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Two Cell Types in Rat Substantia Nigra Zona Compacta Distinguished by Membrane Properties and the Actions of Dopamine and Opioids

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Intracellular recordings were made from 475 rat substantia nigra zona compacta neurons *in vitro*. The region from which recordings were made was rich in catecholamine fluorescence. Two groups of neuron, termed *principal neurons* (95% of the total) and *secondary neurons* (5% of the total) were clearly distinguishable according to one or more of the following 4 electrophysiological properties. *Secondary neurons* (23 cells) (1) fired spontaneous action potentials at frequencies greater than 10 Hz, or were quiescent (30%); (2) had action potentials less than 1 msec in duration; (3) did not show time-dependent inward rectification with step hyperpolarization; and (4) had slope conductances of about 4 nS (between −75 and −90 mV). In contrast, *principal neurons* (1) fired spontaneous action potentials in the range 1–8 Hz, or were quiescent (33%); (2) had action potentials greater than 1 msec in duration; (3) showed pronounced time-dependent inward rectification; and (4) had steady-state membrane slope conductances of around 22 nS (between −75 and −90 mV).

***Secondary cells* were not affected by dopamine but were hyperpolarized by baclofen, GABA, and the μ opioid receptor agonist Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGO). On the other hand, dopamine and baclofen inhibited firing and/or hyperpolarized all *principal* cells tested, but μ or δ opioid receptor agonists had no effect.**

The properties of these 2 cell types broadly correspond with those described by electrophysiological studies *in vivo*, in which case the majority, or *principal*, cells are believed to be dopaminergic. The present findings demonstrate that the dopamine-insensitive *secondary* cells of the substantia nigra zona compacta can be readily distinguished from the dopamine-sensitive *principal* cells on the basis of the electrical properties of their membranes and their sensitivity to opioids.

Neurons in the substantia nigra zona compacta that were inhibited by dopamine receptor agonists were first identified with extracellular single-unit recording *in vivo* (Aghajanian and Bunney, 1973; Bunney et al., 1973). These cells were subsequently considered to be dopamine-containing because cells showing their characteristic triphasic waveform were not seen in rats treated with 6-hydroxydopamine and because intracellular injection of such cells with L-dopa increased their content of catecholamine as evinced by subsequent fluorescence histochemistry (Grace and Bunney, 1980). Guyenet and Aghajanian (1978) additionally identified a second class of neuron in or immediately adjacent to the zona compacta, some of which, like the dopaminergic neurons, could be antidromically activated from the striatum. However, this second cell type fired at higher frequencies, had action potentials of shorter duration, did not respond to dopamine and was unaffected by injections of 6-hydroxydopamine into the medial forebrain bundle.

Extracellular recordings *in vitro* from zona compacta neurons with characteristics similar to the dopaminergic neurons described *in vivo* also showed that they were inhibited by dopamine (Pinnock, 1983). Using intracellular recordings *in vitro*, we have subsequently confirmed the involvement of D_2 dopamine receptors and have shown that this inhibition results from an increase in membrane potassium conductance (Lacey et al., 1987, 1988). During the course of those experiments, recordings were made from a small proportion of cells exhibiting characteristics different from the majority. The present paper reports further experiments in which the electrophysiological and pharmacological properties of this minority of neurons were examined; the properties of the 2 cell types are compared and contrasted.

Materials and Methods

The procedures used for the preparation and *in vitro* maintenance of slices of rat brain containing substantia nigra, as well as techniques for electrophysiological recording and for applying drugs, have been described previously (Lacey et al., 1987). In brief, a 300 μm coronal slice of adult rat mesencephalon containing the substantia nigra was positioned in a recording chamber, submerged in and continuously superfused by a physiological salt solution, buffered with $NaHCO_3$ (26 mM), saturated with 95% O_2 and 5% CO_2 and warmed to 36°C. The superfusion medium also contained (mM): NaCl, 126; KCl, 2.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.3; $CaCl_2$, 2.4; and glucose, 10. Intracellular recordings were made from 475 neurons using glass microelectrodes filled with KCl (2 M). The cells were located in the medial portion of the rostral substantia nigra zona compacta at the level of the medial terminal nucleus of the accessory optic tract. This region was visible *in situ* using a dissection microscope and illumination from above (Fig. 1*A*); following aldehyde fixation (Furness et al., 1977), it exhibited a dense catecholamine fluorescence (Fig. 1*B*).

The composition of the superfusate could be changed to one differing only in its content of drug or salts; there was a delay of 20–30 sec in the perfusion system. The following drugs were used: 4,4a,5,6,7,8,8a,9-octahydro-5-*n*-propyl-2H pyrazolo-3,4-g quinoline (quinpirole, Lilly),

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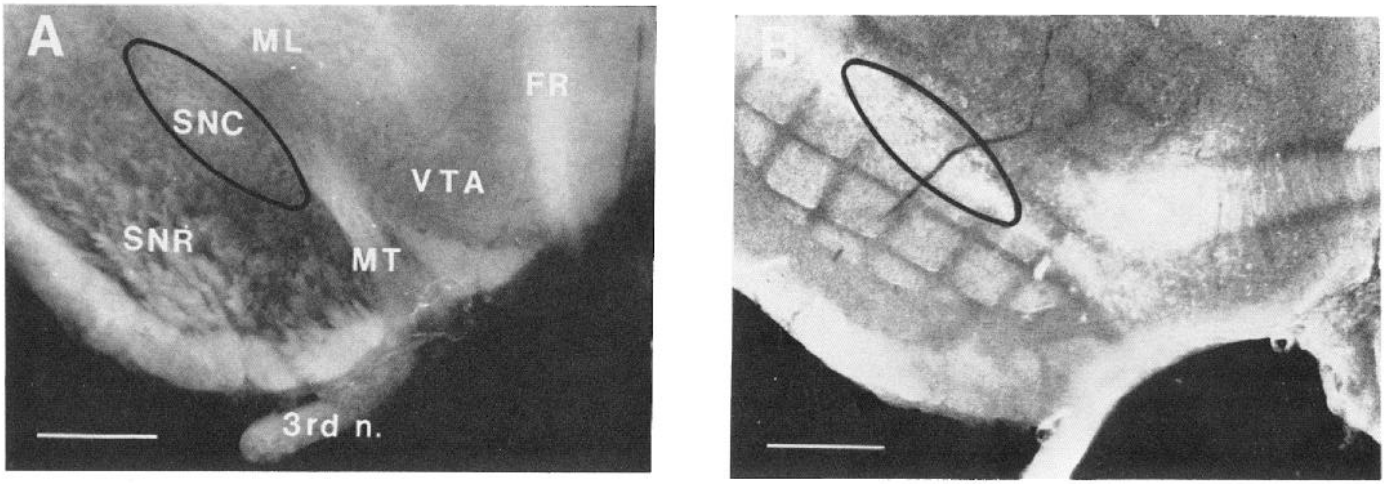

Figure 1. The region of substantia nigra from which recordings were made demonstrated dense catecholamine fluorescence. Photomicrographs of the ventrolateral quadrant of two 300 μm coronal slices of rat mesencephalon delineating the region from which recordings were made. *A*, Slice as seen *in situ* using incident illumination. *B*, Similar slice stained for catecholamines. The 2 distinct regions of fluorescence in *B* correspond to the dopamine-containing ventral tegmental area (A10 region, *VTA*) and the substantia nigra zona compacta (A9 region, *SNC*). The rectangular impressions on the slice in *B* were made by a titanium EM grid used to stabilize slices during recording. Identification of the region in which recordings were made was done by noting the position of the electrode with respect to the grid. In both plates the scale bar is 500 μm, the midline is approximately the righthand edge of the photograph, and the ventral surface of the brain is toward the lower edge. Other abbreviations: *SNR*, substantia nigra zona reticulata; *ML*, medial lemniscus; *FR*, fasciculus retroflexus; *MT*, medial terminal nucleus of the accessory optic tract; *3rd n.*, oculomotor nerve root.

(*p*-chlorophenyl)-γ-butyric acid (baclofen, Ciba-Geigy), trans-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzene-acetamide methanesulphonate (U50488, Upjohn), Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGO) and Tyr-D-Pen-Gly-Phe-D-Pen (DPDPE; both Peninsula), and methionine enkephalin (met-enkephalin), GABA, (−)-bicuculline methiodide, and dopamine (all from Sigma).

Numerical data are expressed as means ± SEM.

Results

The results are based on intracellular recordings of 0.3–9 hr duration from 475 substantia nigra zona compacta neurons. The effects of dopamine, baclofen, or GABA on 322 of these cells have previously been reported in detail (Lacey et al., 1987, 1988). The present paper describes other properties measured while recording from these 322 cells, as well as observations on an additional 153 neurons. An important result of the present experiments is that there exist 2 classes of neurons, which can be distinguished by a number of criteria; we therefore included in our numerical summary the properties of the *entire* population of cells to estimate more accurately the proportion of cells in each class.

Definition of cell types: action potential frequency and duration

The frequency distribution of spontaneous firing rate in 423 cells is shown in Figure 2; values ranged from 0 to 58 Hz, with all but 15 cells firing at less than 10 Hz. (These firing rates were measured after they had become stable following the microelectrode penetration; sometimes 10–30 min was required following impalement for regular spontaneous firing to become established.) This includes 84 cells that did not fire spontaneously during the impalement, and 57 cells that fired irregularly and sporadically at less than 1 Hz throughout the recording period. Passing depolarizing current through the electrode increased cell firing rate, but cells firing spontaneously at rates of 1–10 Hz were unable to produce sustained firing at rates greater than about 15 Hz. In contrast, those cells initially firing at rates greater than 10 Hz were able to sustain depolarization-induced firing rates of 50–70 Hz. Depolarization of the quiescent cells also evoked action potentials, although it was generally not possible to produce sustained regular firing for more than about 1 sec. Spontaneous action potentials were reversibly blocked by TTX (1 μM) whether the initial firing rate was greater or less than 10 Hz.

Action potentials in cells with spontaneous firing rates of less than 10 Hz were 54 ± 1.2 mV in amplitude (range, 41–62.5 mV) and 1.6 ± 0.1 msec in duration (range, 1.1–2.5 msec) in 28 cells (measured from start of the fastest rising phase to the peak of the depolarization or to the equipotential point on the falling phase, respectively); these action potentials had prolonged afterhyperpolarizations (Fig. 3, *A*, *B*). Neurons that fired spontaneously at frequencies less than 10 Hz, or, in the case of quiescent cells, neurons with a spike duration greater than 1 msec, accounted for 95% (452) of the 475 cells (see Table 1). These have therefore been termed *principal* cells.

The cells with spontaneous firing rates greater than 10 Hz had action potentials of amplitude 54 ± 3.3 mV and duration 0.65 ± 0.06 msec (range, 0.4–0.85 msec; 7 cells), clearly shorter than those of the *principal* cells (Fig. 3, *A*, *B*). Seven of the quiescent cells also appeared to be of this type, with action potentials of amplitude 52 ± 1.9 mV and duration 0.57 ± 0.14 msec (3 cells). These cells (total n = 23) were defined as *secondary* cells and represent 5% of the total cell number (Table 1).

Voltage response to hyperpolarizing current

Secondary neurons. Voltage changes in response to hyperpolarizing current pulses did not show time-dependent inward rectification (Fig. 3*C*). Cell input resistance was 219 ± 27 MΩ (17 cells). Steady-state membrane conductance between −75 and −90 mV, estimated in 2 cells from voltage–current plots such as that in Figure 3*D*, was calculated as 3.9 and 4.2 nS, respectively.

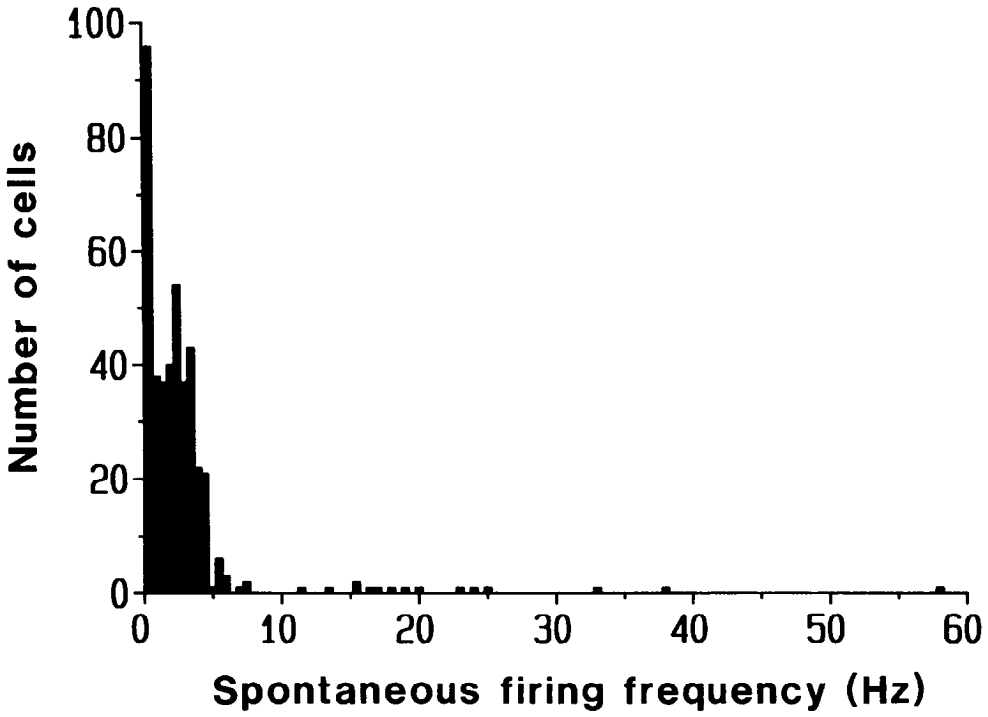

Figure 2. Frequency histogram of spontaneous action potential firing rates of 423 substantia nigra zona compacta neurons. Firing rates are in bins of 0.5 Hz (*horizontal axis*). Regular firing patterns were not observed with discharge frequencies of less than 1 Hz; of the 141 cells in this category, 84 cells were completely quiescent. All cells firing at rates greater than 10 Hz (n = 15) were *secondary* cells. Seven *secondary* cells were quiescent. All remaining cells were *principal* cells.

Principal neurons. Voltage changes produced by hyperpolarizing current pulses exhibited a pronounced time-dependent inward rectification (Fig. 3*C*; see Lacey et al., 1987; Lacey and North, 1988). Estimates of steady-state membrane slope conductance in the region −75 to −90 mV from voltage–current plots such as that in Figure 3*D* gave values of 22.4 ± 1.6 nS (range, 8.7–45.3 nS; 24 cells).

Effects of dopamine

Secondary neurons. Dopamine (10 μM, 3 cells; 30 μM, 3 cells; 100 μM, 6 cells) had no effect on firing rate, membrane potential, or input resistance (Fig. 4*A*). In 2 other cells, dopamine (30 μM) caused a small increase in input resistance accompanied by, in one instance, a 3 mV depolarization and, in another, a 2 mV hyperpolarization.

Principal neurons. In 75 *principal* neurons the response to dopamine (1–300 μM) or the selective D_2 receptor agonist quinpirole (0.1–10 μM) was examined. In all cases a hyperpolarization causing an inhibition of firing (Fig. 5*A*) or (in voltage clamp at −55 to −70 mV) an outward current was observed: this effect was as previously reported in 248 other zona compacta neurons (Lacey et al., 1987, 1988).

Effects of opioids

Secondary neurons. Met^5-enkephalin (10 μM) was applied to 6 cells. In one a 41% reduction in firing rate was observed. In 4 other cells that were either nonfiring or had been hyperpolarized by current injection to prevent firing, a hyperpolarization of 5.4 ± 1.5 mV was observed. No effect was seen in the sixth cell. The μ opioid receptor-selective agonist, DAGO, caused a membrane hyperpolarization (Fig. 4*B*) and/or a reduction of firing rate (Fig. 6) in all 14 cells tested (concentrations were 0.3, 1, 3, and 10 μM); 2 of the cells that were tested were also hyperpolarized by Met^5-enkephalin. The hyperpolarization caused by

Table 1. Comparison of the properties of *principal* and *secondary* cells in rat substantia nigra zona compacta

	Principal cells	Secondary cells
Number recorded	452	23
Spontaneous firing rate[a] (Hz)	<10	>10
Action potential duration (msec)	>1	<1
Time-dependent inward rectification[b]	Present	Absent
Steady-state conductance[c] (nS)	22	4
Dopamine	Hyperpolarizes	No effect
μ Opioids	No effect	Hyperpolarizes
Baclofen (or GABA)	Hyperpolarizes	Hyperpolarizes

[a] One-third of cells of both types were not spontaneously active.
[b] See Lacey and North (1988).
[c] Between −75 and −90 mV.

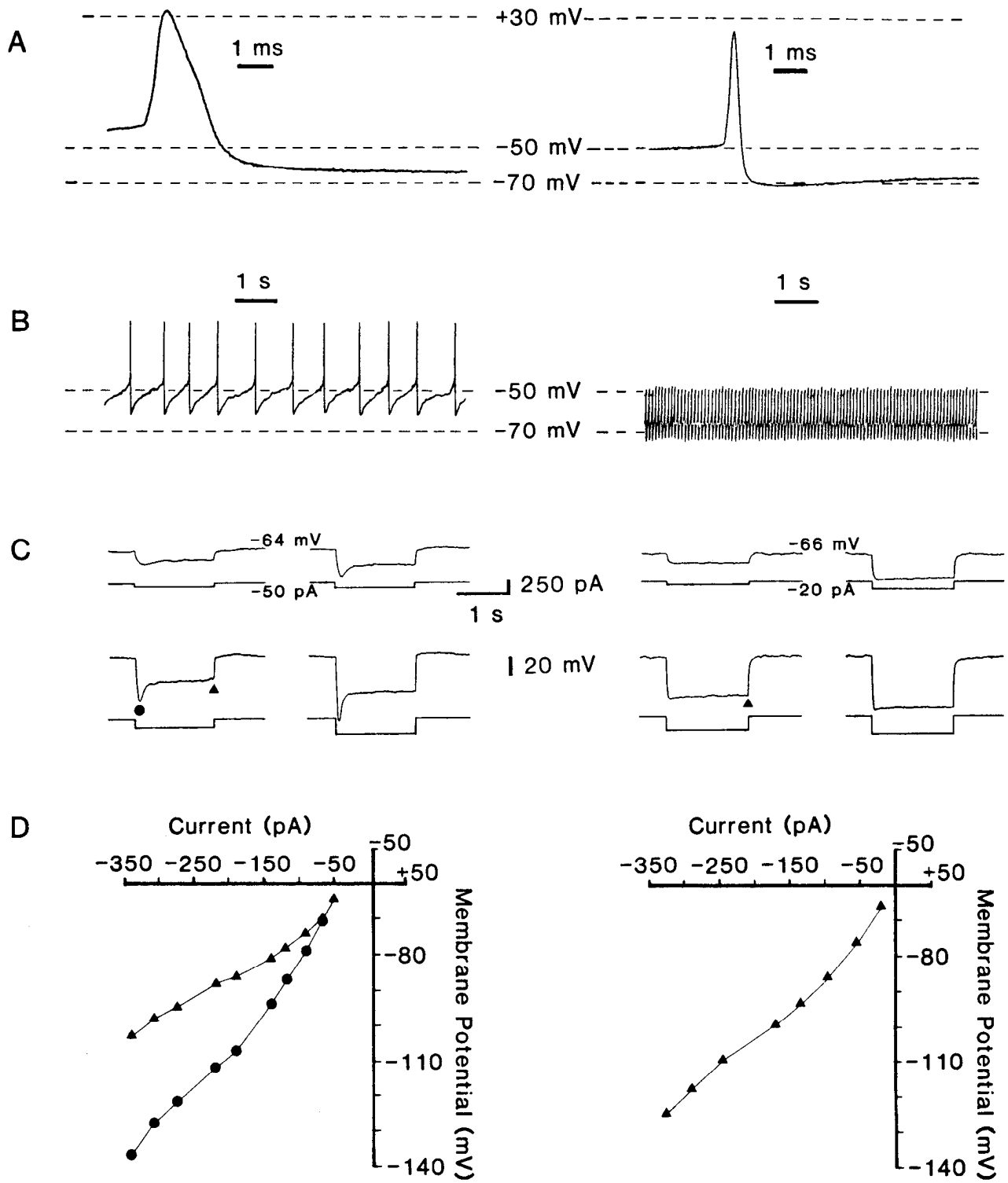

Figure 3. Comparison of some electrophysiological properties of a *principal* cell (*left*) and a *secondary* cell (*right*). *A,* Records of single action potentials from each of the 2 cells, digitized and reproduced at 100 kHz. The duration of the *principal* cell action potential (measured from −37 mV, the point of initiation of its fastest-rising phase) was 1.9 msec, and its amplitude, measured relative to −37 mV, was 61 mV. The *secondary* cell action potential duration was 0.54 msec and the amplitude was 60 mV, measured from its point of initiation at −48 mV. *B,* Chart records of membrane potential at rest showing firing of spontaneous action potentials displayed on the same time scale for each of the 2 cells. The amplitude of fast transients was not fully reproduced due to the limited frequency response of the recorder. The *principal* cell was firing at a rate of 1.3 Hz and the *secondary* cell at 13 Hz. *Dashed lines* represent membrane potential levels of −50 and −70 mV as indicated. *C,* Four paired records for each of the 2 cells showing membrane potential (*top*) and applied current (*bottom*) during passage of hyperpolarizing current pulses 1.7 sec long. Membrane potential of both cells was held hyperpolarized to rest by constant direct current to prevent spontaneous action potential firing. In the *principal* cell, the potential change initially caused by the hyperpolarizing pulse was not maintained for the full duration of the pulse. This time-dependent inward rectification became more pronounced at more hyperpolarized levels. In contrast, time-dependent inward rectification was virtually absent in the *secondary* cell. *D,* Plots of membrane potential attained against the amplitude of 1.7 sec current pulse applied, including those shown in *C,* for both cells. Peak (●) and steady-state potential (▲, at the end of the pulse) are both plotted for the *principal* cell. The steady-state slope conductances between −75 and −90 mV, estimated from these plots, were 9.0 and 4.2 nS for the *principal* and the *secondary* cell, respectively.

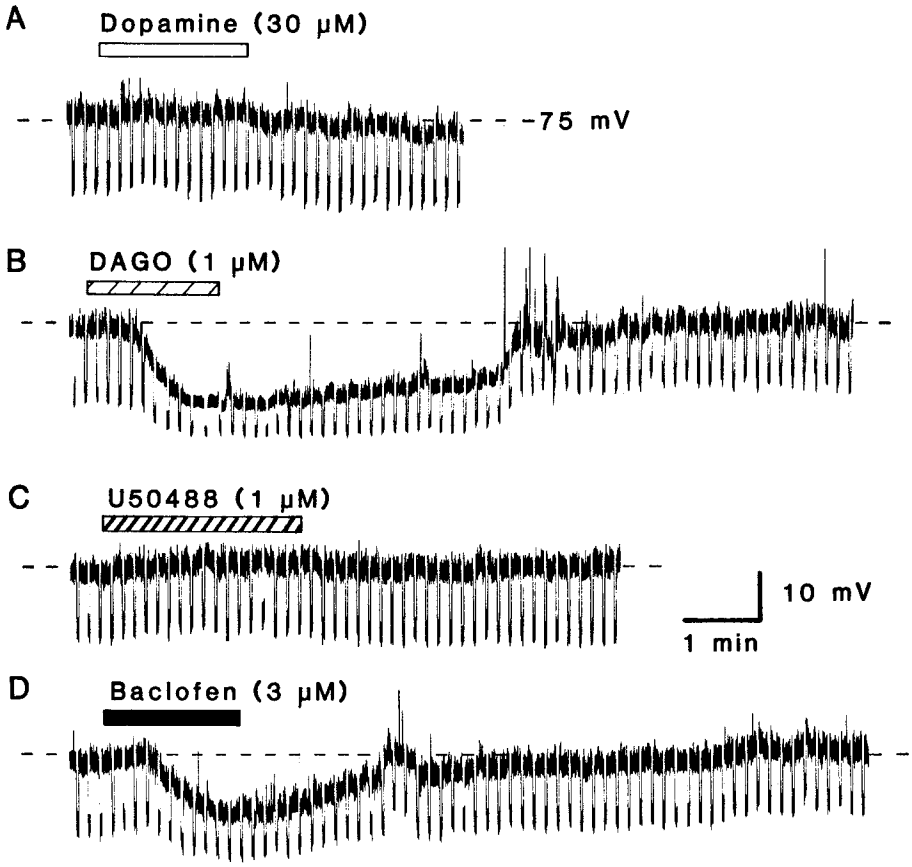

Figure 4. The selective μ opioid receptor agonist DAGO and $GABA_B$ receptor agonist baclofen caused hyperpolarization of *secondary* cells, accompanied by a fall in input resistance; dopamine and the κ opioid receptor-selective agonist U50488 were without effect. Records of membrane potential from a single *secondary* cell with a resting potential of −75 mV (*dashed line*). Transient hyperpolarizations were due to passage of −50 pA current pulses 2 sec long and are proportional to input resistance. Traces show effects of superfusion of (*A*) dopamine, 30 μM (*open bar*); *B*, DAGO, 1 μM (*lightly hatched bar*); *C*, U50488, 1 μM (*heavily hatched bar*); and *D*, baclofen, 3 μM (*filled bar*). Both DAGO (1 μM) and baclofen (3 μM) reversibly hyperpolarized the membrane by 16 and 12 mV, respectively, accompanied by a fall in input resistance.

DAGO (1 μM) was 8.1 ± 1.7 mV (n = 7) in amplitude and was accompanied by a fall in input resistance (6 cells; Fig. 4*B*). This action of DAGO was unaffected by TTX (1 μM, 2 cells). The effects of both Met^5-enkephalin and DAGO reversed when their application was discontinued.

DPDPE (1 μM), an agonist selective for δ opioid receptors, was without effect on membrane potential or input resistance in 3 cells tested, all of which were hyperpolarized by DAGO. U50488 (1 μM), which is a selective agonist at κ opioid receptors, also had no effect on 3 cells that were hyperpolarized by DAGO (Fig. 4*C*).

Principal neurons. Superfusion of Met^5-enkephalin (10 μM, 12 cells) or the μ receptor-selective agonist DAGO (1 μM, 5 cells) was without effect on firing rate, membrane potential, or input resistance in all cells examined (Fig. 5*B*).

Effects of GABA and baclofen

Secondary neurons. GABA (1 mM) was applied to 6 cells, all of which responded with a reversible membrane hyperpolarization and a complete inhibition of firing (4/4 cells). Concomitant application of the $GABA_A$ receptor antagonist bicuculline (10 μM) increased the hyperpolarization and reduced the fall in input resistance produced by GABA (Fig. 7), indicating the presence of both $GABA_A$ and $GABA_B$ receptors on these cells. The $GABA_B$ receptor agonist baclofen (3 μM) inhibited firing and hyperpolarized by 6.5 ± 1.2 mV (3 μM, 4 cells) and 8.5 ± 1.2 mV (10 μM, 4 cells) in all neurons tested. The hyperpolarization was accompanied by a fall in input resistance (Figs. 4*D*, 7).

Principal neurons. GABA (1 mM, 3 cells) and baclofen (3 μM, 10 cells) inhibited firing and caused a hyperpolarization (Fig. 5, *C*, *D*). These findings confirm the effects previously described in detail for 193 other zona compacta neurons (Lacey et al., 1988).

Discussion

Two types of compacta neuron

It is clear from these observations that 2 types of neurons can be distinguished in the substantia nigra zona compacta *in vitro*. The use of firing frequency and spike duration as the main distinguishing criteria is somewhat arbitrary; sensitivity to either dopamine or opioids could equally have been used (Table 1). However, not all cells impaled were tested with opioids or dopamine, nor was the presence of time-dependent inward rectification ascertained or an estimate of steady-state conductance obtained in all cells; thus, it seemed more reasonable to use firing frequency and/or spike duration as the defining characteristics. The main finding of this study is that there exists a strict correlation between certain biophysical properties of the neurons and their expression of D_2 dopamine receptors, and that neurons with μ opioid receptors do not have D_2 receptors.

Principal neurons

The electrophysiological characteristics of these neurons are similar to those described *in vivo* by Grace and Bunney (1983, 1984) and Tepper et al. (1987). The inhibition of these cells by dopamine and their presence within a catecholamine-rich region leaves little doubt that they are of the same type as those considered dopaminergic in previous *in vivo* studies. In 2 respects these cells differ from those described *in vivo*: the pattern of spontaneous action potential discharge and cell input resistance.

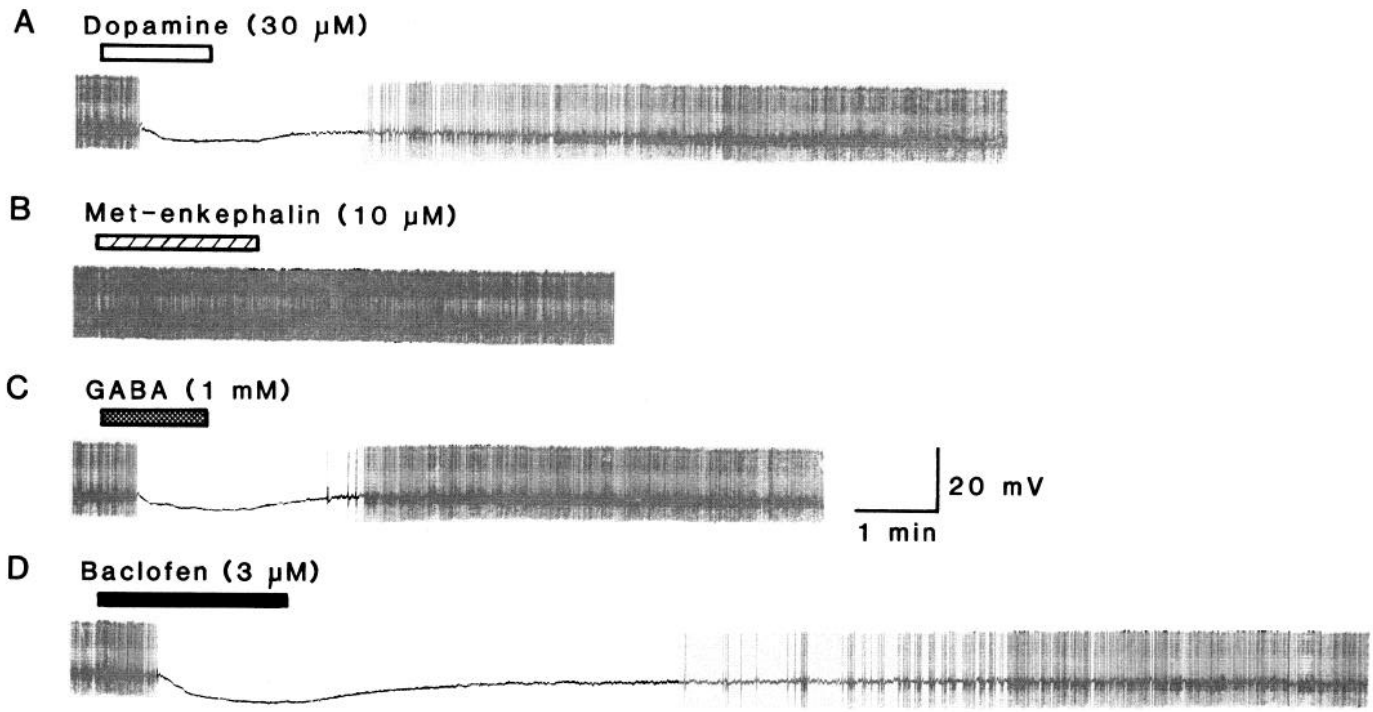

Figure 5. The firing of *principal* cells is inhibited by dopamine, GABA and baclofen, accompanied by a hyperpolarization, but is unaffected by Met^5-enkephalin. Records of membrane potential from a single spontaneously firing *principal* cell (full amplitude of action potentials not reproduced) during superfusion of (*A*) dopamine, 30 μM (*open bar*); *B*, Met^5-enkephalin, 10 μM (*hatched bar*); *C*, GABA, 1 mM (*stippled bar*); and *D*, baclofen, 3 μM (*filled bar*) for the periods indicated by the bars. All effects were reversible on washout of the drug.

The predominant pattern of spontaneous activity in the present study was of a regular "pacemaker" spike discharge in the range of 1–8 Hz. The failure of about one-third of cells to exhibit this firing pattern may be attributable to changes resulting from impalement, because in many cells this pattern was initially absent and took some time to become established. Firing of bursts of action potentials as first reported by Bunney et al. (1973) was not seen under the recording conditions described here; this may be a consequence of synaptic input present *in vivo* but not *in vitro*. The higher input resistances reported here (approximately 5 times higher than those of Grace and Bunney, 1983) may also reflect the absence of synaptic inputs causing conductance increases *in vivo*; alternatively, they may reflect the advantage in stability afforded by the *in vitro* preparation. The *principal* cells of the present study resemble the Type I cells of Guyenet and Aghajanian (1978) in terms of firing rate, spike width, their inhibition by local application of dopamine and GABA, and their presence in the zona compacta.

Secondary neurons

The Type II cells of Guyenet and Aghajanian (1978) resemble the *secondary* neurons described here. They had higher firing rates and shorter spike durations than the Type I cells and were inhibited by GABA, but not by dopamine. Some of their Type II cells, located in zona compacta, could be antidromically activated from the striatum, and this was seen even after 6-hydroxydopamine. However, most Type II cells were in the zona reticulata and did not project to the striatum. The properties of the zona reticulata neurons described by Deniau et al. (1978), some of which projected to the striatum, correspond to the Type II neurons of Guyenet and Aghajanian (1978). Indeed, in some respects the electrical membrane properties of the *secondary* cells resemble those of both the Type I zona reticulata neurons of Nakanishi et al. (1987) and of a presumed nondopaminergic cell type recently described in guinea pig substantia nigra compacta *in vitro* (Matsuda et al., 1987). Although not seen in all cases (such as shown in Fig. 3*C*), the *secondary* cells sometimes exhibited anode break or low-threshold spikes (most readily observed in the quiescent cells), giving rise to short-duration action potentials, similar to those reported by Nakanishi et al. (1987) and Matsuda et al. (1987). Therefore, it is possible that the *secondary* cells of the present study might be considered reticulata neurons. However, it should be stressed that the recording electrode was always positioned under visual control within the substance of the zona compacta (see Fig. 1). Thus, although the nondopaminergic cells of the adjacent zona reticulata (or even cell bodies within the medial lemniscus) are possible candidates for our *secondary* neurons, it seems more likely that these neurons are intrinsic interneurons or projection neurons from the zona compacta. They might represent the cells of origin of a nondopaminergic nigrostriatal pathway, estimated to constitute about 5% of the total number of nigral cells with such a projection (Van der Kooy et al., 1981).

Dopamine receptors are confined to principal neurons and opioid receptors to secondary neurons

The ability of dopamine to inhibit firing or hyperpolarize compacta neurons presumed to be dopaminergic has been reported previously (Aghajanian and Bunney, 1973; Guyenet and Aghajanian, 1978; Pinnock, 1983; Lacey et al., 1987). In contrast, neurons clearly located in the zona reticulata, with a shorter action potential waveform and high firing rate, were not inhibited by dopamine (Bunney et al., 1973; Guyenet and Aghajanian, 1978). Furthermore, approximately one-third to one-half

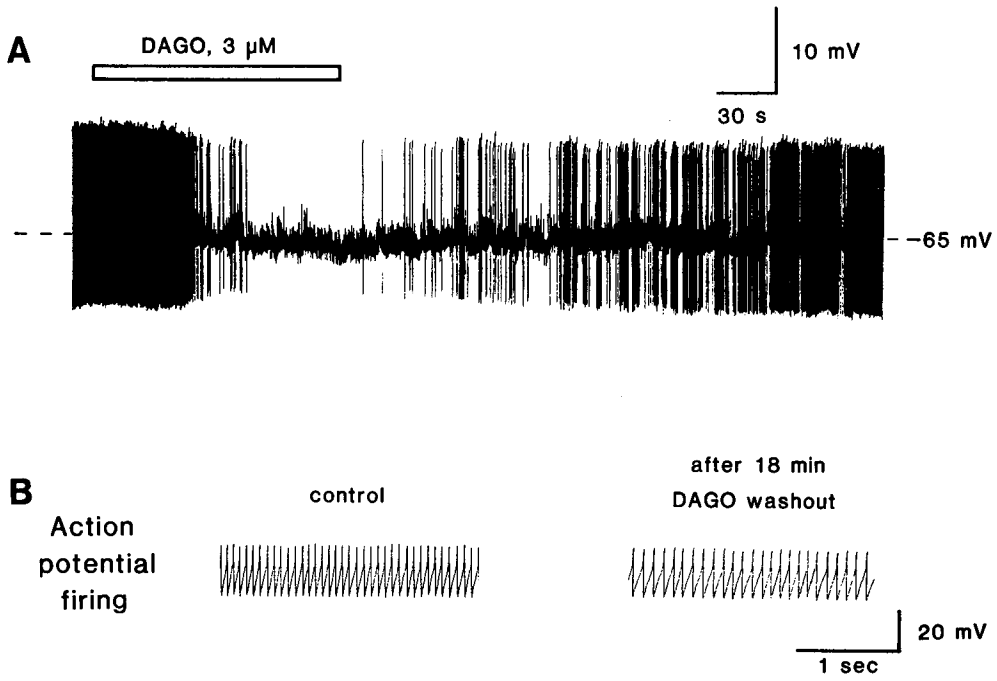

Figure 6. The spontaneous firing of *secondary* cells is reversibly inhibited by DAGO, accompanied by a hyperpolarization. Records of membrane potential from a *secondary* cell at rest, firing spontaneous action potentials (amplitude not fully represented). *A*, DAGO (3 μM), superfused for 4 min where indicated by *hatched bar*, inhibited firing and hyperpolarized the membrane to −67 mV (*dashed line* represents −65 mV). *B*, Records of (attenuated) action potentials at a smaller time scale than in *A* permitting measurement of firing rate before DAGO application (*left*) and 18 min after its washout (*right*). Firing rate had partly recovered to the control level of 15 Hz during the washout period of 18 min.

of neurons in the zona reticulata are excited by dopamine *in vivo* (Ruffieux and Schultz, 1980; Waszczak and Walters, 1983). This also argues against the *secondary* cells being reticulata neurons, since we found no excitatory effects of dopamine.

Previous electrophysiological studies have shown that systemic morphine excites dopaminergic zona compacta neurons *in vivo* (Iwatsubo and Clouet, 1977; Lee et al., 1977; Gysling and Wang, 1983; Matthews and German, 1984; Walker et al., 1987). Iontophoretically applied morphine was either excitatory (Gysling and Wang, 1983; Matthews and German, 1984) or without effect (Pert et al., 1979; Collingridge and Davies, 1982). The excitatory effects of systemic morphine were suppressed or abolished following chronic lesions of either the medial forebrain bundle or the striatum (Kondo and Iwatsubo, 1980; Gysling and Wang, 1983). This observation, combined with reports of inhibition of zona reticulata neurons by systemic (Finnerty and Chan, 1979) and iontophoretic morphine (Hommer and Pert, 1983), support the idea that the facilitatory effect of morphine on dopaminergic cells involves indirect effects *via* inputs from either zona reticulata cells or extranigral afferents or both.

There is independent evidence for the existence of opioid receptors on the striatonigral cells. Lesions of the striatum with kainic acid injection results in reductions of numbers of striatal binding sites for various opioid receptor ligands by 38–70% (Pollard et al., 1978; Abou-Khalil et al., 1984; Van der Kooy et al., 1986; Eghbali et al., 1987). At least some of this (μ and δ) binding appears to be on cells projecting to the substantia nigra because opioid receptor binding in the nigra is also reduced following striatal lesions (Abou-Khalil et al., 1984). Furthermore, section of the nigrostriatal pathway reduces numbers of opioid binding sites in the nigra (Gale et al., 1979) and [^{3}H]etorphine binding accumulates rostral to a nigrostriatal pathway transsection (Van der Kooy et al., 1986).

On the other hand, there are experimental findings that might

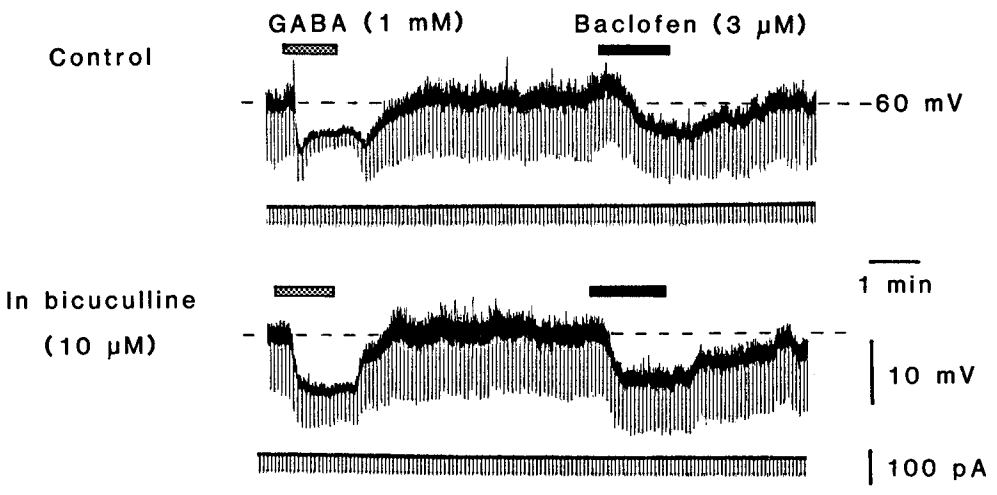

Figure 7. GABA and baclofen both hyperpolarize *secondary* cells: The effect of GABA is not blocked by bicuculline. Paired records of membrane potential (*above*) and current (*below*) from a quiescent cell showing effect of application of GABA (1 mM, *stippled bars*) and baclofen (3 μM, *filled bars*) in control conditions (*upper records*) and in bicuculline methiodide (10 μM, *lower records*). Hyperpolarizing current pulses of 200 msec duration permit estimation of input resistance. The biphasic nature of the response to GABA (1 mM) was abolished by bicuculline (10 μM), and the accompanying fall in input resistance was reduced. The baclofen hyperpolarization and fall in input resistance was little affected by bicuculline. *Dashed lines* indicate −60 mV.

be interpreted to indicate the existence of opioid receptors on the dopamine-containing nigrostriatal cells. ^{3}H-dopamine release from striatal slices is increased selectively by δ receptor agonists, but not by morphine (Lubetzki et al., 1982; see also Arbilla and Langer, 1978). This kind of study provides no evidence that the δ receptors are on the dopamine-containing neurons; the release of dopamine may be affected by other transmitters whose release from the slice is altered by opioids. 6-Hydroxydopamine lesions of either the striatonigral pathway or the substantia nigra reduce opioid receptor numbers in the striatum by 21–34% (Pollard et al., 1978; Llorens-Cortes et al., 1979; Reisine et al., 1979; Eghbali et al., 1987), with μ and δ receptors depleted to an equivalent degree (Eghbali et al., 1987); this could easily result from transsynaptic effects of the lesion. In fact, the effect of similar 6-hydroxydopamine lesions on opioid receptor numbers in substantia nigra, presumably on dopaminergic cells themselves, is equivocal. Llorens-Cortes et al. (1979) report a 20% and Pollard et al. (1978) a 33% reduction in receptor number, while Gale et al. (1979) and Reisine et al. (1979) report reduction of 10% or less. The present findings demonstrate that the *principal* neurons, which are likely to be dopamine-containing, do not possess functional opioid receptors on their cell bodies.

Seventeen of 18 *secondary* cells tested were hyperpolarized or inhibited by Met^5-enkephalin or DAGO. The ineffectiveness of selective δ or κ agonists argues for the existence of only functional μ opioid receptors on these cells. The response is qualitatively similar to that seen in a variety of neurons, including locus coeruleus (Williams et al., 1982), dorsal horn (Yoshimura and North, 1983), hippocampal interneurons (Madison and Nicoll, 1988), and lateral parabrachialis (Christie and North, 1988), in which μ opioid receptor activation has been shown to cause a hyperpolarization due to an increase in membrane potassium conductance. The class of compacta neuron that we have identified (*secondary* neuron) as expressing μ opioid receptors presents one possible target for innervation by the enkephalin-containing terminals within the substantia nigra (Johnson et al., 1980). Additional targets may be reticulata neurons and the terminals of striato/pallidonigral fibers. These cells may therefore play a role in the excitatory (disinhibitory) action of opioids on dopaminergic neurons.

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