Henk J. Groenewegen, and Cyriel M.A. Pennartz. Direct physiological evidence for synaptic connectivity between medium-sized spiny neurons in rat nucleus accumbens in situ. J Neurophysiol 91: 1111–1121, 2004. First published October 29, 2003; 10.1152/jn.00892.2003. Dual whole cell patch-clamp recordings in rat nucleus accumbens, the main component of the ventral striatum, were made to assess the presence of synaptic interconnections between medium-sized spiny neurons, a group of GABAergic and peptidergic neurons that constitute the principal cells of the striatum. Neurons were stained with biocytin for subsequent morphological analysis. Electrical activity of cells was recorded in current- and voltage-clamp mode; the characteristics of medium-sized spiny neurons were confirmed by electrophysiological and morphological properties. Thirteen of 38 medium-sized spiny neuron pairs (34%) showed a synaptic connection. In these pairs, suprathreshold stimulation with current injection evoked a train of action potentials in the presynaptic cell, which in turn elicited depolarizing postsynaptic potentials (dPSPs) in the postsynaptic cell. Twelve of these 13 pairs were connected unilaterally. The onset latency of the postsynaptic response was 1.7 ± 0.7 ms. dPSPs were blocked by 12.5 μM bicuculline, suggesting they were mediated by GABA_A receptors. A linear fit of the current-voltage relationship of GABAergic currents crossed the voltage axis near the value of −20 mV, in agreement with the Cl− equilibrium potential predicted from the composition of the artificial cerebrospinal fluid and pipette medium. No evidence for electrotonic coupling was found. Paired-pulse facilitation and depression were induced when the amplitude of the first IPSC of a pair was relatively small and large, respectively. No clear dependence of paired-pulse facilitation or depression was found on the width of the spike interval, which ranged between 100 and 380 ms. Conversely, 1- to 2-s trains of dPSPs showed marked frequency facilitation at low presynaptic frequencies, but frequency depression at high firing rates. These data show that intra-accumbens synaptic communication between medium-sized spiny neurons exists, is mediated by GABA_A receptors, and exhibits spike train–dependent short-term dynamics.

INTRODUCTION

The nucleus accumbens (Nac) constitutes the most extended part of the ventral striatum and occupies a central position within the meso-cortico-limbic system (Mogenson et al. 1980; Pennartz et al. 1994). It has been implicated in several behavioral and cognitive functions, such as translation of behaviorally relevant stimuli into behavioral responses (Cardinal et al. 2001), instrumental learning, and approach behavior (Baldwin et al. 2002; Balleine and Killcross 1994; Corbit et al. 2001; DiCiano et al. 2001; Hernandez et al. 2002; Parkinson et al. 2002). In general, the Nac can be considered a neuronal interface between sensory and associative brain areas processing and evaluating salient stimuli within their context and the motor and autonomous systems mediating responses to these stimuli (Mogenson et al. 1980; Pennartz et al. 1994).

Anatomical, electrophysiological and behavioral studies have disclosed many aspects of the functional organization of the Nac (Arts and Groenewegen 1992; Cardinal et al. 2002; Chang and Kitai 1986; Groenewegen et al. 1996, 1999; Kelley 1999; Meredith 1999; Pennartz and Kitai 1991; Pennartz et al. 1990, 1991, 1992, 1994, 1999; Sarter et al. 1999; Zahm 1999). The Nac forms an integral part of the striatum, and as in the caudate-putamen region (which forms the dorsal part of the striatum; Bolam et al. 2000; Kawaguchi et al. 1995), about 90–95% of the neuronal population of the Nac consists of medium-sized spiny neurons (MSNs), a class of GABAergic cells. In the Nac, MSNs receive extensive glutamatergic input from different limbic areas such as prefrontal cortex, thalamus, hippocampus, and basolateral amygdala, and project their output to downstream areas of the meso-cortico-limbic system like the ventral pallidum, the lateral hypothalamus, and mesencephalic dopaminergic areas (Chang and Kitai 1985; Groenewegen and Russchen 1984; Nauta et al. 1978).

In addition to the large majority of MSNs, interneurons compose the remaining 5–10% of ventral and dorsal striatal neurons, such as fast spiking interneurons (FS), low-threshold spike interneurons, and cholinergic interneurons (Bracci et al. 2003; Cowan et al. 1990; Hidaka and Totterdell 2001; Kawaguchi et al. 1995; Wilson et al. 1990). Despite our knowledge about these different cell types and their characteristics (Meredith et al. 1990; Pickel et al. 1988; Sesack and Pickel 1990), detailed information about intra-accumbens cellular interconnectivity is still lacking. It has long been known that axons of MSNs project not only outside the Nac but also branch profusely within it (Chang and Kitai 1986; Pennartz et al. 1991). However, it is not known whether MSNs in the Nac are in fact functionally connected to each other and to what extent.

Anatomical evidence supporting the presence of a relatively extended axonal collateralization of MSNs has been provided...
also in the dorsal striatum (Kawaguchi et al. 1990; Preston et al. 1980; Ramon y Cajal 1911; Somogyi et al. 1981; Wilson and Groves 1980; Yung et al. 1996). Together with these anatomical data, early electrophysiological studies suggested the existence of GABAergic, bicuculline-sensitive inhibition in the dorsal striatum in vivo (Bernardi et al. 1975; Katayama et al. 1981; Park et al. 1980) and in vitro (Lighthall and Kitai 1983; Misgeld et al. 1982). However, direct evidence of GABAergic inhibition between MSNs in striatal slices has been provided only recently. Contrary to an earlier study suggesting the absence of lateral inhibition (Jaeger et al. 1994), Tunstall et al. (2002) and Czubayko and Plenz (2002), using dual intracellular and whole cell recordings in rat slices and organotypic cultures, recently demonstrated the existence of bicuculline-sensitive GABAergic connectivity between pairs of MSNs in the dorsal striatum. In addition, FS interneurons also provide GABAergic inhibition onto MSNs in the dorsal striatum (Koos and Tepper 1999).

In the Nac, previous studies have demonstrated GABAergic transmission in MSNs by means of local or afferent electrical stimulation (Chang and Kitai 1986; Pennartz and Kitai 1991; Pennartz et al. 1991), but no demonstration of GABAergic lateral inhibition between pairs of ventral striatal MSNs has been provided so far. We do not consider it warranted to extrapolate the very recent evidence for lateral inhibition from the dorsal striatum to the ventral striatum, because several major physiological differences between these areas have been revealed, such as the presence versus lack of a dopamine receptor–mediated attenuation of fast excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) in the ventral and dorsal striatum, respectively (Nicola and Malenka 1998). Also, dopamine has been reported to gate corticostriatal long-term potentiation (LTP) and long-term depression (LTD) of glutamatergic EPSPs in the dorsal (Calebresi et al. 2000) but not ventral striatum (Pennartz et al. 1993). Thus the existence, extent, and functional properties of lateral inhibition in the ventral striatum are no trivial matters that can be simply extrapolated from findings in the dorsal striatum. Several model studies have indicated that lateral GABAergic inhibition in the striatum might be an important feature in selecting proper output patterns, as part of a coherent response to environmental stimuli encoded and evaluated by the prefrontal cortex and other glutamatergic areas afferent to the striatum. Since different MSNs may receive overlapping inputs from multiple common sources, this selection function has been proposed to be implemented by a “Winner-take-all” (WTA) strategy, which would ensure the detection of salience differences by rapidly solving conflict between competing stimuli, preventing distortion of proper outputs, and avoiding rapid switching between closely matched competitive states. These characteristics may endow the striatum with the attributes of a competitive network for pattern classification, subserving selection of behavioral strategies (Redgrave et al. 1999) and cognitive functions (Beiser and Houk 1998). Moreover, GABAergic inhibitory postsynaptic potentials may significantly delay the occurrence of postsynaptic action potentials, possibly causing disruption of the temporal relationship (coincidence detection) between excitatory synaptic inputs and backpropagating spikes that are thought to lie at the basis of LTD or LTP (Markram et al. 1997; Stuart and Hauser 2001; see also Plenz 2003).

Given these comparative and theoretical considerations, the general aim of this study was to assess whether lateral inhibition does exist in the Nac, and if so, to assess its electrophysiological properties. We show that MSNs in acutely prepared slices of rat Nac have a relatively high probability of being synaptically interconnected. This chemical neurotransmission is mediated by GABA_A receptors and shows forms of frequency facilitation and depression. No evidence for electrical coupling between MSNs was found. These findings advance our fundamental understanding of intra-accumbens communication and provide empirical constraints on biologically plausible network models of the basal ganglia.

**METHODS**

**Slice preparation**

Wistar rats (23–30 days of age) were anesthetized with an intraperitoneal injection of sodium pentobarbital sodium (60 mg/kg) and decapitated. Adequate measures were taken to minimize pain or discomfort of the animals. The experiments were carried out in accordance with the European Community Council Directive 86/609/EEC and approved by the National Committee on animal experiments.

Slices containing the Nac were prepared as described previously (Taverna and Pennartz 2003). Briefly, brains were removed from the skull, and 280-μm-thick coronal slices were cut in artificial cerebrospinal fluid (ACSF) at 4°C using a vibratome (VT1000S, Leica). The slices were allowed to recover for at least 1 h in ACSF at room temperature. Individual slices were submerged in a recording chamber in which ACSF was continuously flowing (1 ml/min) at 32°C. The composition of ACSF was as follows (in mM): 124 NaCl, 3.5 KCl, 1 NaH₂PO₄, 2.5 CaCl₂, 26 NaHCO₃, 1.3 MgSO₄, and 10 d-glucose, saturated with 95% O₂-5% O₂ (pH 7.3). NBQX (5 μM) was added to ACSF at the beginning of experiments.

Patch-clamp glass pipettes (5–7 MΩ) contained the following solution (in mM): 60 K-glucurate, 58 KCl, 10 HEPES, 0.5 EGTA, 1 MgCl₂, 2 Na₂-ATP, 0.3 Na₂-GTP, 20 Na₂-phosphocreatine, 0.1 leupeptin, and 26 biocytin (pH 7.2, adjusted with KOH). We chose this composition to obtain a relatively depolarized reversal potential for Cl⁻ ions (~20.8 mV at 32°C according to the Nernst equation) compared with the resting membrane potential and thus to enhance the amplitude of Cl⁻-mediated ionic currents evoked at resting level. A liquid junction potential of ~3 mV between intra- and extracellular solution was corrected for. At the end of recording sessions, slices were fixed in formalin and stored at +4°C.

**Electrophysiology**

Voltage- and current-clamp dual-cell recordings were made using a MultiClamp 700A amplifier (Axon Instruments). Signals were filtered at 1–3 kHz, digitized at 10 kHz, and stored on a computer using pClamp 8.0 software (Axon Instruments). Whole cell configurations were established in voltage-clamp (VC) mode simultaneously in two cells, chosen under visual control using an Axioskope upright microscope (Zeiss) equipped with Hoffman modulation contrast. The series resistance was compensated (40–60%), and electrode capacitance was canceled. We subsequently switched to current-clamp (CC) mode, and after bridge-balance compensation, assessed the intrinsic membrane properties of both cells by applying positive and negative rectangular current pulses (range: ±100/–400 pA, 2 s) that were also used to calculate the membrane input resistance (Table 1). The time constant was calculated by fitting the initial 150 ms of the membrane potential response to a small (20–50 pA) depolarizing current injection using a single exponential equation. Action potential threshold was measured at the point of clear-cut up rise of membrane potential.
(i.e., spike outbreak). Action potential amplitude was measured as the difference between peak and threshold values.

Synaptic connectivity between two cells was detected in CC mode by applying a rectangular current step (100–200 pA, 2 s) to one neuron to elicit a train of action potentials, while the response of the second neuron was recorded (Fig. 3). If a connection was present, presynaptic firing evoked a series of depolarizing postsynaptic potentials (dPSPs) in the second neuron. In paired-pulse experiments, we recorded the membrane current of the postsynaptic neuron in VC mode, whereas the presynaptic neuron was stimulated in CC mode with short current pulses (150 ms).

Notably, a minor amount of cross-talk was detected between the two input channels of the A/D interface (DigiData 1200, Axon Instruments). This was initially confused with electrical coupling between cell pairs due to the presence of putative gap junctions. Subthreshold current injections causing sizable tonic membrane depolarization (20–30 mV) in a given cell were reliably accompanied by small tonic postsynaptic passive membrane depolarizations in the other recorded cell, with an estimated coupling factor of approximately 0.007. Due to its small size, the coupling signal from the nonstimulated cell was not visible in individual traces, but could be revealed by averaging 50–100 traces. Simultaneous hyper- or depolarization is often accounted for by gap junction mediated electrical coupling (Czubayko and Plenz 2002; Galarreta and Hestrin 2001; Gibson et al. 1999; Koos and Tepper 1999; Tamas et al. 2000). However, we were unable to block the current-induced depolarization in the paired cell with the putative inhibitor of gap junctions octanol (1 mM). Furthermore, similar electrical coupling was found when two model cells were mounted in cell-mode configuration on the two headstage inputs of the amplifier. This latter effect was the ultimate hint for an electrical artifact due to cross-talk between input channels. Such artifact was detected in all recorded pairs, but did not interfere with the detection of synaptic transmission between cells.

Morphology

Following fixation, the 280-μm slices were cryoprotected by storage for 1 h at room temperature in a mixture of 20% glycerin and 2% dimethyl sulfoxide (DMSO) in aqua bi-dest or 0.1 M Na₂HPO₄·2H₂O/KH₂PO₄ [phosphate buffer (PB)], pH 7.4. Unless stated otherwise, all reagents used for morphological analysis were obtained from Merck (Darmstadt, Germany). The slices were rapidly frozen in 30% w/w sucrose in aqua bi-dest or PB onto the stage of a sliding microtome. The slices were then stored at −80°C, all reagents used for morphological analysis were obtained from Gibco (Invitrogen). Mounted sections were dehydrated through an ascending series of alcohol (50%, 70%, 80%, 2 times at 96%, and 2 times at 100%) and coverslipped from xylene using Entellan.

Analysis of pre- and postsynaptic signals recorded in CC and VC mode was accomplished using the statistical and fitting analysis tools of Clampfit 8.0 (Axon Instruments) and Origin 5.0 (Microcal Software).

Inhibitory postsynaptic current (IPSC) onset latency was measured as the time interval between the peak of the first derivative of the presynaptic action potential and the onset of the postsynaptic response. Peak amplitudes of postsynaptic events were measured by averaging values within a time window of 0.5–2 ms for IPSCs and 3–5 ms for dPSPs. Baseline references were taken by averaging values within a 2- to 5-ms time window. The decay phase of IPSCs could be fitted well with a single exponential time constant. A failure was defined as the lack of a postsynaptic event in response to a presynaptic action potential. In particular, a presynaptic current or potential was considered absent when its value, measured within a 20-ms time window starting 0.5 ms after the peak of the presynaptic action potential, failed crossing an empirically determined threshold value that was set as twice the standard deviation (SD) of the baseline mean value, measured within a 20-ms time window preceding the presynaptic action potential.

To quantify frequency potentiation and depression, postsynaptic CC traces were integrated across the first 300 ms departing from the onset of the 2-s lasting presynaptic pulse and across the final 300 ms before the offset of the presynaptic pulse. Averaged areas under the curve within the two time windows were plotted in histograms.

For paired pulse recordings, peak amplitude values of the first IPSC (IPSC₁) and second IPSC (IPSC₂) were measured and averaged across four different pairs. The ratio between averages for each interspike interval was calculated and plotted (Kim and Alger 2001). This method yields one single ratio value per interspike interval; thus no standard error (SE) was calculated. Failures were included in the analysis.

We also sorted IPSC₁s of each cell pair according to their amplitude and normalized them relative to the largest value (maximal normalized value was set to 1). Data were grouped into 0.1-sized bins, and across-pair averages (±SE) of normalized values were calculated within each bin and plotted against paired-pulse ratios (these were calculated by averaging IPSC₂ amplitudes within the same bin interval as for the normalized IPSC₁ and computing the ratio between mean IPSC₂ and mean IPSC₁). Note that, in Fig. 6, some bins remain empty, because no averaged value fell within those bins. The relationship between the normalized amplitude of IPSC₁ and paired-pulse ratios was fit with a hyperbolic function (see Debanne et al. 1996).

For statistical analysis, we used Wilcoxon’s matched-pairs signed-rank test, Student’s t-test, and one-way ANOVA (STATISTICA). Results are given as means ± SE.

Drugs

Bicuculline methochloride and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f][1]quinoxaline-7-sulfonamide disodium salt (NBQX) were obtained from Tocris Cookson. Biocytin was obtained from Sigma.

R E S U L T S

General properties of recorded neurons

Seventeen slices with electrophysiologically identified pairs of neurons were available. After immunohistochemical processing, 4 of these 17 slices failed to show any stained neuron, whereas in 9 slices, only one of the neurons of a pair could be anatomically visualized. In six of these nine slices, this neuron could be unequivocally identified as a medium-sized spiny cell. In the remaining four slices, both cells of a pair could be...
identified and characterized as MSNs. Two of these cell pairs exhibited extensive filling of both axons and dendrites (Fig. 1A). A few close appositions between axons of the stimulated neuron and dendrites or cell body of the responsive neuron were identified.

The basic electrophysiological properties of the cells included in this study are shown in Fig. 1B and summarized in Table 1. Most cells were characterized by relatively negative resting membrane potentials, inward rectification, high-input resistance, a slow depolarizing ramp at potential levels just below firing threshold, regular firing of action potentials when stimulated over threshold, and prominent spike afterhyperpolarizations (AHPs). Some neurons showed firing rate adaptation. These features are consistent with the characteristics commonly ascribed to MSNs (Kita et al. 1985; Pennartz et al. 1991) and their morphological properties as described above. Other cell types encountered during the recording sessions will be presented elsewhere.

We recorded a total of 38 pairs of MSNs, of which 13 (34.2%) were connected unidirectionally and 1 (2.6%) was connected bidirectionally (Table 1). We did not find significant differences between the intrinsic properties of connected versus unconnected MSNs (Table 2).

The estimated distance between somata of cells recorded simultaneously ranged between 2 and 50 μm. Within this range, no significant correlation between the intersomatic dis-

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<th>TABLE 1. Electrophysiological properties of medium-sized spiny neurons recorded in the nucleus accumbens</th>
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<tr>
<td>Connected (n = 26)</td>
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<tr>
<td>-------------------------------------</td>
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<tr>
<td>Non-connected (n = 48)</td>
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Values are means ± SE. The “Connected” group includes both presynaptic and postsynaptic cells. With “Non-connected,” we denote the group of paired recordings not revealing any connectivity. This, however, does not imply that the recorded cells were not connected to non-recorded cells. Note that action potential amplitude was quantified with reference to spike threshold level. Differences between values of each group were not statistically significant (P > 0.1, t-test for independent values). Rmp, resting membrane potential; Rin, input resistance; τ, membrane time constant; Ap, action potential.

FIG. 1. Basic morphological and electrophysiological properties of medium-sized spiny neurons (MSNs). A: photomicrograph of a pair of MSNs in the nucleus accumbens core. a: extended focus photomicrograph of 2 synaptically connected neurons. Cell body indicated with 1 belongs to the postsynaptic neuron; the one indicated with 2 belongs to the presynaptic neuron. Dendrites of neurons are densely packed with spines (inset c, part of the dendrite of cell 1). To distinguish dendrites and axon collaterals of the stimulated from the recorded neuron, dendritic arborizations and axon collaterals in 3 successive sections were traced using a 63× oil immersion objective and reconstructed with a Neurolucida system (MicroBrightField). This reconstruction yielded close appositions between axons of cell 1 and dendrites of cell 2 (arrowhead, inset b). In total, 3 possible contacts were identified between neuron 1 and neuron 2. B: Top: response of a different MSN to hyper- and depolarizing current steps (bottom traces). Negative membrane potentials are characterized by inward rectification. A ramp-like depolarization is visible at subthreshold level (arrow), whereas a train of action potentials is elicited by suprathreshold stimuli. Bottom: current-voltage curve obtained by plotting steady-state membrane potential values against amplitudes of injected current. Note the deviation from linearity (indicated by the dashed line) at hyperpolarized levels.
distance and the probability of finding a connected pair was detected. We often found no connection between neurons with somata very close or adjacent to each other, whereas connections were found in some cases between neurons placed approximately 50 μm apart.

Synaptic communication between MSNs: basic properties and GABAergic nature

Figure 2 shows an example of a dual-cell recording from two synaptically connected neurons. AMPA receptor–mediated synaptic transmission was blocked by 5 μM NBQX throughout all experiments to prevent spontaneous, AMPA receptor–mediated synaptic potentials that may interfere with evoked GABAergic synaptic potentials. In VC mode, unitary inward currents (IPSCs) followed presynaptic spikes closely in time, although not every spike elicited a response. The average IPSC peak amplitude was −31 ± 11 pA (range, −8 to −75 pA). Table 3 summarizes the properties of IPSCs evoked by a single presynaptic action potential. A failure was defined as a lack of a postsynaptic IPSC in response to a presynaptic action potential and was not included in the computation of other parameters.

In addition to single-spike evoked IPSCs, clear indications for synaptic connectivity were obtained from responses to spike trains. A train of depolarizing postsynaptic potentials (dPSPs) was evoked in one neuron by suprathreshold current injection into the other neuron. In all four pairs tested, the evoked synaptic response was completely and reversibly blocked by 12.5 μM bicuculline (Fig. 3), which indicates that dPSPs were mediated by GABA A receptors. The positive polarity of these synaptic potentials was due to the direction of the electromotive force on Cl− ions, determined by the difference between membrane resting potential and reversal potential (−83 and −20 mV, respectively).

The GABAergic nature of the postsynaptic response was further confirmed by dual recordings in which a single action potential was evoked in the presynaptic cell in CC mode, while the postsynaptic cell was voltage-clamped at different levels with steps of 10 mV, starting from −73 mV (n = 4 pairs, Fig. 4). A linear fit of the current-voltage relationship reversed at −22 mV, which is close to the expected value of the reversal potential for Cl− ions in our experimental conditions. These findings are consistent with the MSN-to-MSN synaptic response being an inhibitory, GABA A receptor–mediated postsynaptic current (IPSC) (cf. Chang and Kitai 1986; Czubayko and Plenz 2002; Misgeld et al. 1982; Pennartz and Kitai 1991; Tunstall et al. 2002).

TABLE 2. Summary of connectivity data in the recorded pairs

<table>
<thead>
<tr>
<th>Cell Pair Configuration</th>
<th>n</th>
<th>%</th>
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<tbody>
<tr>
<td>Connected</td>
<td>13/38</td>
<td>34.2</td>
</tr>
<tr>
<td>MSN → MSN (unilateral)</td>
<td>12</td>
<td>31.6</td>
</tr>
<tr>
<td>MSN ↔ MSN (bilateral)</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Non-connected</td>
<td>25/38</td>
<td>65.8</td>
</tr>
</tbody>
</table>

MSN, medium-sized spiny neuron.

TABLE 3. Properties of evoked unitary inhibitory postsynaptic currents

<table>
<thead>
<tr>
<th>Response parameter</th>
<th>Mean</th>
</tr>
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<tbody>
<tr>
<td>Amplitude (pA)</td>
<td>31 ± 11</td>
</tr>
<tr>
<td>Conductance (nS)</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>10–90% Rise time (ms)</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Decay (ms)</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Failure rate (%)</td>
<td>31 ± 3</td>
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</table>

Values are mean ± SE. Data are based on 6 cell pairs, yielding a total of 96 evoked inhibitory postsynaptic currents.

Fig. 2. Dual recordings revealed a synaptic connection between MSNs detected in voltage clamp mode. A: presynaptic action potential (bottom) evokes a fast unitary inward current (average of 10 sweeps, failures excluded) in a postsynaptic MSN. B: original superimposed traces from a different postsynaptic cell, showing both inward currents and failures.

Fig. 3. GABAergic nature of depolarizing postsynaptic potentials (dPSPs) evoked by presynaptic stimulation. A train of presynaptic action potentials elicited by current injection (bottom) induces depolarizing postsynaptic potentials that are fully and reversibly blocked by the GABA A receptor antagonist bicuculline (top).

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Frequency-dependent plasticity of postsynaptic dPSPs

We wondered whether intrastriatal GABAergic synapses show properties of short-term, activity-dependent plasticity, which could be revealed by differential patterns of presynaptic stimulation. Figure 5 illustrates short-term plastic properties of evoked dPSPs, studied with dual CC recordings. When the presynaptic cell was stimulated with relatively low amounts of current (+80 to +150 pA), its firing rate ranged between 8 and 15 Hz; at these rates, frequency facilitation (FF) of the postsynaptic response occurred (n = 9 of 11 pairs), as the amplitude of evoked dPSPs progressively augmented during the presynaptic spike train despite fluctuations in individual dPSP amplitude (Fig. 5A, left). This augmentation could not be attributed to frequency adaptation of the presynaptic spike trains, since in all connected pairs tested presynaptic cells fired trains of action potentials in a regular spiking or nonadapting fashion. Conversely, when presynaptic firing was evoked at higher rates (≥20 Hz) by means of larger current injections, the train of postsynaptic responses was markedly switched into frequency depression (FD; Fig. 5A, right). Intermediate firing rates (16–20 Hz) evoked trains of dPSPs having largely stable amplitudes. In 9 of 11 pairs tested with high presynaptic firing rates, the first dPSP was the largest in amplitude, followed by depression of dPSPs amplitude down to a relatively stable value within the first 200–300 ms (Fig. 5A). The amplitude of the first dPSP in ≥20-Hz trains was significantly larger than the one in 8- to 15-Hz and 16- to 20-Hz trains (6 ± 1, 4 ± 1, and 4 ± 1 mV, respectively; P < 0.05, Wilcoxon’s matched-pairs signed-rank test).

The time course of postsynaptic response amplitudes evoked by this high-frequency firing could be crudely approximated with a single exponential; the time constant of depression was 128 ± 19 ms. On a longer time scale, it was noted that the amplitude of the first dPSP, apparently boosted by a strong presynaptic current pulse, returned to control levels character-

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**FIG. 4.** Current-voltage relationship of postsynaptic GABAergic currents. 
A: unitary inhibitory postsynaptic currents (IPSCs) were evoked at different holding potentials by a single presynaptic spike (bottom). Polarity of the IPSC peak reversed at −23 mV. B: normalized peak amplitudes of unitary IPSCs were averaged across cell pairs (n = 4) and plotted against postsynaptic holding potentials. Data points were interpolated with a linear fit which crossed the x axis at −22 mV.

**FIG. 5.** Short-term plasticity of dPSP trains. A: left: a 2-s train of presynaptic spikes at 11 Hz evoked a train of dPSPs showing a gradual increase in amplitude. Right: at 20 Hz, the dPSP train of the same pair underwent rapid frequency depression until a steady state was reached. Note that the amplitude of the 1st dPSP is the largest in the train (inset). B: areas under the initial and final 300-ms portions of curves (A, horizontal bars) are plotted as histograms for both presynaptic firing rates. Differences between the values of 1st vs. last 300-ms trace portions are statistically significant (*P < 0.02, Wilcoxon’s matched-pairs signed-rank test).
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istic of low-frequency responses, indicating that no “memory”
effect was involved.

FF and FD were quantified by computing the integral values
for the first 300 ms and last 300 ms of the dPSP train response.
These were 192 ± 66 and 631 ± 292 ms · mV, respectively, for
presynaptic trains of 8–15 Hz (Fig. 5B, left: n = 9 pairs; FF
was significant at P < 0.02). For trains at ≥20 Hz, values were
549 ± 221 and 384 ± 168 ms · mV, respectively (Fig. 5B,
right: n = 9 pairs; FD was significant at P < 0.02).

When low-rate and high-rate stimulations were alternately
repeated, postsynaptic responses of FF and FD, respectively,
were also alternately evoked, showing that these phenomena
are specific to presynaptic firing rate and readily reversible.

**Paired-pulse recordings**

Responses to paired pulse stimuli are a useful tool to study
the dynamic properties of short-term plasticity. Characteristics
of paired-pulse response patterns are thought to reflect presyn-
aptic dynamics in particular (Katz and Miledi 1968; Zucker
1989). We further characterized intra-accumbens GABAergic
inhibition by determining whether an IPSC evoked by a pre-
synaptic spike was able to influence the amplitude of a subse-
quently IPSC induced by a second presynaptic spike, with time
intervals ranging between 120 and 380 ms from the first spike
in 20-ms steps. The ratio of the mean amplitude of IPSC2
relative to the mean amplitude of IPSC1 was plotted against the
interspike interval (see METHODS and Fig. 6A). The time course
did not reveal a clear dependence of paired-pulse facilitation or
depression on the interspike interval; no significant difference
was found when averages of IPSC1 and IPSC2 amplitudes were
compared for each time point (P > 0.05, paired r-test).

Large amplitude variability in the evoked responses may
have dampened clear paired-pulse effects as a function of
interspike interval. Thus other independent variables affecting
paired-pulse dynamics were examined. Paired-pulse depres-
sion (PPD) and facilitation (PPF) were induced depending on
whether the amplitude of IPSC1 was small or large, respect-
ively. This observation was quantified by plotting paired-pulse
ratios against mean normalized values of IPSC1, as described
in METHODS (Fig. 6B). Paired-pulse ratios relative to mean
normalized values of IPSC1 of 0.12 ± 0.04 and 0.9 ± 0.1 were
3.3 and 0.71, respectively. Averaged amplitudes of IPSC1s and
IPSC2s from different cell pairs (−6 ± 2 and −21 ± 5 pA,
respectively) were significantly different at the lowest mean
normalized IPSC1 tested (0.12 ± 0.04; P < 0.02). These data
suggest a major dependence of paired-pulse dynamics on the
amplitude of the first IPSC.

Lack of a consistent paired-pulse effect, other than due to the
amplitude of IPSC1, reflected the apparent randomness of the
relationship between pairs of dPSPs evoked by trains of pre-
synaptic action potentials (Fig. 5). To quantify this latter ob-
ervation, the ratio between the second and the first dPSP in a
train was calculated at two different presynaptic firing rates
(8–15 Hz and ≥20 Hz); as described above, these frequency
ranges induced FF and FD, respectively. Average paired-pulse
ratios were 0.94 ± 0.10 and 0.91 ± 0.10 at 8–15 Hz and ≥20
Hz, respectively (n = 9, P = 0.8, paired t-test). No significant
correlation between this measure of PPF and FF or between
PPD and FD was found (r = −0.22, P = 0.27, n = 26 trains);
for example, the second dPSP in a facilitating train at 8–15 Hz
could be smaller in amplitude than the first one, but facilitation
would still occur later during the train (Fig. 5A).

In summary, these results indicate that trains of GABAergic
dPSPs in the Nac show marked frequency-dependent facilita-
tion and depression and that paired-pulse effects are primarily
governed by the relative amplitude of IPSC1 rather than by
interspike intervals.

**Discussion**

The main result of our study is the demonstration that fast
GABA_A receptor–mediated synaptic interconnections between
MSNs are present in acutely prepared slices of Nac. MSNs
were recognized by their typical electrophysiological and mor-
phological properties: a prominent inward rectification at hy-
perpolarized membrane potentials, a ramp-like depolarization
at subthreshold levels, and regular or adapting firing of action
potentials when stimulated over threshold. Dendritic spines
and axonal collateralization were visible in photomicrographs
of electrophysiologically identified MSNs obtained after DAB
staining, although this morphological evidence was not ob-
tained for every recorded cell. Dual patch-clamp recordings
in the presence of the AMPA-receptor antagonist NBQX revealed
fast dPSPs in MSNs in response to spikes elicited in presyn-
aptic MSNs, with a relative incidence of about 34%, almost all
pairs being unidirectionally connected. Postsynaptic potentials
evoked by presynaptic action potentials had short latencies
(1.7 ± 0.7 ms), confirming a monosynaptic configuration, and
were fully blocked by bicuculline, suggesting they were me-
diated by GABA_A receptors. This was further confirmed by the
reversal potential of evoked currents lying around −20 mV,
which is the expected value for Cl− ions in our experimental
conditions. No evidence for electrotonic coupling between
MSNs was found.
Depending on the strength of presynaptic current injection setting the presynaptic firing rate, GABAAergic synaptic potentials showed different types of short-term plasticity: frequency facilitation (FF) at 8–15 Hz and frequency depression (FD) at ≥20 Hz. Paired-pulse facilitation or depression was detected when IPSC was relatively small or large, respectively. Dependence of paired-pulse dynamics on presynaptic spike interval was not apparent.

Besides being the first demonstration, to our knowledge, of GABAAergic lateral inhibition between MSNs in the Nac in situ, the data presented in this study extend similar results recently obtained in dorsal striatal neurons by means of double recordings (Czubayko and Plenz 2002; Tunstall et al. 2002; but see Jaeger et al. 1994). Tunstall et al. (2002) reported for the first time unitary postsynaptic GABAergic IPSPs induced by stimulation of single presynaptic striatal cells, with a 20% probability of finding a connected pair. This relatively low probability may be explained by a difference in postsynaptic signal-to-noise ratio relative to our study. Czubayko and Plenz (2002) showed GABAergic transmission between MSNs in cultured slices of the rat neostriatum, with an approximately 38% probability of finding connections. In the same study, GABAergic connections between pairs of MSNs in acutely prepared slices from rats aged 11 days were also reported, but with substantially lower probability (~13%). In culture, GABAergic transmission showed short-term plasticity properties, i.e., FF or FD at different presynaptic firing rates and paired pulse depression at interpulse intervals <100 ms.

In our results, we found some similarities as well as differences with the results of Czubayko and Plenz (2002). First, the approximately 34% probability of finding a connected pair in our study is comparable to the probability reported for cultured slices but higher than that found in acutely prepared slices of dorsal striatum in their study. It is possible that different experimental procedures account for this discrepancy between their study and ours, e.g., the difference in rat age (p11 vs. p23–28, respectively) and the recording temperature (~22°C vs. 31–33°C, respectively). In cultured slices of Czubayko and Plenz, in which cells were recorded at 35 ± 0.5°C, the probability of finding connected pairs was similar to ours, but it should be noted that the authors found a relatively high fraction of mutually interconnected MSNs (8/26, 30.7%), while we found only 1 reciprocally connected pair out of 13 (7.7%). Besides a possible difference between dorsal and ventral striatum that may explain this discrepancy, organotypic culturing may lead to re-patterning of axonal connections between neurons.

Furthermore, Czubayko and Plenz reported some pairs to be connected by electrical synapses, contrary to our study in which no electrical synapses between MSNs were detected. Importantly, we found that cross-talk between the two input channels of our analog/digital interface could be mistaken for electrical coupling among recorded cells (see Methods for details). Therefore our results suggest that gap junctions between MSNs are not present or occur with very low probability in the Nac in situ.

Altogether, these observations suggest that GABAergic transmission between MSNs of the ventral striatum shares certain properties with that in the dorsal striatum. Nonetheless, some differences with recent studies in the dorsal striatum are noted, which may reflect different experimental approaches or a genuine structural and physiological divergence between the two areas.

**Functional implications and mechanistic aspects**

GABAergic postsynaptic potentials shown in our results were depolarizing because we used a high intracellular Cl– concentration, which was useful to increase the amplitude of synaptic responses. Considering the average conductance of 0.6 nS obtained here, it is possible to estimate the amplitude of GABAergic IPSCs at a more physiological reversal potential for Cl– ions (E_Cl–). An estimate of E_Cl– (~71 mV) was obtained in a study using recording pipettes containing potassium methylsulphate (Pennartz and Kitai 1991).

Considering this value for E_Cl– and applying Ohm’s law I_Cl– = G_Cl– × (E − E_Cl–) (where I_Cl– is the chloride current, G_Cl– is the GABA_A receptor conductance, and E is the membrane potential), GABAergic IPSCs occurring at a membrane potential of ~80 mV would be ~5.4 pA. Assuming an input resistance of 100–200 MΩ, this yields a dPSP value of approximately 0.5–1.1 mV. At membrane potentials less negative than E_Cl–, such as those resembling the “up states” of MSNs (from ~60 to ~40 mV), GABAergic IPSCs will be outward, and corresponding PSPs will be hyperpolarizing, indicating that GABAergic IPSPs arising from lateral contacts may significantly counteract firing of action potentials. This is consistent with the common view that GABA_A receptors act to stabilize the membrane potential around the Cl– reversal potential and to lower the membrane resistance, yielding an overall inhibitory effect (Shepherd 1994). Nevertheless, our data do not rule out additional GABA_A effects that may be excitatory (cf. Plenz 2003).

What is the physiological role of frequency-dependent plasticity of intrastriatal inhibitory transmission, and which mechanisms of action underlie it? Answers to these questions are still to be given, but the presently available findings permit us to advance several ideas that can be tested by future experiments.

FF may be suitable for efficacious silencing of the postsynaptic cell, probably accompanied by a capacity to suppress simultaneous potentiation of glutamatergic synapses onto it. Indeed, GABA_A receptors have been shown to inhibit LTP induction of limbic inputs to Nac (Pennartz et al. 1993).

FD occurring at relatively high presynaptic rates (~20 Hz) reflected a negative feedback on dPSPs amplitude to a stable level after the first dPSP in the train had reached a large amplitude. Figure 5 illustrates that the dPSP evoked by the first action potential had a large amplitude and was followed by smaller dPSPs elicited by subsequent action potentials. Notably, the first two or three dPSPs of a train evoked by high presynaptic rates were significantly larger than the first dPSP of a train evoked by low or intermediate rates. We denote this phenomenon as the “first dPSP effect.” The mechanism underlying this phenomenon—which is logically independent from sequence-associated dPSP changes because it is already observed in the first dPSP—is presently unknown. However, an explanation must be sought in those parameters in the dual-cell configuration that differ between high- and low-frequency trains and precede the first dPSP. Thus it is reasonable to hypothesize that the rate of current-induced depolarization in the presynaptic cell and the first spike latency are involved.
such that short-latency firing during presynaptic depolarization results in a larger dPSP. Tentatively, one may speculate that an inhibitory (K⁺ or Cl⁻) current, which slowly activates on depolarization and affects vesicle release probability, is at play here. A possible candidate may be the persistent potassium current (I_{Kpa}), characterized by Nisenbaum et al. (1996) in neostriatal slices and dissociated neurons. If, indeed, the “first dPSP” effect described here would be mediated at the level of the presynaptic terminal, it may well affect the probability and amplitudes of subsequent dPSPs in the train, thus contributing to FD. As such, the effect may lead to the depletion of the releasable pools of synaptic vesicles (Betz 1970; Dobrunz and Stevens 1997; Stevens and Tsujimoto 1995). Other factors regulating FD in the Nac may comprise the action of GABA_B receptors, which are known to inhibit GABA_A receptor-mediated currents in Nac (Uchimura and North 1991) and dorsal striatum (Calabresi et al. 1991; Nisenbaum et al. 1993). Alternatively, desensitization of postsynaptic GABA_A receptors may occur (Jones and Westbrook 1995, 1996; Numann and Wong 1984).

Regardless of the precise mechanism of action involved in FD, we noted that FF could be restored by subsequent return to lower presynaptic rates, indicating that the induction of depression is reversible and not maintained by long-lasting mechanisms.

**Synaptic dynamics and Winner-take-all model**

Ventral striatal MSNs project their axons to extrastriatal structures like the ventral pallidum, substantia nigra pars reticulata and compacta, lateral hypothalamus, and ventral tegmental area, where they are thought to exert GABAergic synaptic inhibition (Groenewegen et al. 1993; Mogenson et al. 1983; Nauta et al. 1978; Walaas and Fonnum 1980; Yim and Mogenson 1980). According to anatomical results that revealed specific convergence of prefrontal cortex, hippocampus, amygdala, and thalamic projections to ventral striatal compartments and the maintenance of such topographic specificity in striatofugal projections (Groenewegen et al. 1999), it was postulated that the ventral striatum is organized into a mosaic-like pattern of neurochemically defined compartments or “ensembles” of cells processing functionally distinct patterns of information in a modular fashion (Pennartz et al. 1994). An immediate question arising from this hypothesis concerns the functional relationship between ensembles, as well as the role they may play in mediating aspects of cognition and behavior such as motivated approach behavior, instrumental conditioning, and responding for delayed rewards (Apicella et al. 1991; Cardinal et al. 2002; Hernandez et al. 2002; Kelley et al. 1997). In addition, dysfunctional communication between cells and cell groups in Nac has been proposed to contribute to pathological states such as schizophrenia (Swedlow and Koob 1987).

Although these findings address neither the definition nor the nature of ensembles, they are relevant to the question as to how ensembles or modules within the Nac interact, to ensure that behaviorally relevant stimuli exert an appropriate, well-timed effect on behavior.

The ensemble hypothesis of Nac function, which attributes a major role to lateral inhibition between cell groups, can be considered in the light of more generally applicable WTA models. The WTA models are designed to solve the problem of how a given neuron or population of neurons is selected among others for engagement in information processing tasks and mediating behavioral output (Fukai and Tanaka 1997; Redgrave et al. 1999). If different MSNs subserve integration of separate pieces of information supplied by the neocortex and other glutamatergic afferent areas, some form of selection is required to avoid incoherent or unstable processing and generation of an excess of potentially conflicting output patterns.

Lateral inhibition between MSNs provided by GABAergic synapses, as we have described in this paper, is a good candidate for a mechanism achieving such output selectivity (Groves 1983). Axonal collaterals of MSNs that spread within the Nac ensure fast GABAergic inhibition of nearby cells. Thus action potential firing of a given MSN engaged by particular cues or environmental conditions may be hypothesized to dampen concurrent, glutamate-induced depolarization of connected “competitor” cells or ensembles, ensuring sharp selection of output channels and restricted targeting of extra-accumbens areas by the axonal projecting branches.

Despite the clear demonstration of lateral inhibition in the Nac, a number of theoretically relevant questions remain to be answered. First, some of the original papers speculating on striatal lateral inhibition departed from the assumption of reciprocal GABAergic connections between MSNs (e.g., Groves 1983), and it is far from clear what consequences primarily unidirectional lateral inhibition would have for selection (WTA type) processes operating at the level of single neurons. If single MSNs are indeed organized in functionally distinct ensembles, it is less problematic to see how aggregates of individual GABAergic outputs from a given ensemble may translate into an overall “weighting factor” of lateral inhibition of that ensemble onto a competing ensemble characterized by its own reciprocal weighting factor.

Second, because the current study was limited to cells lying in each other’s immediate vicinity (up to approximately 50 μm), the spatial extent of lateral inhibition remains to be examined. Preliminary anatomical tracing findings indicate that recurrent axon collaterals of MSNs may extend up to several millimeters in the Nac (Y. Van Dongen and H. Groenewegen, unpublished observations).

GABAergic lateral inhibition between MSNs within the Nac is only one part of the complex synaptic organization of this brain area, since other neurotransmitters like glutamate, acetylcholine, and numerous peptides also exert important functions. However, due to the fact that these cells contribute a large majority in Nac, GABAergic lateral inhibition is likely to have a crucial impact on striatal signal integration, and consequently, on behavioral functioning.

**Conclusions**

We showed that lateral inhibition between MSNs in acute slices of rat nucleus accumbens exists, has a relatively high incidence (approximately 34%), and is mediated by GABA_A receptors. This form of intrastriatal communication is characterized by FF and FD at relatively low and high presynaptic firing rates, respectively.

These data support the hypothesis that lateral inhibition between MSNs may provide a fast and efficacious mechanism for selecting neuronal ensembles competitively involved in...
processing the heterogeneous flows of information that converge to the Nac from different areas of the meso-cortico-limbic system.

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