Neuron, Vol. 15, 637–647, September, 1995, Copyright © 1995 by Cell Press

Axonal Initiation and Active Dendritic Propagation of Action Potentials in Substantia Nigra Neurons

Michael Häusser,* Greg Stuart, Claudia Racca,† and Bert Sakmann
Abteilung Zellphysiologie
Max-Planck-Institut für medizinische Forschung
Jahnstrasse 29
69120 Heidelberg
Federal Republic of Germany

Summary

The site of action potential initiation in substantia nigra neurons was investigated by using simultaneous somatic and dendritic whole-cell recording in brain slices. In many dopamine neurons, action potentials were observed first at the dendritic recording site. Anatomical reconstruction showed that in these neurons, the axon emerged from the dendrite from which the recording had been made. Action potentials showed little attenuation in the dendritic tree, which in dopamine neurons was shown to be due to recruitment of dendritic sodium channels and may be related to the dendritic release of dopamine. We conclude that in substantia nigra neurons, the site of action potential initiation, and thus the final site of synaptic integration, is in the axon. As the axon can originate from a dendrite, up to 240 μm away from the soma, synaptic input to the axon-bearing dendrite may be privileged with respect to its ability to influence action potential initiation.

Introduction

To understand how incoming synaptic signals are integrated to generate an action potential, it is important to determine where in the neuron the action potential is initiated. Evidence that the dendritic membrane contains active conductances (Llinas, 1988) has lent support to the long-standing proposal (Andersen, 1960; Spencer and Kandel, 1961; reviewed by Adams, 1992; Regehr and Armstrong, 1994) that action potentials may be initiated in the dendrites. Simultaneous recordings from two locations in the same neuron have shown that in neocortical pyramidal cells and cerebellar Purkinje cells, initiation occurs in the axon (Stuart and Sakmann, 1994; Stuart and Häusser, 1994). Whether these findings are applicable to other types of neurons, many of which have morphologically and functionally different dendritic trees, remains to be established.

The substantia nigra is centrally involved in the control of movement, and the degeneration of the dopamine neurons in this brain area is thought to be largely responsible for the symptoms associated with Parkinson's disease (reviewed by Graybiel, 1993). The dendritic tree of these neurons is sparse, with few, relatively unbranched dendrites, and shows little polarization. In contrast with γ-aminobutyric acid (GABA) neurons in the substantia nigra, dopamine neurons have been shown to generate rhythmic bursts of action potentials in vivo (reviewed by Grace, 1988). In addition, there is evidence that dopamine neurons are able to release dopamine from their dendrites (Cheramy et al. 1981), although the mechanism by which this might occur remains unclear. Given the importance of these neurons for brain function and the possibility that dopamine neurons may release transmitter from their dendrites, we have investigated the site of initiation of action potentials and their spread within the dendritic tree for both dopamine and GABA neurons in the substantia nigra by using simultaneous somatic and dendritic recordings.

Results

Identification of Dopamine and GABA Neurons

Dopamine neurons were identified by their electrophysiological features (Grace and Onn, 1989; Yung et al., 1991). These included a prominent sag of the membrane potential back toward the resting membrane potential during hyperpolarization (average steady-state to peak sag ratio of 0.46 ± 0.12, n = 18) and broad, often multiphasic action potentials (Figures 1A and 1C). These features contrasted markedly with the other population of neurons in the substantia nigra, which showed little or no sag (average sag ratio of 0.80 ± 0.04, n = 5), and briefer monophasic action potentials (Figures 1B and 1D). On the basis of their electrophysiological characteristics (Tepper et al., 1987; Grace and Onn, 1989; Yung et al., 1991), the latter cells are likely to be GABA neurons, which represent the other major neuronal population in the substantia nigra (Mugnaini and Oertel, 1985). The input resistance of dopamine and GABA neurons was not significantly different ($p > 0.05$), with the average values being 394 ± 21 MΩ (n = 18) and 476 ± 99 MΩ (n = 7), respectively. There were also no marked differences in somatodendritic morphology between dopamine and GABA neurons detected with infrared differential interference contrast (IR-DIC) microscopy, consistent with previous morphological observations (Grace and Onn, 1989; Yung et al., 1991), except that GABA neurons were more often observed to have a bipolar dendritic tree and a fusiform soma.

Initiation of Action Potentials

To study the site of action potential initiation, simultaneous whole-cell recordings were made under visual control from the somata and dendrites of dopamine and GABA neurons. In many of the neurons identified as dopaminergic, the action potential was observed to occur first at the dendritic recording site (n = 18; Figure 1C), whereas in other dopamine neurons, the action potential was observed to occur first at the soma (n = 13), or else no clear difference

*Present address: Laboratoire de Neurobiologie, Ecole Normale Supérieure, 46 rue d'Ulm, 75005 Paris, France.

†Present address: Laboratoire de Biologie Cellulaire de la Synapse, Ecole Normale Supérieure, 46 rue d'Ulm, 75005 Paris, France.

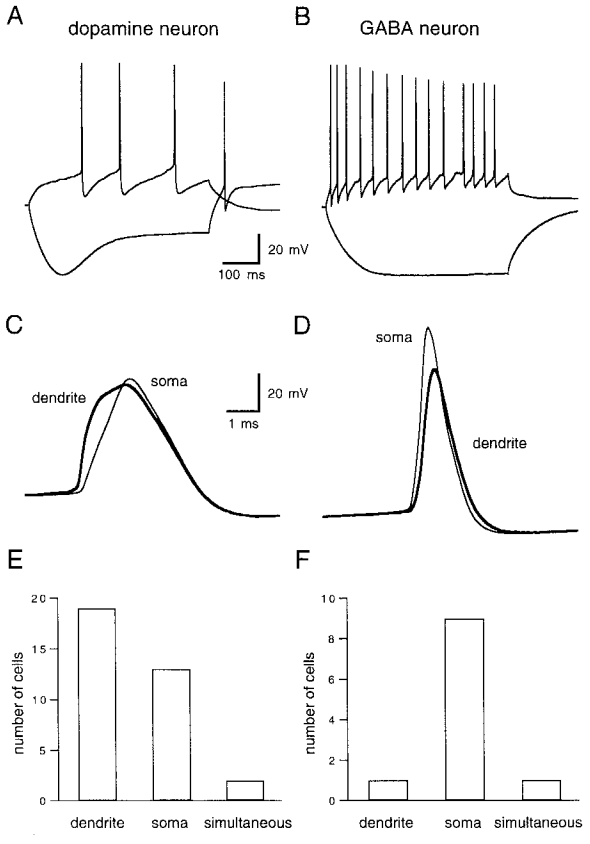

Figure 1. Electrophysiological Characteristics of Dopamine and GABA Neurons Measured with Patch-Pipette Recording in Brain Slices

(A and B) Somatic recordings from a dopamine and a GABA neuron, respectively, showing the responses of the neurons to +0.1 nA and −0.1 nA current pulses. Note the characteristic differences in the hyperpolarizing response and firing pattern shown by the two different types of neuron. Scale bar in (A) also applies to (B).

(C and D) Simultaneous somatic and dendritic recording from a dopamine and a GABA neuron, respectively. The action potential, activated by a depolarizing current pulse, was observed first at the dendritic pipette in the dopamine neuron (dendritic recording site 65 μm from the soma) and first at the somatic pipette in the GABA neuron (dendritic recording site 68 μm from the soma). Note the characteristic differences in action potential shape and width in the two cell types. Scale bar in (C) also applies to (D).

(E and F) Histograms showing the site where the action potential was observed to occur first during simultaneous somatic and dendritic recordings from different dopamine and GABA neurons, respectively. Where no latency difference could be detected, the action potential was classified as arriving simultaneously at both recording sites.

in latency could be detected (n = 2). By contrast, in the large majority of GABA neurons (n = 9), the action potential was observed to occur first at the soma (Figure 1D). In only one GABA neuron did the action potential occur first at the dendritic recording site. The sites where the action potentials were observed to occur first in dopamine and GABA neurons are summarized in Figures 1E and 1F.

For both dopamine and GABA neurons, in any one cell the action potential was always observed to occur first at a particular recording site regardless of whether action potentials were elicited by somatic or dendritic depolarizing current pulses, by synaptic stimulation, by antidromic activation, or during spontaneous firing of action potentials, suggesting that the site of initiation remained constant for all methods of stimulation. In those cases where it could be detected, the latency difference between the action potentials depended on the distance between the recording pipettes. In dopamine neurons, when the action potential occurred first at the dendritic recording site, this latency difference was usually greatest at the onset of the action potential but sometimes became less at the peak (e.g., Figure 1C), with the peaks occasionally appearing to occur simultaneously at the time resolution used in these experiments (20 μs).

To determine whether action potential initiation is similar at more physiological temperatures, several experiments were carried out at 34°–35°C. In 6 of 9 dopamine neurons, the action potential was observed to occur first at the dendritic recording site, while in the three others, the action potential occurred first at the somatic pipette. By contrast, in all five GABA neurons, the action potential occurred first at the soma. As at room temperature, the recording site where the action potential was observed first remained consistent with all methods of stimulation.

Correlation with Morphology

One possible explanation for the finding that the action potentials were observed to occur first in dopamine neuron dendrites could be that in these neurons, the axon emerges from the dendrite from which the recording has been made, since it has been reported previously that the axon of dopamine neurons can emerge from a dendrite (see Juraska et al., 1977; Preston et al., 1981; Grace and Bunney, 1983; Tepper et al., 1987). Unfortunately, it was not possible to see the axon of substantia nigra neurons by using IR-DIC optics, possibly owing to the small diameter of the axon. Therefore, neurons were filled with biocytin during recording and subsequently stained by use of the avidin-horseradish peroxidase (HRP) reaction. The axon was identified by using several criteria (see Experimental Procedures), and the dendritic recording site was determined by comparison of the stained cell with an IR-DIC image of the neuron made during recording.

The results from two such experiments are illustrated in Figure 2. In Figure 2A, recordings are shown from a dopamine neuron in which the action potential was observed to occur first at the dendritic recording site, 195 μm away from the soma. When the morphology of the neuron was reconstructed, the axon was found to emerge close to the dendritic recording site, 215 μm away from the soma. Figure 2B shows traces from a GABA neuron where the action potential occurred first at the somatic recording site. Upon reconstruction of this neuron, the axon was found to emerge near the soma. These findings suggest that it is the location of the axon that determines where the action potential will be observed first.

This hypothesis was confirmed in all other recordings where the axon could be identified by subsequent morphological reconstruction, as summarized in Figure 3. In every case where the action potential was observed to occur first in the dendrite, the axon of the neuron was found to emerge from the dendrite from which the recording had

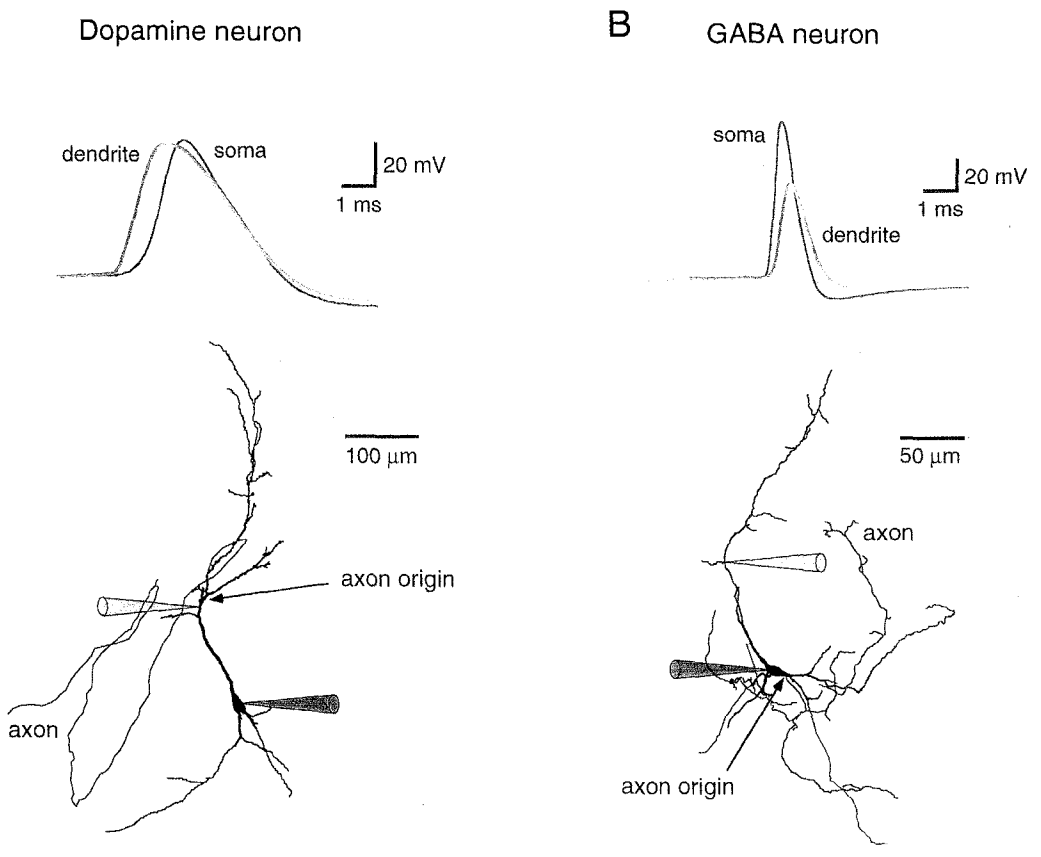

Figure 2. Correlation of Observed Action Potential Initiation Site with Location of the Axon Origin

For each neuron, an action potential recorded simultaneously at the soma (blue) and dendrite (green) is displayed in the top panel, and the morphological reconstruction of the filled neuron is shown below, with the location of the somatic (blue) and dendritic (green) pipettes and axon origin indicated. The axon is shown in red. In the dopamine neuron (A), the action potential was observed to occur first at the dendritic recording site, 195 μm from the soma; the axon of this cell was found to emerge from the dendrite from which the dendritic recording had been made, 215 μm from the soma. In contrast, in the GABA neuron (B), the action potential was observed first at the somatic pipette (dendritic pipette 110 μm from the soma), and the axon was also found to emerge from the soma.

been made. When the action potential was observed to occur first at the soma, the axon was found to originate either from the soma or from a dendrite other than the one from which the dendritic recording had been made. Finally, in cases where the action potential appeared to be simultaneous at the somatic and dendritic recording site, the axon was found to emerge from the dendrite in between the two recording pipettes. These findings indicate that the site of action potential initiation is always in the axon, and the observation that action potentials were often found to occur first at the dendritic recording site in dopamine neurons is due to the axon originating from the dendrite from which the dendritic recording had been made.

To obtain quantitative information about the site of origin of the axon, a large number of dopamine and GABA neurons were filled with biocytin and the location of the axon identified. As shown in Figure 4, in dopamine neurons the axon was found to emerge from a dendrite in the majority of cells (76%). The distance of the axon origin from the soma was quite variable in dopamine neurons, ranging from only a few micrometers to 240 μm in the most extreme case, with the mean distance being 74.5 ± 11.2 μm (n = 28). In GABA neurons the dendritic origin of the axon was somewhat less common, with 54% of the axons emerging from the soma (Figure 4); furthermore, the mean distance of axon origin was closer to the soma (52.4 ± 9.6 μm; n = 6). To determine whether there was a tendency for the axon to emerge from a particular dendrite, the dendrites were categorized as apical (toward the pia), basal (away from the pia), medial, or lateral. In dopamine neurons, the most common dendrite of origin for the axon was medial (35% of cells), with the basal, lateral, and apical dendrites bearing the axon in 23%, 23%, and 19% of the cells, respectively. By contrast, in five of the six GABA neurons where the axon emerged from a dendrite, the origin was basal; in the remaining neuron, the axon emerged from a medial dendrite. In both cell types, the axon usually appeared to arise from the dendrite with the largest diameter, often at a branch point, as this dendrite split into two or three thinner branches (see Figure 2A). Since it is easiest to see and record from the largest dendrites, recordings from dopamine neurons where the action potential occurred first in the dendrite were more com-

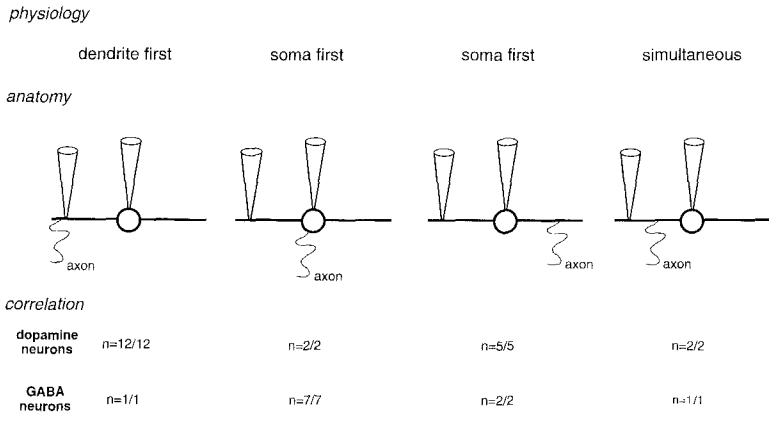

Figure 3. Summary of Experiments Correlating Electrophysiology and Morphology

The top row lists the various possible outcomes of the simultaneous somatic and dendritic recording experiments, and the second row shows a schematic representation of the possible anatomical configuration of the axon and recording pipettes. The bottom rows show the number of cells for which the correlation between the physiology and anatomy was achieved in the different cases.

mon than those where the action potential occurred first at the soma. In a set of experiments where somatic recordings were made from dopamine neurons with no obvious large dendritic processes, a higher proportion of these neurons had axons emerging close to or from the soma (43% versus 15% for double recordings).

Components of the Action Potential in Dopamine Neurons

In many dopamine neurons, a clear inflection was observed on the rising phase of the action potential, suggesting that it may be composed of several components (Figure 5A). Work on motoneurons has shown that the somatic action potential triggered by antidromic stimulation can be decomposed into two components: an initial segment (IS) axonal spike and a somato-dendritic (SD) spike (Coombs et al., 1957; Fuortes et al., 1957). A similar decomposition of the somatic action potential of dopamine neurons during antidromic activation has previously been observed in vivo (Grace and Bunney, 1983; Tepper et al., 1987). We have found that hyperpolarization of dopamine neurons during antidromic stimulation could, in an all-or-none fashion, block simultaneously a component of both the somatic and the dendritic action potentials, suggesting that the dendritic action potential is also composed of an IS and an SD component (Figures 5B and 5C). By subtrac-

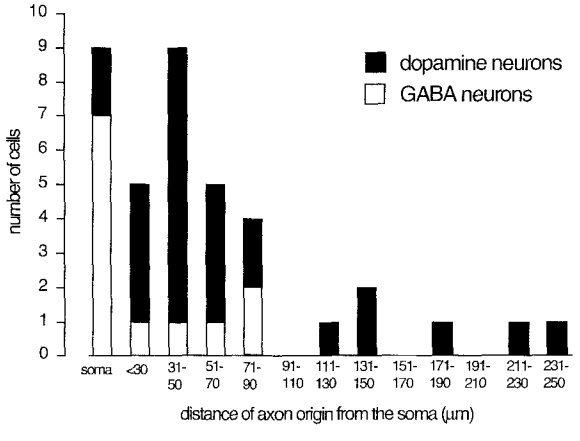

Figure 4. Origin of the Axon in Dopamine and GABA Neurons

Histogram of the distance of axon origin from the soma in dopamine (solid bars) and GABA (open bars) neurons. Data include cells filled during simultaneous somatic and dendritic recordings as well as cells filled with somatic recordings alone.

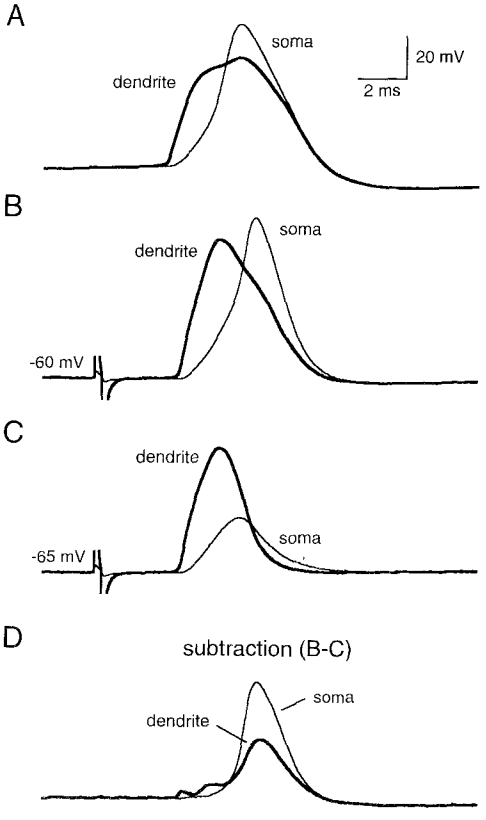

Figure 5. Components of the Action Potential in Dopamine Neurons

Simultaneous somatic and dendritic recordings from the same dopamine neuron; dendritic recording site, 195 μm from the soma.

(A) Action potential elicited by a dendritic current pulse.

(B and C) All-or-none action potential elicited by antidromic stimulation of the axon. In (C), the neuron was hyperpolarized to −65 mV, which prevented activation of the second component of the action potential. Note that in all of these records, the action potential was recorded first by the dendritic pipette.

(D) The second component of the action potential has been isolated by subtracting trace (C) from trace (B); this component appeared to be simultaneous at both dendritic and somatic recording sites.

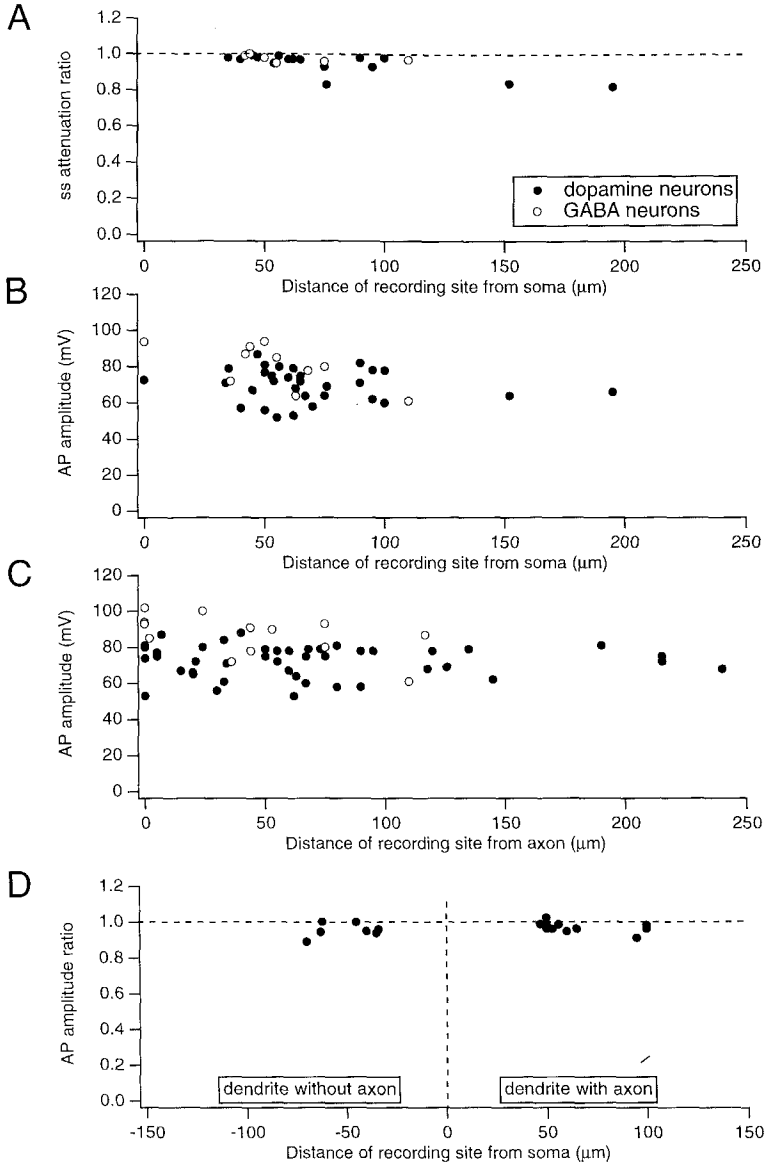

Figure 6. Attenuation of Steady-State Voltage and Action Potentials in the Dendrites of Dopamine and GABA Neurons

Data are from simultaneous somatic and dendritic recordings from dopamine neurons and GABA neurons, represented by closed and open circles, respectively.

(A) Attenuation of steady-state (ss) somatic voltage in response to a small somatic hyperpolarizing current pulse (expressed as the ratio of the dendritic/somatic ss voltage) with distance from the soma.

(B) Relationship of the dendritic action potential (AP) amplitude with distance of the recording site from the soma. The mean somatic action potential amplitude is also plotted for each cell type; the SEM bars are symbol size.

(C) Relationship of the action potential amplitude with distance of the recording site from the axon origin, determined by subsequent morphological reconstruction. Dendritic and somatic recordings have been pooled, as there was no difference in their individual relationships with distance from the axon.

(D) Comparison of the amplitudes of the somatic and dendritic action potentials in dopamine neurons at different distances from the soma, expressed as the ratio of the peak of the dendritic/somatic action potential amplitude. The data are plotted to allow comparison between dendrites bearing the axon (right) and those lacking an axon (left).

tion of the antidromic response at the hyperpolarized membrane potential from that at rest, the second (SD) component of the somatic and dendritic action potentials could be isolated. Interestingly, the SD component appeared to occur simultaneously at the somatic and dendritic recording sites (Figure 5D). This finding may explain why in some recordings the peak of the full-blown action potential often occurred simultaneously at somatic and dendritic recording sites, despite a clear difference in onset latency. In GABA neurons, a difference between the onset and peak latency of the somatic and dendritic action potentials was not observed, and it was also not possible to resolve two components of the action potential in these neurons.

Comparison of Action Potential and Steady-State Attenuation

To determine the attenuation of steady-state voltage in substantia nigra neurons, long-duration small hyperpolarizing somatic current pulses were applied, and the resulting membrane voltage was compared at somatic and dendritic recording sites. As found in cerebellar Purkinje cells (Stuart and Häusser, 1994), the steady-state somatic voltage was attenuated by less than 20% even at the most distal dendritic recording sites in both dopamine and GABA neurons (Figure 6A), suggesting that these neurons are electrically compact under steady-state conditions (note that these values represent an overestimate of the attenuation that one would expect under truly passive conditions, owing to the presence of sag conductances).

Attenuation of voltage in a dendritic tree is frequency dependent (Rall, 1977; Spruston et al., 1994), and therefore one would expect a rapid signal such as an action potential to attenuate much more severely than steady-state voltage under passive conditions. The amplitude of dendritic action potentials is plotted against the distance of the recording site from the soma in Figure 6B, and the

amplitude of the somatic and dendritic action potentials at different distances from the axon is plotted in Figure 6C. In both dopamine and GABA neurons, there was no clear relationship between somatic or dendritic action potential amplitude and the distance of the recording site from the soma or axon. To compare attenuation in dendrites bearing an axon with that in other dendrites of dopamine neurons, the dendritic action potential amplitude was normalized against the somatic action potential amplitude from the same cell and plotted against distance from the soma (Figure 6D) for dendrites bearing the axon (right) and dendrites without the axon (left). There was no obvious difference in action potential attenuation in the two types of dendrites at the distances studied.

Active Propagation of Action Potentials in the Dendritic Tree

As shown above (Figure 6), there was surprisingly little difference in the attenuation of action potentials and steady-state voltage in the dendrites, contrary to what one would expect if the action potential spreads passively through the dendritic tree (see Stuart and Häusser, 1994). To determine whether the dendrites of dopamine neurons contain voltage-activated sodium channels that could support the propagation of action potentials, outside-out patches were excised from somatic and dendritic membrane. Neurons were confirmed to be dopamine neurons in whole-cell mode before withdrawal of the pipette to form an outside-out patch, and similar-sized patch pipettes were used for somatic and dendritic recordings in order to obtain a similar membrane patch area.

In response to depolarizing voltage steps, rapid inward currents followed by slowly inactivating outward currents were recorded in patches from both somatic and dendritic membrane (Figure 7A). The inward currents were reversibly blocked by 500 nM tetrodotoxin (TTX), indicating that they represent the activation of voltage-dependent sodium channels. The average peak sodium current in dendritic patches, 3.6 ± 1.6 pA (n = 7), was not significantly different from that in somatic patches, 5.0 ± 1.3 pA (n = 8; $p > 0.05$), suggesting that somatic and dendritic membranes possess a similar sodium channel density.

To determine whether dendritic sodium channels increase the amplitude of the action potential in the dendrites of dopamine neurons, we compared the attenuation of an action potential with that of a somatic voltage waveform having the same shape and amplitude as a somatic action potential in the presence of TTX. To generate this action potential waveform, a previously recorded somatic action potential was used as the voltage-clamp command during somatic voltage clamp. Subsequent recording from the soma of the same cell with a second pipette showed that this somatic action potential voltage command was faithfully reproduced.

In all such experiments (n = 8; Figure 7B), the attenuation of the action potential in the presence of TTX was greater than that of the evoked action potential, with this difference being more pronounced for dendritic recordings at greater distances from the soma. These results suggest that action potentials actively propagate into the dendrites of dopamine neurons via activation of dendritic sodium channels.

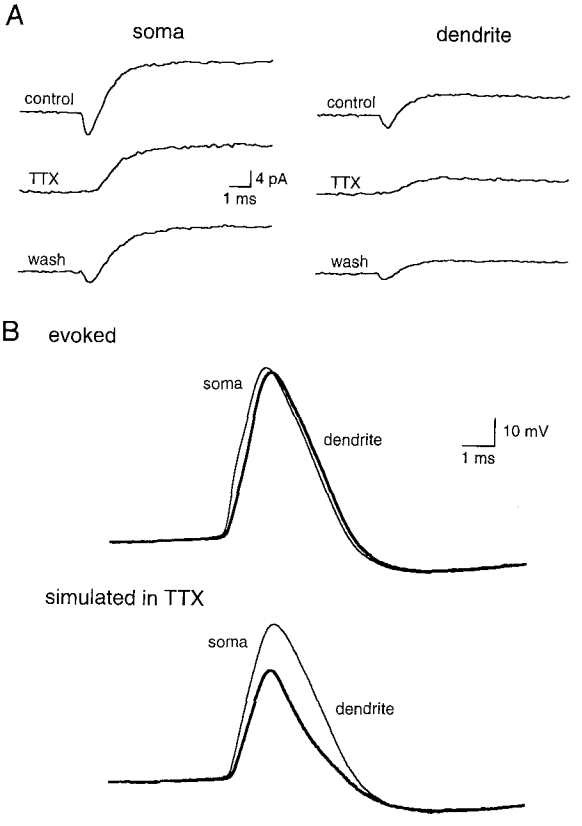

Figure 7. Active Propagation of Action Potentials in the Dendritic Tree of Dopamine Neurons

(A) Voltage-activated currents evoked by depolarizations to −10 mV from a prepotential of −120 mV in outside-out patches excised from the soma (left) and a basal dendrite of the same dopamine neuron, 45 μm from the soma. The rapid inward current present in both patches was reversibly blocked by application of 500 nM TTX.

(B, top) Somatic and dendritic action potentials evoked by a depolarizing dendritic current pulse (dendritic recording 100 μm from the soma).

(B, bottom) Voltage recorded with the same dendritic pipette in response to a simulated somatic action potential in the presence of 1 μM TTX, shown together with the somatic response to the same simulated somatic action potential subsequently recorded with a second somatic recording pipette.

Discussion

Simultaneous somatic and dendritic recordings from substantia nigra neurons showed that in the majority of dopamine neurons, action potentials occurred first at the dendritic recording site. We demonstrate that this is a consequence of a morphological characteristic of these neurons, namely that the axon, the site of initiation of the action potential, emerges not from the soma but rather from a dendrite.

Dendritic Origin of Action Potentials Is Due to the Dendritic Emergence of the Axon

The fact that the recording site at which the action potential was observed to occur first was always closest to the sub-

sequently determined location of the axon strongly suggests that the action potential was initiated in the axon. These findings confirm the hypothesis that action potential initiation occurs in the axon (Coombs et al., 1957; Fuortes et al., 1957) and are in agreement with axonal recordings from neocortical pyramidal and cerebellar Purkinje cells (Stuart and Sakmann, 1994; Stuart and Häusser, 1994). It is interesting that the site of action potential initiation is the same in these different neuronal types, despite the large differences in their dendritic morphology, and this suggests that axonal initiation of the action potential is a feature that is highly conserved across neuronal cell types.

These experiments were carried out using whole-cell recording from neurons in a brain slice preparation, where spontaneous synaptic input is presumably reduced compared with in vivo. The decrease in membrane resistance associated with a high level of spontaneous synaptic activity may change the integrative properties of neurons (Bernander et al., 1991; Rapp et al., 1992), and it is possible, although unlikely, that with certain patterns of synaptic activity the site of initiation may shift from the axon into other regions of the cell (see also Stuart and Sakmann, 1994, and Spruston et al., 1995). The possibility that whole-cell recording may lead to washout of membrane conductances (particularly calcium conductances) and affect the present results was judged to be unlikely on the basis of several observations. First, there were no obvious changes in action potential shape or the electrophysiological properties of neurons with time during whole-cell recording, arguing against washout being a major problem. Second, the shape of the action potential measured with whole-cell recording was similar to that measured with microelectrodes. Finally, studies on neocortical and hippocampal pyramidal cells have shown that the site of action potential initiation is the same whether determined with whole-cell or cell-attached recordings, where washout is avoided (Spruston et al., 1995; G. S., unpublished data).

The predominantly dendritic origin of the axon in dopamine neurons reported here confirms and extends similar observations made in previous morphological studies of these cells (Juraska et al., 1977; Preston et al., 1981; Grace and Bunney, 1983; Tepper et al., 1987). The axon preferentially emerged from medial dendrites in dopamine neurons and had a strong preference for emerging from basal dendrites in GABA neurons, often emerging from the thickest dendrite at a branching point. The emergence of the axon from a dendrite is not a feature unique to substantia nigra neurons, as GABA neurons in various other parts of the brain have been reported to have axons emerging from the dendritic tree, often at considerable distances from the soma (Palay and Chan-Palay, 1974; Amaral, 1978; Feldman and Peters, 1978; Peters and Kimerer, 1981; Peters and Regidor, 1981; Gulyas et al., 1992). Principal or excitatory neurons, such as pyramidal cells in the cortex (Palay et al., 1968; Peters et al., 1968; Sloper and Powell, 1979) or granule cells in the cerebellum (Palay and Chan-Palay, 1974), can also have axons originating from a dendrite, although in these cases the axon usually originates less than ~30 μm from the soma. What appears to be special about nigral dopamine neurons is the distance from the soma at which the axon can emerge from a dendrite, which can be as far as 240 μm.

Functional Consequences of the Dendritic Origin of the Axon

The emergence of the axon from a dendrite rather than from the soma has several interesting consequences. First, it reverses the normal direction of propagation of the action potential, in that the action potential will travel from the dendritic tree toward the soma. Consequently, the dendrite bearing the axon will experience the action potential before it spreads into the soma and other dendrites; this may be relevant if the precise timing of the action potential in the dendritic tree is important to its integrative properties (see below). Second, since synaptic potentials must spread to the axon before action potential initiation can occur, in these neurons the final site of synaptic integration prior to the axon itself will not be at the soma, but rather in the dendrites at the point where the axon emerges. Since synaptic potentials attenuate in the dendritic tree (Rall, 1977; Jack et al., 1983), this suggests that synapses made on the axon-bearing dendrite will be in an electrotonically privileged position. We have tested this possibility with a simple cable model of a neuron with the axon emerging from a dendrite, 50 μm from the soma (Figure 8A). Simulations showed that excitatory postsynaptic potentials (EPSPs) recorded at the axon origin were always larger when generated by synapses located on the axon-bearing dendrite compared with those generated by synapses located on another dendrite at a similar distance from the soma. This was also true for synapses located at similar distances from the axon origin (Figures 8B and 8C). These findings were robust for a wide range of values of the model parameters. The degree by which EPSPs generated by synapses on the axon-bearing dendrite were larger at the axon origin than those generated by synapses located on another dendrite at an equivalent distance from either the soma or axon origin was most dependent on the dendritic diameter used in the model, with the largest differences being found for small dendrites (Figure 8D). The results of the simulations also apply to inhibitory synaptic potentials. Given that inhibition located on the path between the synapse and the axon is particularly potent (Jack et al., 1983; Koch et al., 1983), the section of dendrite between the soma and the site of axon origin also represents a particularly effective location for inhibitory inputs. Taken together, these considerations suggest that the dendrite bearing the axon represents a privileged dendrite, since its synaptic input will have a disproportionate effect on the output of the neuron relative to inputs made onto the other dendrites. The concept of the privileged dendrite may be applicable to other neurons in the central nervous system, since as described above, many neuronal types have been found where the axon originates not from the soma but from a dendrite. Further study will be necessary to determine whether there are any special features of the synaptic input to the axon-bearing dendrite.

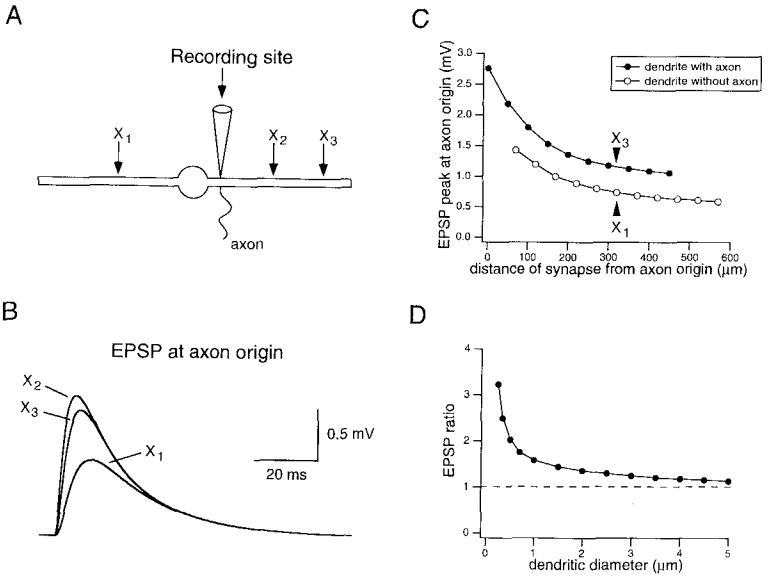

Figure 8. Consequences of the Dendritic Origin of the Axon on Synaptic Integration

(A) Schematic representation of a model neuron with the axon emerging 50 μm from the soma. The location of three synapses is shown, at X2 and X3 on the axon-bearing dendrite and X1 on another dendrite. The location of the synapses was such that the physical distance of the synapse from the soma is the same for X1 and X2 (250 μm), and the physical distance of the synapse from the axon origin is the same for X1 and X3 (320 μm). See Experimental Procedures for model parameters.

(B) EPSPs recorded at the site of axon origin (50 μm from the soma) generated by the same synaptic conductance (500 pS, τ_{on} = 0.3 ms, τ_{off} = 3 ms) at the three sites indicated in (A).

(C) Peak amplitude of EPSPs at the axon origin generated by identical synaptic conductances on either the axon-bearing dendrite or one of the other dendrites as a function of the distance of the synapse from the axon. Note that EPSPs generated by synapses on the axon-bearing dendrite are always larger in amplitude at the axon origin than identical synapses made on the other dendrites at similar distances from the axon.

(D) Effect of changing dendritic diameter on the relative size, measured at the axon origin, of EPSPs generated by a synapse at X3 compared with a synapse at X1, expressed as a ratio of EPSP amplitude.

Minimal Attenuation of Action Potentials in the Dendritic Tree of Substantia Nigra Neurons

The action potential in substantia nigra neurons has a similar amplitude in different regions of the dendritic tree, comparable to the slight attenuation demonstrated for steady-state voltage. We have shown that the dendrites of dopamine neurons express a similar density of sodium channels to the soma, and that these channels are involved in increasing the amplitude of the action potential as it propagates in the dendritic tree. The similarly modest attenuation of action potentials in GABA neurons suggests that action potentials may also propagate actively in the dendrites of these neurons. Evidence also exists for active propagation of action potentials in the dendrites of neocortical (Stuart and Sakmann, 1994) and hippocampal pyramidal cells (Miyakawa and Kato, 1986; Turner et al., 1989; Spruston et al., 1995) and hippocampal granule cells (Jeffreys, 1978). Interestingly, the attenuation of the action potential in dopamine cells was similar in dendrites bearing the axon compared with that in dendrites lacking an axon (Figure 6D), suggesting that the sodium channel density is similar in all parts of the dendritic tree. A striking feature of the dopamine neurons that may reduce attenuation of action potentials in the dendritic tree is that their action potentials have a relatively broad shape (duration at half amplitude of approximately 2–3 ms). Consistent with this, the dendritic attenuation of the dopamine neuron action potential in the presence of TTX (Figure 7B, bottom) was much less severe than that experienced under the same conditions by the Purkinje cell action potential, which is very brief (see Stuart and Häusser, 1994).

The minimal attenuation of the action potential in the dendritic tree may have important functional consequences. In effect, the action potential acts as a signal to the dendritic tree that the axon has fired. The transient conductances activated by the action potential will briefly shunt the dendritic tree and suppress ongoing synaptic integration. On the other hand, the transient depolarization may also serve to relieve the voltage-dependent block of NMDA receptor channels and amplify the signals mediated by these channels. Furthermore, since the action potential of dopamine neurons has a pronounced calcium component on its falling phase (Yung et al., 1991), this provides for a means of transducing the electrical signal into a chemical signal of longer duration (Ross and Werman, 1987; Jaffe et al., 1992; Markram et al., 1995; Schiller et al., 1995).

Finally, the back-propagating action potential may be important for the dendritic release of dopamine. Dopamine is stored in the dendrites of dopamine neurons and has been shown to be released within the substantia nigra both spontaneously (Lacey et al., 1990) and in response to electrical and chemical stimulation (Geffen et al., 1976; Korf et al., 1976; Cheramy et al., 1981; Johnston and North, 1992; Rice et al., 1994). The origin of the released dopamine is thought to be from the dendrites, as the axons of the dopamine neurons lack collaterals or plentiful varicosities within the substantia nigra. Although the mechanism of dopamine release is unclear, it appears to be Ca^{2+}- and voltage-dependent (Cheramy et al., 1981; Rice et al., 1994) and is blocked by TTX (Santiago et al., 1992), suggesting that the back-propagating action potential may

provide the stimulus. The released dopamine can have at least two possible effects: hyperpolarization of the same dopamine neuron, or its immediate neighbors, or both, via activation of D_2 autoreceptors (Lacey et al., 1987; Lacey et al., 1990), and presynaptic enhancement of synaptic inhibition mediated by $GABA_B$ receptors (Cameron and Williams, 1993). The action potential propagating actively in the dendritic tree may therefore be the signal that mediates the negative feedback of the dopamine neuron on its own activity.

Experimental Procedures

Recording from Visualized Neurons

Recordings were made from substantia nigra neurons in the pars compacta region, which contains the highest density of dopamine cell bodies, as well as from the region of the pars reticulata immediately underlying the pars compacta, in coronal slices, 300 μm thick, from the midbrain of 14- to 25-day-old Wistar rats. Neurons were visualized with IR-DIC optics (Stuart et al., 1993). Simultaneous whole-cell recordings were made from the soma and dendrites of the same neuron under direct visual control by using previously described techniques (Stuart and Sakmann, 1994; Stuart and Häusser, 1994). Recordings were only made from cells where it was possible to follow the dendrite from the soma to the site of the dendritic recording, and the distance of dendritic recordings from the soma was measured directly from the video monitor by using the Argus 10 system (Hamamatsu, Hamamatsu City, Japan). The location of the dendritic pipette was then recorded by taking a picture of the imaged neuron with a video printer (P68E, Mitsubishi, Tokyo, Japan) for later comparison with biocytin staining of the same cell (see below). Slices were perfused continuously with an oxygenated Ringer's solution containing the following: 125 mM NaCl, 25 mM $NaHCO_3$, 25 mM glucose, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ (pH 7.4 with 5% CO_2), and experiments were performed at room temperature (22°–24°C) unless otherwise indicated. Current-clamp recordings were made with two identical microelectrode amplifiers (Axoclamp 2A, Axon Instruments, Foster City, CA) by using bridge balance and capacitance neutralization; recordings were terminated if the access resistance exceeded 100 MΩ. Voltage was filtered at 10 kHz and sampled at 50 kHz with a VME bus computer (Motorola Delta series 1147, Tempe, AZ). For whole-cell current-clamp recordings, patch pipettes (4–7 MΩ for somatic recordings, 8–10 MΩ for dendritic recordings) were filled with a K-gluconate-based intracellular solution (120 mM K-gluconate, 20 mM KCl, 10 mM HEPES, 10 mM EGTA, 2 mM $MgCl_2$, and 2 mM Na_2-ATP [pH 7.3]), and inhibitory synaptic potentials were usually blocked by 20–40 μM bicuculline methochloride (Sigma); no significant differences were noted when inhibition was left intact. All values given in the text are quoted as mean ± SEM.

Neurons were usually spontaneously active and were hyperpolarized to −55 to −60 mV by application of holding current. Action potentials were evoked by depolarizing current pulses and their amplitude measured from a baseline set at threshold. Activation of excitatory postsynaptic potentials was achieved by applying brief voltage pulses (200 μs, up to 50 V amplitude) to an extracellular patch pipette (tip diameter, 5–10 μm) filled with Ringer's solution or a tungsten wire bipolar stimulating electrode. Antidromic stimulation was achieved with the same electrodes, sometimes in the presence of cadmium (200 μM) to prevent synaptic transmission; care was taken to prevent direct dendritic stimulation by placing the stimulation pipette far from the recorded neuron. The degree of sag of the membrane potential back toward the resting potential during hyperpolarization was expressed as a ratio of the steady-state versus peak voltage during a large (0.3–0.5 nA) hyperpolarizing somatic current pulse. For measurement of steady-state voltage attenuation, long (500–1200 ms), small hyperpolarizing somatic current pulses (50 or 100 pA) were used and recordings only accepted if accurate bridge balance (within 5%) could be made at the soma; the accuracy of the bridge balance could be confirmed independently by simultaneous double somatic recording.

Outside-Out Patch Recording

Both somatic and dendritic outside-out patch-clamp recordings were made by using a patch-clamp amplifier (EPC-7, List, Darmstadt, Federal Republic of Germany) with 8–10 MΩ patch pipettes filled with the K-gluconate-based intracellular solution (see above). Voltage-activated currents were evoked by depolarizing steps to a test potential of −10 mV from a prepotential of −120 mV to evoke maximal sodium current (patches were held at −80 mV and stepped for 50 ms to −120 mV prior to stepping to the test potential). Leak and capacitive currents were subtracted on-line and the peak inward current measured from the average of 10–20 sweeps.

Simulated Action Potential Experiment

To preserve the normal direction of action potential propagation, experiments where a simulated action potential waveform was generated at the soma were only performed in recordings where the action potential occurred first at the somatic pipette. Somatic voltage recordings were made with a patch-clamp amplifier (EPC-7), using low-resistance (1–3 MΩ) patch pipettes, and dendritic recordings with a microelectrode amplifier (Axoclamp 2A). Somatic and dendritic action potentials were first evoked by a depolarizing current pulse. The slice was then perfused with 1 μM TTX to block all voltage-activated sodium channels and the previously recorded somatic action potential used as the voltage command during somatic whole-cell voltage clamp. In voltage-clamp mode, access resistances (≤10 MΩ) were compensated by more than 70%. The actual voltage change during this simulated somatic action potential was later recorded at the soma with a second somatic patch pipette (n = 4), and the peak amplitude was found to be within 10%–15% of the amplitude of the evoked somatic action potential.

Filling and Morphological Reconstruction

Neurons were filled with biocytin by including 5 mg/ml biocytin in the pipette solution. At the end of the recording, the pipettes were withdrawn from the neuron to form outside-out patches, and the slices were immediately fixed in cold 4% paraformaldehyde in phosphate buffer solution (0.1 M [pH 7.2]). Biocytin-filled neurons were visualized by using the avidin-biotinylated HRP complex reaction (Vectastain ABC Elite kit, Vector Laboratories) with 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma) as a chromogen. Visualized cells were reconstructed by using a light microscope (Axioplan, Zeiss) with a camera lucida attachment (Zeiss). Axons were identified independently by three investigators on the basis of a variety of characteristics (see below). The identification was carried out without knowledge of the dendrite from which the recording had been made. The axon was the thinnest process (≤0.5 μm), nontapering after an initial taper. In dopamine neurons, it was usually also the longest process emerging from the neuron and followed a wiggly, often tortuous path before exiting from the slice. Occasional varicosities were detected, and the spines or filaments sparsely studding the dopamine neuron dendrites were absent. The axon of dopamine neurons was always nonbranching, whereas that of GABA neurons often branched extensively close to the axon origin, forming a dense network of axon collaterals (see Figure 2); such a pattern of arborization is typical of GABA neurons in other brain areas.

Simulations

Modeling of the effects of the dendritic origin of the axon on the amplitude of synaptic potentials arriving at the axon from synapses located at different dendritic sites was performed by use of the compartmental modeling program NEURON (Hines, 1993) running on a Sun SPARC station 2 (SunOS 4.1.2). A simple passive model (six compartments) was used with the axon (200 μm long, 1 μm in diameter) emerging 50 μm from the soma from one of the four dendrites (each 500 μm long, 1 μm in diameter). The soma was modeled as a compartment with a diameter of 20 μm and a length of 20 μm. Specific membrane resistivity (R_m), internal resistivity (R_i), and capacitance were usually set to 20,000 Ωcm², 200 Ωcm, and 1 μF/cm², respectively, yielding an input resistance (measured at the soma) of 306 MΩ and a membrane time constant of 20 ms. The resting membrane potential was set to −65 mV. EPSPs were simulated by a conductance change (reversal potential of 0 mV) with a time course defined by rising and decaying

exponentials of 0.3 and 3.0 ms, respectively. Synapses were located on either the axon-bearing dendrite or one of the other dendrites. To determine how robust the findings were, R_m was varied over the range from 5000 to 50,000 Ωcm^2, R_i was varied from 50 to 300 Ωcm, and dendritic diameters were varied between 0.5 μm and 5.0 μm.

Acknowledgments

We thank Yitzhak Schiller and Nelson Spruston for their comments on the manuscript. We would also like to thank Wing-Ho Yung, whose visit to the lab inspired this project. M. H. and G. S. are grateful to the Alexander von Humboldt Stiftung for financial support.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *"advertisement"* in accordance with 18 USC Section 1734 solely to indicate this fact.

Received May 30, 1995; revised July 5, 1995.

References

Adams, P. R. (1992). The platonic neuron gets the hots. Curr. Biol. *2*, 625–627.

Amaral, D. G. (1978). A Golgi study of cell types in the hilar region of the hippocampus in the rat. J. Comp. Neurol. *182*, 851–914.

Andersen, P. (1960). Interhippocampal impulses. II. Apical dendritic activation of CA1 neurons. Acta Physiol. Scand. *48*, 178–208.

Bernander, O., Douglas, R. J., Martin, K. A., and Koch, C. (1991). Synaptic background activity influences spatiotemporal integration in single pyramidal cells. Proc. Natl. Acad. Sci. USA *88*, 11569–11573.

Cameron, D. L., and Williams, J. T. (1993). Dopamine D1 receptors facilitate transmitter release. Nature *366*, 344–347.

Cheramy, A., Leviel, V., and Glowinski, J. (1981). Dendritic release of dopamine in the substantia nigra. Nature *289*, 537–542.

Coombs, J. S., Curtis, D. R., and Eccles, J. C. (1957). The interpretation of spike potentials of motoneurons. J. Physiol. *139*, 198–231.

Feldman, M. L., and Peters, A. (1978). The forms of non-pyramidal neurons in the visual cortex of the rat. J. Comp. Neurol. *179*, 761–794.

Fuortes, M. G. F., Frank, K., and Becker, M. C. (1957). Steps in the production of motoneuron spikes. J. Gen. Physiol. *40*, 735–752.

Geffen, L. B., Jessel, T. M., Cuello, A. C., and Iversen, L. L. (1976). Release of dopamine from dendrites in rat substantia nigra. Nature *260*, 258–260.

Grace, A. A. (1988). In vivo and in vitro intracellular recordings from rat midbrain dopamine neurons. Ann. NY Acad. Sci. *537*, 51–76.

Grace, A. A., and Bunney, B. S. (1983). Intracellular and extracellular electrophysiology of nigral dopaminergic neurons. II. Action potential generating mechanisms and morphological correlates. Neuroscience *10*, 317–331.

Grace, A. A., and Onn, S. P. (1989). Morphology and electrophysiological properties of immunocytochemically identified rat dopamine neurons recorded in vitro. J. Neurosci. *9*, 3463–3481.

Graybiel, A. M. (1993). Functions of the nigrostriatal system. Clin. Neurosci. *1*, 12–17.

Gulyas, A. I., Miettinen, R., Jacobowitz, D. M., and Freund, T. F. (1992). Calretitinin is present in non-pyramidal cells of the rat hippocampus. I. A new type of neuron specifically associated with the mossy fibre system. Neuroscience *48*, 1–27.

Hines, M. (1993). NEURON: a program for simulation of nerve equations. In Neural Systems: Analysis and Modelling, F. Eeckman, ed. (Norwell, Massachusetts: Kluwer Academic Publishers), pp. 127–136.

Jack, J. J. B., Noble, D., and Tsien, R. W. (1983). Electric Current Flow in Excitable Cells, Second Edition (Oxford: Oxford University Press).

Jaffe, D. B., Johnston, D., Lasser-Ross, N., Lisman, J. E., Miyakawa, H., and Ross, W. N. (1992). The spread of Na^+ spikes determines the pattern of dendritic Ca^{2+} entry into hippocampal neurons. Nature *357*, 244–246.

Jefferys, J. G. R. (1979). Initiation and spread of action potentials in granule cells maintained in vitro in slices of guinea-pig hippocampus. J. Physiol. *289*, 375–388.

Johnston, S. W., and North, R. A. (1992). Two types of neurone in the rat ventral tegmental area and their synaptic inputs. J. Physiol. *450*, 455–468.

Juraska, J. M., Wilson, C. J., and Groves, P. M. (1977). The substantia nigra of the rat: a Golgi study. J. Comp. Neurol. *172*, 565–600.

Koch, C., Poggio, T., and Torre, V. (1983). Nonlinear interactions in a dendritic tree: localization, timing, and role in information processing. Proc. Natl. Acad. Sci. USA *80*, 2799–2802.

Korf, J., Zieleman, M., and Westerink, B. H. C. (1976). Dopamine release in the substantia nigra. Nature *260*, 257–258.

Lacey, M. G., Mercuri, N. B., and North, R. A. (1987). Dopamine acts on D2 receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta. J. Physiol. *392*, 397–416.

Lacey, M. G., Mercuri, N. B., and North, R. A. (1990). Actions of cocaine on rat dopaminergic neurones in vitro. Br. J. Pharmacol. *99*, 731–735.

Llinas, R. R. (1988). The intrinsic electrophysiological properties of mammalian neurons: insight into central nervous function. Science *242*, 1654–1664.

Markram, H., Helm, P. J., and Sakmann, B. (1995). Dendritic calcium transients evoked by single back-propagating action potentials in rat neocortical pyramidal neurones. J. Physiol. *485*, 1–20.

Miyakawa, H., and Kato, H. (1986). Active properties of dendritic membrane examined by current source density analysis in hippocampal CA1 pyramidal neurons. Brain Res. *399*, 303–309.

Mugnaini, E., and Oertel, W. H. (1985). An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry. In Handbook of Chemical Neuroanatomy, Volume 4, A. Björklund and T. Hökfelt, eds. (Amsterdam: Elsevier), pp. 436–622.

Palay, S. L., and Chan-Palay, V. (1974). Cerebellar Cortex (Berlin: Springer-Verlag).

Palay, S. L., Sotelo, C., Peters, A., and Orkand, P. M. (1968). The axon hillock and the initial segment. J. Cell Biol. *38*, 193–201.

Peters, A., and Kimerer, L. M. (1981). Bipolar neurons in rat visual cortex: a combined Golgi–electron microscope study. J. Neurocytol. *10*, 921–946.

Peters, A., and Regidor, J. (1981). A reassessment of the forms of nonpyramidal neurons in area 17 of cat visual cortex. J. Comp. Neurol. *203*, 685–716.

Peters, A., Proskauer, C. C., and Kaiserman-Abramof, I. R. (1968). The small pyramidal neuron of the rat cerebral cortex. The axon hillock and initial segment. J. Cell Biol. *39*, 601–619.

Preston, R. J., McCrea, R. A., Chang, H. T., and Kitai, S. T. (1981). Anatomy and physiology of substantia nigra and retrorubral neurons studied by extra- and intracellular recording and by horseradish peroxidase labelling. Neuroscience *6*, 331–344.

Rall, W. (1977). Core conductor theory and cable properties of neurons. In Handboook of Physiology: The Nervous System, Volume 1, E. Kandel, ed. (Bethesda, Maryland: American Physiological Society), pp. 39–97.

Rapp, M., Yarom, Y., and Segev, I. (1992). The impact of parallel fibre background activity on the cable properties of cerebellar Purkinje cells. Neural Comput. *4*, 518–533.

Regehr, W. G., and Armstrong, C. M. (1994). Where does it all begin? Curr. Biol. *4*, 436–439.

Rice, M. E., Richards, C. D., Nedergaard, S., Hounsgaard, J., Nicholson, C., and Greenfield, S. A. (1994). Direct monitoring of dopamine and 5-HT release in substantia nigra and ventral tegmental area in vitro. Exp. Brain Res. *100*, 395–406.

Ross, W. N., and Werman, R. (1987). Mapping calcium transients in the dendrites of Purkinje cells from the guinea-pig cerebellum in vitro. J. Physiol. *389*, 319–336.

Santiago, M., Machado, A., and Cano, J. (1992). Fast sodium channel dependency of the somatodendritic release of dopamine in the rat's brain. Neurosci. Lett. *148*, 145–147.

Schiller, J., Helmchen, F., and Sakmann, B. (1995). Spatial profile of dendritic calcium transients evoked by action potentials in rat neocortical neurones. J. Physiol., in press.

Sloper, J. J., and Powell, T. P. S. (1979). A study of the axon initial segment and proximal axon of neurons in the primate motor and somatic sensory cortices. Phil. Trans. Roy. Soc. (Lond). B *285*, 173–197.

Spencer, W. A., and Kandel, E. R. (1961). Electrophysiology of hippocampal neurons. IV. Fast prepotentials. J. Neurophysiol. *24*, 272–285.

Spruston, N., Jaffe, D. B., and Johnston, D. (1994). Dendritic attenuation of synaptic potentials and currents: the role of passive membrane properties. Trends Neurosci. *17*, 161–166.

Spruston, N., Schiller, Y., Stuart, G., and Sakmann, B. (1995). Activity-dependent action potential invasion and calcium influx into hippocampal CA1 dendrites. Science *268*, 297–300.

Stuart, G. J., Dodt, H.-U., and Sakmann, B. (1993). Patch-clamp recordings from the soma and dendrites of neurons in brain slices using infrared videomicroscopy. Pflügers Arch. *423*, 511–518.

Stuart, G., and Häusser, M. (1994). Initiation and spread of sodium action potentials in cerebellar Purkinje cells. Neuron *13*, 703–712.

Stuart, G. J., and Sakmann, B. (1994). Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. Nature *367*, 69–72.

Tepper, J. M., Sawyer, S. F., and Groves, P. M. (1987). Electrophysiologically identified nigral dopaminergic neurons intracellularly labelled with HRP: light microscopic analysis. J. Neurosci. *7*, 2794–2806.

Turner, R. W., Meyers, D. E. R., and Barker, J. L. (1989). Localization of tetrodotoxin-sensitive field potentials of CA1 pyramidal cells in the rat hippocampus. J. Neurophysiol. *62*, 1375–1387.

Yung, W. H., Häusser, M. A., and Jack, J. J. B. (1991). Electrophysiology of dopaminergic and non-dopaminergic neurones of the guinea-pig substantia nigra pars compacta in vitro. J. Physiol. *436*, 643–667.