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REVIEW

THE SIGNIFICANCE OF DENDRITIC RELEASE OF TRANSMITTER AND PROTEIN IN THE SUBSTANTIA NIGRA

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CRITIQUES

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Abstract—The transmitter dopamine and the protein acetylcholinesterase are released within the substantia nigra from the dendrites of nigrostriatal neurons. These phenomena do not correspond to the familiar events involved in information transfer at the classic axonal synapse. It is possible that both dopamine and acetylcholinesterase, released in the substantia nigra, are acting in a novel, perhaps synergistic, fashion as "neuromodulators". A hypothetical mechanism of neuromodulation by dendritically released material is discussed, in the light of recent findings on the morphology and physiology of nigrostriatal neurons.

I. OVERVIEW

Within the last ten years, the concept of dendritic release has rapidly gained credence. Studies describing this phenomenon have been most intensively pursued in a particular neuronal population in the brain; dopamine (DA)-containing nigrostriatal cells. Since degeneration of these neurons is so closely associated with Parkinson's Disease, there is a cogent need to understand the mechanisms by which nigrostriatal cells function and participate in signal transfer. It could be the case that dendritic release underlies one or more such mechanism.

In 1975, Bjorkland and Lindvall reported the presence of DA in nigrostriatal cell dendrites and postulated that there might be a "hitherto unknown function of DA as a transmitter in a dendro–dendritic synapse". Interestingly enough, this hypothesis was based on a novel finding by Kreutzberg *et al.* (1974, 1975). These workers had provided morphological evidence that dendritic secretion could occur from neurons of the guinea pig facial nucleus; even more surprisingly, the material released from the dendrites was, in this case, the normally membrane-bound enzyme, acetylcholinesterase (AChE). The substantia nigra is also rich in AChE, even though there is very little acetylcholine (ACh) or choline acetyltransferase [ChAT] (Silver, 1974); indeed there is increasing evidence that in brain areas such as the substantia nigra, AChE could have a second, non-cholinergic function (Greenfield, 1984a). A single soluble form of AChE is released from the substantia nigra (Greenfield and Shaw, 1982). Since release of AChE is not a normal or necessary feature of the cholinergic synapse, this event could underlie a second role for the enzyme (Greenfield, 1984b).

It is possible that this secretion of AChE occurs, like DA, from nigrostriatal cell dendrites (Greenfield *et al.*, 1983a). As we shall see later, certain similarities can be drawn between dendritic release of AChE and DA, and it is possible that the secreted protein and transmitter interact. Nonetheless, studies on dendritic release of DA alone, are both more established and extensive: we shall explore first the conclusions arising from this work.

The evidence that DA is released from nigrostriatal cell dendrites is compelling (Cuello and Iversen, 1978; Glowinski and Cheramy, 1981; Cheramy *et al.*, 1981). However, in characterizing dendritic release of DA it seems that there are certain problems: dendritic release of DA is not directly comparable with the familiar release of transmitter from the classic axonal terminal. This descrepancy has made workers cautious in ascribing a function to DA released from dendrites in the substantia nigra, hence "... DA could be released from dendritic ramifications of

nigral neurons in a more unspecific fashion . . ." (Bjorklund and Lindvall, 1975). "These observations make it unlikely that DA released from dendrites exerts its effects on typical synapses, but rather favours a more general role, affecting a larger area," (Hefti and Lichtensteiger, 1978); ". . . by their dendrites, the dopaminergic neurons may modulate information delivered by striato- or pallidonigral fibres to nigral neurons . . ." (Glowinski and Cheramy, 1981); ". . . dendrites belonging to the dopaminergic neurons of the nigrostriatal pathway, in addition to their main role in the reception of signals from other neurons, may have other functions involving the release of dopamine. This does not necessarily imply the presence of specialized dendrodendritic or dendroaxonic synapses, but may involve other types of neuronal interaction which have not been established," (Cuello and Iversen, 1978).

Clearly, these diverse workers are suggesting that DA released from dendrites of nigrostriatal neurons is acting in an uncharacteristically non-defined manner more typical of a "neuromodulator": we are, however no nearer to understanding the nature of this modulation.

The one attempt at ascribing a function for dendritic release of DA was made earlier, in 1975, by Groves *et al.*: the "autoreceptor hypothesis". When DA agonists are administered systemically (Bunney *et al.*, 1973) or microinfused into the substantia nigra (Groves *et al.*, 1975), the firing rate of nigrostriatal cells is reduced. Application of DA antagonists has the reverse effects (Groves *et al.*, 1975). It has thus been postulated that nigrostriatal neurons themselves possess inhibitory DA receptors, "autoreceptors". These autoreceptors could mediate a local negative feedback within the substantia nigra, independent of efferent and afferent connections (Groves *et al.*, 1975). In a physiological situation, this local negative feedback i.e. activation of autoreceptors, could be produced by DA released from dendrites (Fig. 1).

However two critical questions are still unanswered; (1) what are the physiological conditions for dendritic release of DA? (2) Once released, does the transmitter act on specific autoreceptors of one or more particular target neurons, as at the classic axonal synapse, or does it inhibit whole populations of nigrostriatal neurons indiscriminately? Until these questions are answered, the "autoreceptor theory"

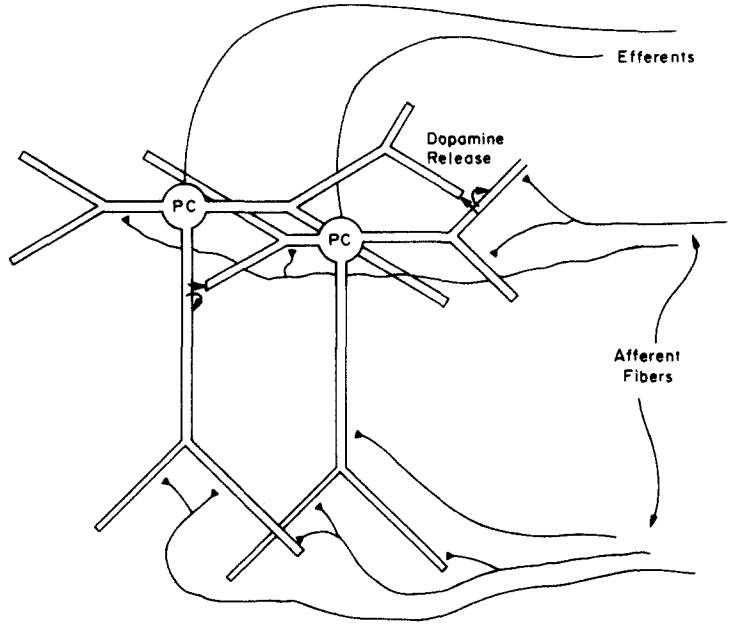

Fig. 1. A schematic diagram representing the influence of dopamine released from the dendrites of dopaminergic neurons of pars compacta of the substantia nigra (PC). Dopamine is theorized to be released from points of dendritic synaptic apposition with accompanying membrane specializations and accumulations of synaptic vesicles. Its presynaptic and postsynaptic actions are theorized to be inhibitory. From Groves *et al.* (1979). Reproduced by permission of Pergamon Press.

does not give an adequate account of the role of dendritic DA release in nigrostriatal cell functioning. In an attempt to discover the significance of dendritic release of DA, and indeed AChE, we will consider the phenomena in the light of what we now know concerning the morphology and physiology of nigrostriatal neurons.

II. CELLULAR AND SUBCELLULAR LOCALISATION OF DA AND AChE IN THE SUBSTANTIA NIGRA

It is well established that DA is contained in nigrostriatal neurons (Dray, 1979). The somata of these cells are distributed mainly in the dorsal layer, "pars compacta", of the substantia nigra (Fig. 2A). Although some dendrites emanating from the cell bodies are confined to the pars compacta, others project for long distances (as much as 1000 μ) down into the central zone of the substantia nigra, the "pars reticulata" (Juraska *et al.*, 1977). Curiously enough, virtually all dopaminergic nigrostriatal cells contain AChE (Fig. 2B) even though cells in this region do not contain ChAT (Kimura *et al.*, 1982; Levey *et al.*, 1983) and there is no evidence for cholinergic afferents (Butcher and Woolf, 1982; Greenfield, 1984a). Non-dopaminergic neurons in the pars reticulata also contain AChE (Fig. 2B) but ultrastructural studies reveal a difference in subcellular localization. In pars reticulata neurons, AChE is present only in the RER and at the nuclear envelope; however in nigrostriatal neurons the enzyme is, in addition, detectable in the Golgi apparatus and at the plasma membrane (Fig. 3).

Since, in cultured quail muscle, AChE acquires complex sugars in the Golgi apparatus prior to externalization (Rotundo, 1984), these morphological observations would seem to indicate that AChE could be released only from nigrostriatal neurons. In fact, these cells are characterized by large amounts of RER, which suggests an appropriately high rate of protein synthesis (Domesick *et al.*, 1983). Studies on the adrenal gland have shown that soluble, i.e. releasable AChE, is stored in SER (Somogyi *et al.*, 1975) and AChE is indeed present in the SER of nigral cell dendrites (Henderson and Greenfield, 1984).

The storage of DA in nigral cell dendrites is surprisingly different from that at the axon terminal, in that it does not appear to be contained in vesicles (Wassef *et al.*, 1983): instead, studies using 5-OHDA suggest that, like AChE, dendritic DA is contained in SER (Mercer *et al.*, 1979). Although the SER has already been shown to be an alternative storage organelle for catecholamines in sympathetic nerve axons (Tranzer, 1972), no one is sure why dendritic DA should be non-vesicular in the substantia nigra. Even if the SER represents an "immature" compartment from which vesicles are subsequently formed (cf. Droz *et al.*, 1975), we should question why vesicles are detectable in terminals but not dendrites of nigrostriatal neurons. It seems that there would have to be at least a quantitative, if not qualitative, difference release of DA from the terminal compared to the dendrites (cf. Wassef *et al.*, 1981; Cuello and Iversen, 1978). If, however, the SER actually is the final compartment from which DA is released, at least two speculations are possible. First, dendritic release of DA may not be "quantal" but perhaps involve a more sustained process for which the SER is a more appropriate storage compartment. This idea corresponds well to the neurochemical observations of Cheramy *et al.* (1981) "... we already know that the changes in the dendritic release of DA are generally of long duration, even when they are induced by physiological treatments such as sensory stimuli. Such a phenomenon explains the prolonged modification of the activity of the dopaminergic cells observed in several situations". Secondly, since AChE possibly destined for release is also stored in the SER of nigral cell dendrites (Henderson and Greenfield, 1984), it may be necessary for an as yet unknown reason, that the transmitter and protein be co-stored to be co-released (cf Greenfield and Shaw, 1982). Nonetheless, although the non-vesicular nature of the storage of dendritic DA is puzzling, it should perhaps be regarded not as a problem, but as a clue in furthering our understanding of dendritic release: we should not be too surprised if properties of the release process itself are not comparable with events at the classic axonal synapse.

III. THE RELEASE PROCESS OF DA AND AChE IN THE SUBSTANTIA NIGRA

Release of DA from the substantia nigra has been observed in both superfusates of nigral slices (Geffen *et al.*, 1976; Cuello and Iversen, 1978) and via "push–pull" cannulae in the anaesthetized animal (Glowinski and Cheramy, 1981). Using similar preparations, release of AChE has also been observed from the substantia nigra both *in vitro* (Cuello *et al.*, 1981) and *in vivo* (Greenfield and Shaw, 1982; Greenfield *et al.*, 1983a).

Evidence that release of DA in the substantia nigra is probably dendritic can be summarized as follows: (1) Nigrostriatal cell dendrites are capable of DA

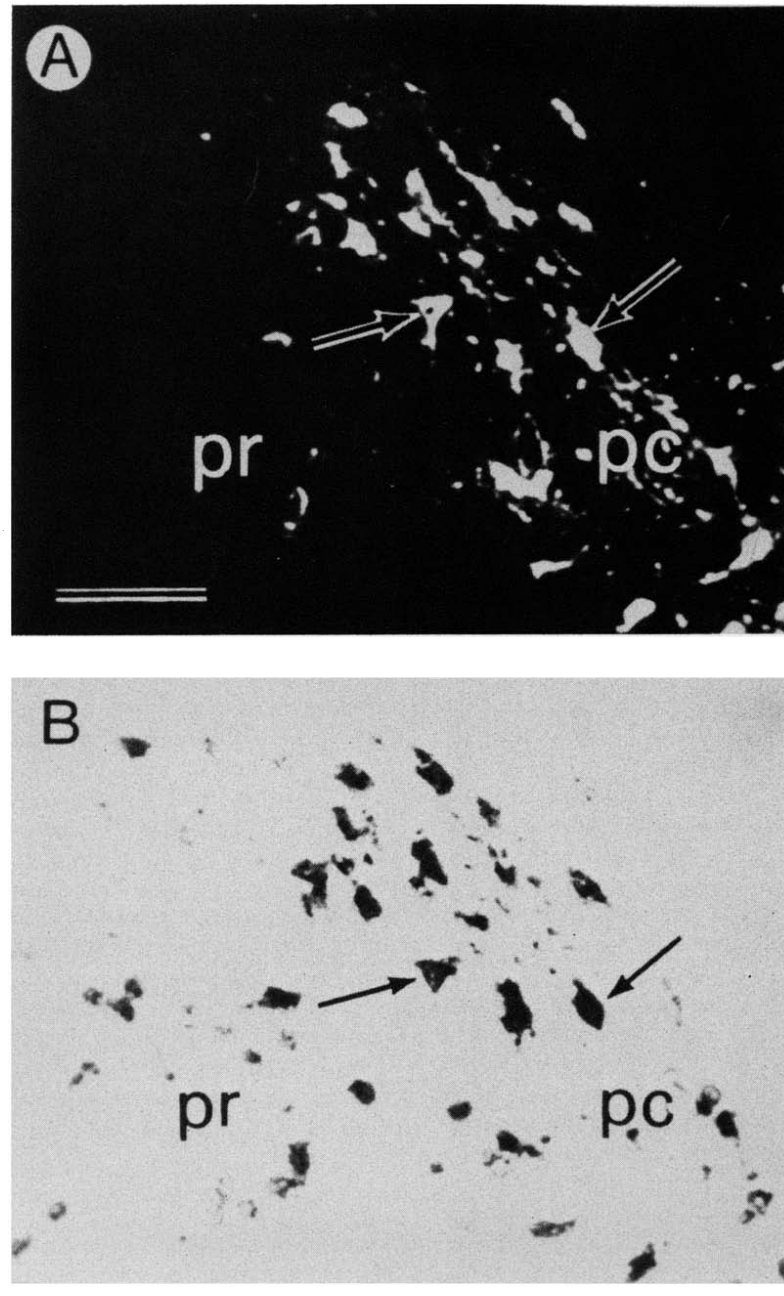

Fig. 2. Catecholamine (A) and AChE-containing (B) somata in the substantia nigra demonstrated sequentially on the same transverse brain section. Arrows point to corresponding cell bodies. Catecholamines demonstrated with glyoxylic acid (de la Torre and Surgeon, 1976). Pharmohistochemical regimen for AChE (Butcher and Bilezikjian, 1975; Butcher Talbot and Bilezikjian, 1975). Rat sacrificed 4 h after DFP. pc, pars compacta; pr, pars reticulata. Scale, 100 μm. From Butcher and Woolf (1982). Reproduced by permission of ANKHO International Inc.

uptake (Bjorklund and Lindvall, 1975; Cuello and Iversen, 1978). (2) There are no dopaminergic axon terminals or collaterals in the substantia nigra (Wassef *et al.*, 1981). (3) In the isolated pars reticulata, which contains only dopaminergic dendrites, there is a more marked release of DA than in the

somata-rich pars compacta (Cuello and Iversen, 1978). (4) Although it is K^+-evoked and Ca^{2+}-dependent, DA release in the substantia nigra is not decreased by agents that block action potentials in nigrostriatal neurons i.e. tetrodotoxin [TTX] (Cheramy *et al.*, 1981) or γ-butyrolactone (Hefti and Lichtensteiger, 1978).

A single soluble isoenzyme of AChE is also released from the substantia nigra (Greenfield and Shaw, 1982) in a K^+ evoked, Ca^{2+}-dependent manner (Greenfield *et al.*, 1983b). Following a large 6-OHDA lesion of the nigrostriatal pathway, this release is virtually abolished (Greenfield *et al.*, 1983a). Hence it seems that, despite the presence of AChE in pars reticulata neurons, release of the enzyme occurs exclusively from dopaminegic nigrostriatal neurons—a finding corroborated by the more recent ultrastructural observations (Fig. 3). Hence, as argued above, the absence of dopaminergic axon terminals and collaterals in the substantia nigra, would leave only the somata and dendrites as possible origins for released AChE. Additional reasons for believing that the enzymes release is at least in part dendritic are that firstly, nigrostriatal cell dendrites contain AChE (Butcher and Woolf, 1982; Henderson and Greenfield, 1984) and, secondly, as in the case of DA, release is not blocked by TTX or γ-hydroxybutyrate (Weston and Greenfield, 1985).

In view of the inefficacy of TTX it seems that dendritic release of neither DA nor AChE can be directly linked to somatofugal action potentials, either intrinsically or synaptically generated. The relay of synaptically transmitted signals from soma to dendrite is in any case unlikely as there is a paucity of synaptic boutons on nigrostriatal cell somata (Rinvik and Grofova, 1970 and Fig. 3). What then could trigger release of DA and AChE from dendrites? One hypothesis could be simply that release of the transmitter and protein is not involved with individual events in nigral neuronal networks and that the phenomenon occurs as an unmodifiable, exocytosis. This theory seems very unlikely: studies using drugs related to endogenous nigral transmitter systems, have shown that release of DA and AChE can be modified within the substantia nigra (Table 1). It is particularly striking, however, that these drug-induced modifications in release do not parallel in any consistent fashion, changes in nigrostriatal cell firing rate following application of the respective drugs (Table 1). This observation is not particularly surprising in the light of the resistance of nigral DA and AChE release to TTX, and further indicates another important difference between dendritic and axonal release.

It seems very likely therefore that, in a physiological situation, dendritic release of AChE and DA is governed by a Ca^{2+} dependent yet Na^+-independent process. By means of intracellular recording from nigrostriatal neurons *in vitro*, we have localised a conductance in the distal segment of the dendrites (Llinas *et al.*, 1984). This conductance is carried by Ca^{2+} ions and is resistant to TTX. In a physiological situation, this conductance may possibly be activated by synaptic input. There are no reports tracing the distribution of boutons along the entire length of identified dopaminergic dendrites. Nonetheless, we know that nigrostriatal cell somata have very few boutons in contact with their surface membrane (Fig. 3), (Grofova and Rinvik, 1970) and that identified nigral dopaminergic dendrites are the target for striatal afferent inputs (Wassef *et al.*, 1981). Furthermore, Rinvik and Grofova (1970), claim "In appropriate sections it seems that a rapidly increasing portion of the dendritic surface is covered by boutons

Table 1. Effects of various substances on firing rate of nigrostriatal cells and on release of DA or AChE in the substantia nigra

Substance	Firing rate	Release DA	Release AChE
Tetrodotoxin	↓[1]	↑[8]	No effect [11]
Gammahydroxybutyrate	↓[2]	No effect [9]	↑[11]
Amphetamine	↓[3]	↑[8]	↑[12]
Apomorphine	↓[4]	—	No effect [11]
Haloperidol	↑[3]	—	↑[11]
ACh	↑[5]	↑[10]	*in vivo* [11] no effect *in vitro* [13]
GABA	↓[6]	No effect [10]	No effect (K^+-stimulated) [13]
Substance P	↑[5]	↓[10]	No effect [13]
5-HT	↓[7]	↑[10]	—

References: [1] Llinas and Sugimori (1980); [2] Walters *et al.* (1973); [3] Groves *et al.* (1975); [4] Bunney *et al.* (1973); [5] Walker *et al.* (1976); [6] Scarnatti and Paccitti (1982); [7] Dray *et al.* (1976); [8] Cheramy *et al.* (1981); [9] Hefti and Lichtensteiger (1978); [10] Glowinski and Cheramy (1981); [11] Weston and Greenfield (1985); [12] Greenfield and Shaw (1982); [13] Cuello *et al.* (1981).

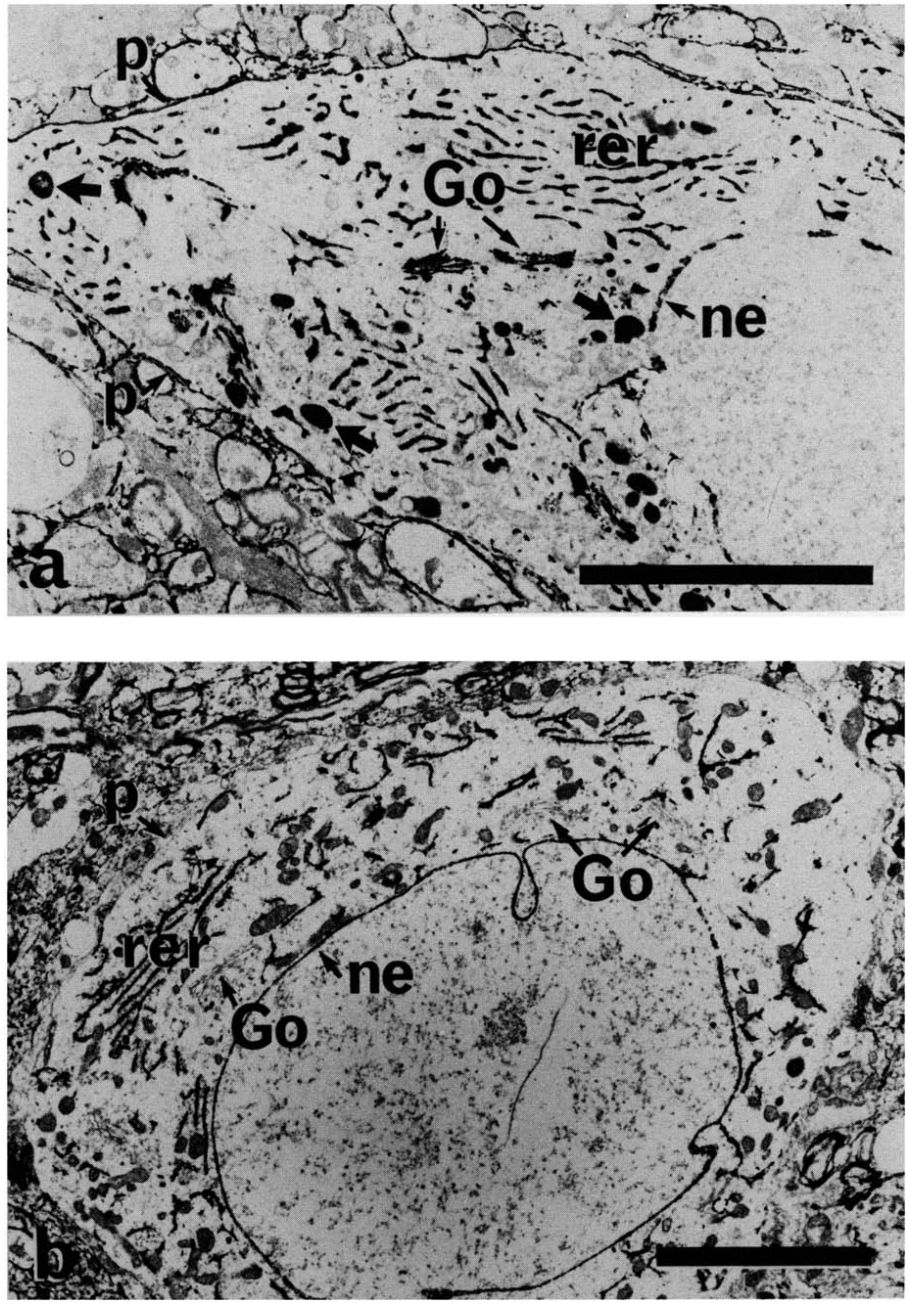

Fig. 3. (a) Electron micrograph of rat nigrostriatal cell stained for HRP (broad arrows) and AChE. AChE is localized within the nuclear envelope (ne), rough endoplasmic reticulum (RER), Golgi apparatus (Go) and at the plasma membrane (p). HRP labelling is in lysosomes (this section was not stained with lead citrate). Calibration bar—5 μm. (b) Cell in zona reticulata of rat substantia nigra, stained for AChE. There is no AChE in the Golgi apparatus (Go) and little at the plasma membrane (p). Calibration bar—5 μm. Note absence of synaptic boutons in both (a) and (b) and abundant RER in (a) compared to (b). From Henderson and Greenfield (1984). Reproduced by permission of Alan R. Liss Inc.

as one proceeds from the cell body". It seems then that synaptic activation of the TTX-resistant Ca^{2+} conductance could indeed occur in the more distal dendrites of the pars reticulata, which are electrically remote from the soma (Llinas *et al.*, 1984).

Since dendritic release is also TTX-resistant and Ca^{2+} dependent (see above), it is tempting to associate it with this conductance. Indeed, there is suggestive morphological evidence to believe that AChE, at least, could be released from nigral dendrites receiving dense synaptic contacts (Fig. 4; Henderson and Greenfield, 1984). Depolarizing synaptic signals would activate the Ca^{2+} conductance and thus facilitate dendritic release, without the signal being transmitted along the dendrite to the soma. This hypothesis would explain how dendritic release of DA and AChE can be modified by endogenous transmitter systems, but is at the same time unrelated to nigrostriatal cell discharge.

IV. FATE OF DENDRITICALLY RELEASED DA AND AChE

A third important difference between classic synaptic transmission and dendritic release appears to be that material released from nigral cell dendrites in the pars reticulata would not act on specific post-synaptic target sites. Although Wilson *et al.* (1977) reported the existence of dendro–dendritic synapses in the pars reticulata, a more quantitative study revealed that dopaminergic dendrites bearing a typical "active zone" amounted to less than 0.2% of the dendritic population (Wassef *et al.*, 1981). This view was further supported by Cuello (1982) who found "no obvious ultrastructural signs for dendro–dendritic synapses except for casual appositions".

If, as seems to be the case, dendritically released compounds do not act via classic synapses, then what is their fate? This issue is crucial to our understanding of the physiology of dendritic release: until the latency, duration and locality of the action of dendritically released compounds is understood, it will be very difficult to ascribe to them a precise function. We will consider first the diffusion rates of DA and AChE through the extracellular space, and second the location of possible target sites.

DA is a relatively small molecule (mol. wt 153.18). Using the formula for diffusion of a small molecule, i.e. that time taken (in ms) would be the square of the distance (in μm), we would expect DA to diffuse very approx at 100 μ/10 s, 500 μ/4 min and 1000 μ/17 min. Myers and Hoch (1978) microinjected various volumes of ^{14}C DA into the pars compacta of the rat substantia nigra. They found that after a 15 min period, ^{14}C activity was detectable at "0.5 or 1 mm" from the injection site; this observation agrees with the rough calculation above. This study also raises an issue central to the function of DA in the extracellular space, namely the consideration that the transmitter will be degraded/taken up/metabolised. Myers and Hoch (1978) found that whatever the volume of ^{14}C DA injected, approximately half the injected dose was lost from the brain within a minute. Hence endogenous DA released from nigral dendrites would either act on a target site very close to its site of release, or if it were to diffuse further to a relatively remote receptor, it would have to be protected in some way from the familiar mechanisms that normally remove it from the extracellular space.

There are at least three possible target sites for DA in the substantia nigra: (1) "autoreceptors" on the somata and dendrites of nigrostriatal neurons (Bunney and Aghajanian, 1973). [^{3}H]Spiroperidol binding (not coupled to DA-adenylate cyclase) is reduced following a 6-OHDA lesion (Reisine *et al.*, 1979; Quick *et al.*, 1979); (2) Pre-synaptic receptors on striatonigral afferent terminals, coupled to DA adenylate cyclase. Following a 6-OHDA lesion, adenylate cyclase activity is unaffected, but on the other hand, is reduced following destruction of striatonigral inputs (Spano *et al.*, 1976; Gale *et al.*, 1977). It seems likely that these presynaptic DA receptors are located on GABA but not Substance P containing striatal afferents, since exogenous DA elicits GABA release in nigral slices, but release of Substance P is unaffected (Reubi *et al.*, 1978). (3) Post-synaptic receptors (not cyclase linked) on non-dopaminergic, particularly nigrothalamic, output neurones in the pars reticulata. Following a 6-OHDA lesion, some [^{3}H]spiroperidol binding (not cyclase linked) remains (Reisine *et al.*, 1979; Quick *et al.*, 1979). In addition, it is known that dopaminergic dendrites ramify close to the neuropil of non-dopaminergic pars reticulata neurons (Deniau *et al.*, 1978): more significantly, iontophoresis of exogenous DA excites these cells (Ruffieux and Shultz, 1978). It is probable that this effect is directly via a DA receptor. The alternative explanation, that DA acts indirectly via release of transmitter from afferent axon terminals is unlikely: as far as we know, GABA is the only transmitter released by a pre-synaptic action of DA (Reubi *et al.*, 1978) and yet GABA has an opposite effect (i.e. strongly inhibitory) to DA, when applied to non-dopaminergic pars reticulata cells, (Grace and Bunney, 1979). In addition, it has been shown that DA can in fact *attenuate* the action

of GABA on pars reticulata neurons (Waszezak and Walters, 1983).

Via these three target sites, dendritically released DA could have various actions in the substantia nigra: inhibition of nigrostriatal neurons (Bunney and Aghajanian, 1973); increased release of GABA from striatonigral afferent terminals (Reubi *et al.*, 1978); excitation of nigrothalamic cells (Ruffieux and Shultz, 1978). However, as mentioned earlier there are very few dendrodendritic synapses in the pars reticulata, and released DA will be rapidly removed from the extracellular space. These considerations imply that, in a physiological situation, dendritic DA could only act on a very close target site e.g. the presynaptic receptor, unless the autoreceptors are near to the site of release. However there is evidence to suggest that DA released from dendrites *does* diffuse and have an action remote from its site of release.

It has been shown that amphetamine (administered systemically or via microinfusion) has a profound depressant action on nigrostriatal cells. This action is probably due to the drugs displacement of endogenous DA, since alpha-methylparatyrosine blocks the effect (Bunney and Aghajanian, 1973). Furthermore we know that the effect observed is due to direct action of DA since DA receptor blockers also prevent the inhibition. Surprisingly however, when iontophoresed onto a nigrostriatal neuron, amphetamine, unlike DA or apomorphine, has only weak inhibitory actions. There are two possible explanations for the discrepancy in action of amphetamine on nigrostriatal cell discharge, when it is administered iontophoretically or otherwise. (1) Amphetamine, unlike DA or apomorphine acts via a nigrostriato–nigral loop. Evidence for this idea comes from the finding that destruction of the connecting pathways prevents the inhibitory action of amphetamine administered systemically (Bunney and Aghajanian, 1973). However, following such a lesion, nigrostriatal neurons exhibit abnormally high firing rates and it is possible that this modification has changed the physiology of the neurons such that they are resistant to the inhibitory action of their autoreceptors. More importantly perhaps, this theory would not account for the potent inhibition seen following infusion of amphetamine locally in the substantia nigra (Groves *et al.*, 1975) (2) Nigrostriatal neurons display strong tachyphylaxis to repeated administration of DA agonists (Aghajanian and Bunney, 1973): if amphetamine was iontophoresed after DA, it might thus be expected to have an attenuated effect (Groves *et al.*, 1979). This explanation is unsatisfactory since it has been shown that amphetamine iontophoresed on an untreated cell, still has only weak actions (Bunney and Aghajanian, 1973) compared to when it is given systemically or microinfused.

A far more likely explanation for these puzzling results seems to be that the site of release of dendritic DA is remote from that of its action. Hence, if amphetamine is microinfused into the substantia nigra, endogenous DA will be displaced and then diffuse to act on the autoreceptors: however if the drug is administered by the far more localised method of iontophoresis, the target receptor may be very near (as shown by the efficacy of application of DA itself) but its site for displacing endogenous DA must be too far away for the drug to have any effect over the brief period of recording. In the case of apomorphine however, this problem would not apply, as the drug acts directly on auto receptors: hence iontophoresis of apomorphine will, as shown, have a potent inhibitory effect (Bunney and Aghajanian, 1973). If, then, dendritically released DA acts via autoreceptors to inhibit nigrostriatal cell firing, as proposed by the autoreceptor theory, the transmitter must in some way be protected, as suggested earlier.

The diffusion of AChE through the extracellular space has been investigated by Kreutzberg and Kaija (1974). Rats and guinea pigs were pretreated with DFP so that the brain tissue was depleted of endogenous enzyme. Exogenous AChE was then injected into the ventricular system. It was found that AChE could readily penetrate the ependyma and diffuse through the extracellular space at "one to two millimetres an hour". This rate is, as befits a large molecule (mol. wt approx. 260,000), much slower than that of DA. If, therefore, dendritically released AChE has any function at all, it would be most appropriate in the transmission of slow, sustained signals. A further consideration is that AChE would probably not be destroyed as readily as DA, and could thus diffuse further.

It is, at this stage, impossible to discuss target sites for dendritically released AChE: if we accept that a soluble form of the enzyme is secreted within the substantia nigra in a non-cholinergic capacity (Greenfield, 1984a, b), then we would clearly need to know its second mode of action, before we could identify the site of that action. Nonetheless there are several, probably non-exhaustive, possibilities: (1) AChE could exert its effects via the breakdown of an, as yet, unknown substrate (Chubb 1984); (2) AChE could act on nigral ACh receptors: nigrostriatal neurons are cholinoceptive (Walker *et al.*, 1976) and recent evidence suggests that AChE can interact with

the ACh receptor, independent of the hydrolysis of ACh (Fossier *et al.*, 1983); (3) either the turnover or affinity of non-cholinergic receptors, such as DA autoreceptors, could be modified by AChE; (4) AChE could act, not at the level of a receptor, but rather directly on an ion channel or, less specifically, change the properties of the cell membrane; (5) AChE could act as a carrier protein to protect DA until the transmitter has reached its higher affinity receptor. This last possibility is particularly attractive in the light of the problem raised earlier concerning the stability of dendritically released DA in diffusing for relatively long distances.

V. ACTION OF DA AND AChE RELEASED IN THE SUBSTANTIA NIGRA

The original finding (Bunney *et al.*, 1973) that DA inhibits nigrostriatal cell firing, is now well established (Dray, 1979). More recent evidence indicates that this inhibition i.e. activation of autoreceptors is accompanied by hyperpolarization of the neuron (Grace and Bunney, 1983). AChE also inhibits the firing of these cells, independent of ACh hydrolysis (Greenfield *et al.*, 1981). It is possible therefore that AChE may be released to "amplify" the DA signal (cf. Greenfield and Shaw 1982), in a way or ways such as those postulated at the end of the last section.

What could be the functional significance of this inhibitory effect of DA and AChE? It is well known that rats will exhibit circling behaviour if there is a disparity in the functional amount of DA in the two striata (Pycock, 1980) i.e. following a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway, rats turn in a direction away from the intact side, towards the side depleted of DA (Ungerstedt, 1971). Hence, one would expect that when DA agonists or AChE are infused into one substantia nigra, rats would circle towards the side of injection. Curiously, however, both the DA receptor agonist, ADTN (Andrews and Woodruff, 1982) and AChE, playing a non-cholinergic role (Greenfield *et al.*, 1984) cause circling behaviour *away* from the side of injection. These findings suggest that despite their inhibitory action on firing rate, DA and AChE in the substantia nigra are ultimately causing an increase in DA release from the striatal nerve terminals. Indeed it seems that AChE injected into the substantia nigra causes changes in striatal DA receptors consistent with a sustained increase in striatal DA release (Greenfield *et al.*, 1984). Furthermore, analogies can be drawn with the actions of GABA and taurine in the substantia nigra. These compounds inhibit nigrostriatal cell discharge (Scarnatti and Pacitti, 1982; Dray and Straughan, 1978) yet cause turning away from the side of injection (Coward, 1982; Kaakkola and Kaariainen, 1980) consistent with an increase in DA release from striatal axon terminals (Cheramy *et al.*, 1978). Hence DA and AChE released in the substantia nigra have an action locally, that appears similar in both electrophysiological and behavioural studies. It is yet another mysterious feature of dendritic release, however, that direct inhibition at the level of the perikaryon appears to result in a net increase in transmitter release at the nerve terminal.

VI. SIGNIFICANCE OF DENDRITIC RELEASE OF DA AND AChE

The features of dendritic release of DA and AChE, discussed in the preceding sections, are summarized in Table 2. Two features seem immediately apparent: (1) there are close parallels between release of DA and AChE; (2) dendritic release differs from that at the axonal terminal. The general pattern that emerges is of a process whereby a transmitter and protein are mediating long-lasting and non-specific inhibitory signals to nigrostriatal neurons, that nonetheless result in increased activity in the region of the nerve axons and terminals. The "Autoreceptor Theory" of undefined and simple negative feedback does not easily embrace all these features.

It is, however, possible to postulate an alternative theory that takes account of what we now know of the morphology and physiology of nigrostriatal neurons. This theory hinges on a further discovery made during intracellular recordings from nigrostriatal cells. If these neurons are hyperpolarized, a second slow Ca^{2+} conductance, again resistant to TTX, is de-inactivated i.e. made ready to occur. Actual activation is expressed when depolarization occurs from this hyperpolarized level (Fig. 5). This slow Ca^{2+} conductance is located in the proximal segment of

Table 2. Features of dendritic release of DA and AChE in the substantia nigra

1. Not stored in vesicles but in SER of dendrites.
2. Release not blocked by TTX or gamma-butyrolactone.
3. Release is K^+-evoked, Ca^{2+}-dependent.
4. Release is not related to firing frequency.
5. Paucity of dendro-dendritic synapses in pars reticulata.
6. Approximate diffusion rates through extracellular space: (a) DA (mol. wt 153.18): 17 min/1000; 4 min/500 μ; 10 s/100 μ. (b) AChE: 1 or 2 mm/h.
7. DA and AChE depress firing frequency.
8. Direct activation of DA receptors hyperpolarizes cell membrane.
9. Unilateral microinjections of potent DA agonst (ADTN) or AChE into the substantia nigra causes contraversive rotation.

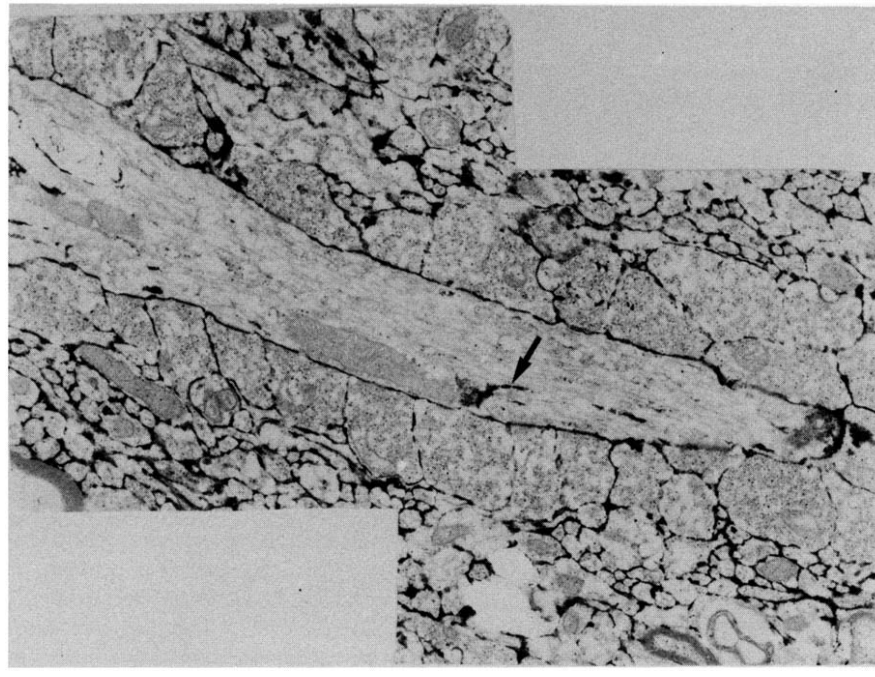

Fig. 4. Electron micrograph of dendrite in pars reticulata of guinea pig substantia nigra, stained for AChE. AChE is present in SER (arrowed), at the plasma membrane and in the extracellular space. Note high number of synaptic boutons. From Henderson and Greenfield (unpublished).

nigrostriatal cell dendrites and, when activated, facilitates the relaying of signals from the distal end of the dendrite to the soma (Fig. 5; Llinas *et al.*, 1984). Hence, if the neuron is hyperpolarized, the sensitivity of the soma to events at the distal dendrites would be improved. This observation can be incorporated to build a new theory of the function of dendritic release, which is described in Fig. 6.

As can be seen from the scheme in Fig. 6, dendritically released DA, and perhaps AChE, could hyperpolarize groups of nigrostriatal neurons that will then become sensitive to any further synaptic signals arriving at the dendrites of neurons within that group. Only in the presence of subsequent continuing signals would the increase in sensitivity have any value i.e. the dendritically released substances could be mediating a "low pass filter". If no other signals are transmitted, DA and AChE will eventually be degraded and the neurons depolarize.

The "low pass filter" theory would explain several puzzles:

(1) The paradox that DA and AChE cause inhibition of nigrostriatal neurons, monitored electrophysiologically, but net excitation when assessed behaviourally, could be interpreted as follows. Most *in vivo* electrophysiological experiments are performed in the anaesthetized or curarized preparation: in behavioural experiments the animals are, of course, freely moving. In both types of experiment DA, and possibly AChE, would cause hyperpolarization (see in extracellular recordings as inhibition of discharge) and the neurons become more potentially sensitive to incoming signals. However, in the anaesthetized or curarized preparations, signals concerning movement would be far less than in the behaving animal, hence the change in sensitivity would not be exploited. In the conscious animal, hyperpolarization-induced increases in sensitivity to a continued bombardment of movement related signals, would result in an increase in the activity of the nigrostriatal pathway.

(2) The unfamiliar slow time course of dendritic release and action has already been pointed out by Cheramy *et al.*, (1981); "This time dimension is another peculiar feature of the signals mediated by dendritic DA and provides further evidence for a modulatory role of the nigrostriatal dopaminergic neurons in the transfer of information in the substan-

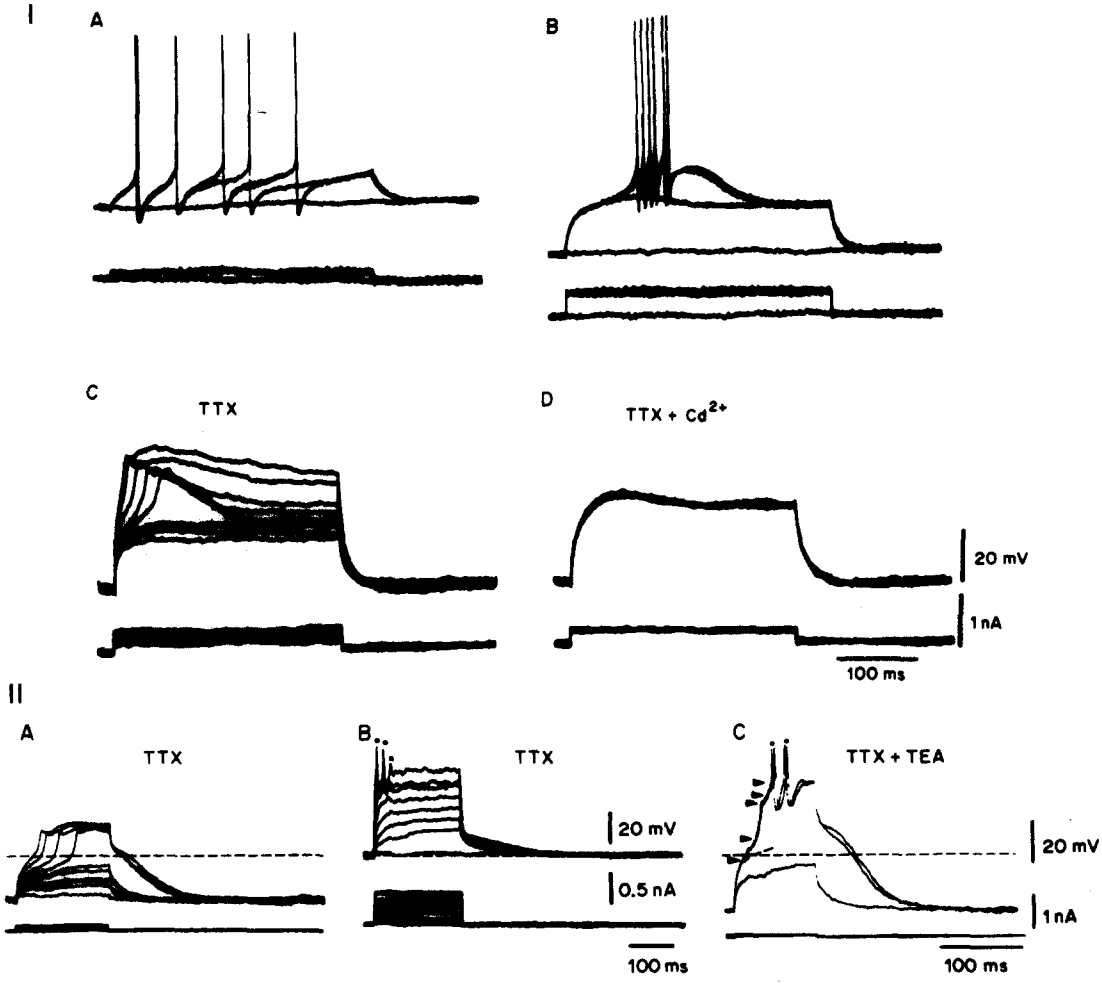

Fig. 5. Intracellular recordings obtained from a guinea pig pars compacta neuron *in vitro*. I. (A) Direct activation from rest membrane potential at −60 mV produces repetitive firing of the cell (3 superimposed traces). (B) d.c. hyperpolarization of the membrane by 20 mV produced, during a square pulse depolarization, a burst of action potentials. (C) Application of TTX to the bath removed the fast action potential seen in (A) and (B) but a slow all-or-nothing broad spike remains. (D) Addition of Cd^{2+} following TTX intoxication blocks the activation of TTX-insensitive low threshold spike (LTS). II. (A) Low threshold spikes obtained following 25 mV d.c. hyperpolarization by the injection of outward square current pulses of increasing amplitude. The all-or-none nature of the LTS can be observed. With superathreshold stimuli the LTS moves to the left but does not increase its amplitude. (B) Record in same cell as in (A) activated from rest membrane potential, demonstrating absence of low threshold spikes. At a membrane depolarization of 40 mV high threshold spikes (HTS) are generated (dots). (C) In another cell following TEA and TTX application to the bath with a d.c. hyperpolarization of 20 mV, a direct stimulation generates many levels of low threshold spiking (arrows) including high threshold action potentials (dots). From Llinas *et al.* (1984). Reproduced by permission of Elsevier Science Publishers, B.V.

tia nigra and striatum". The "low pass filter" theory is consistent with these remarks; namely that dendritic DA and AChE could modulate the sensitivity of nigrostriatal neurons to signals received over a relatively long time period.

(3) The function of AChE in the substantia nigra has long been puzzling (cf Lehmann and Fibiger, 1978). Furthermore, the role of a soluble form of the enzyme, released in an apparently non-cholinergic capacity, has been regarded as "obscure" (Butcher

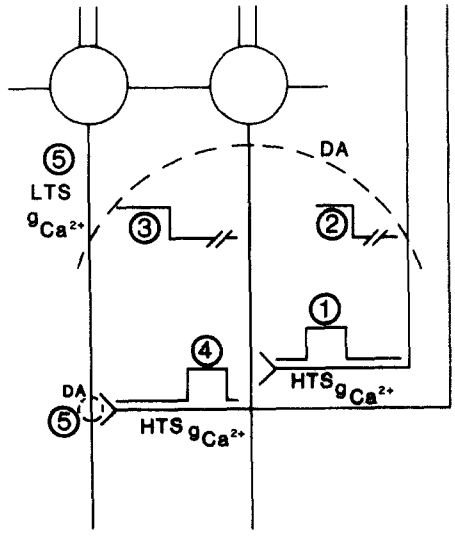

Fig. 6. Schematic diagram of how dendritically released DA could underlie a "low pass filter" mechanism. (1) Synaptic signal arriving at distal segment of dendrite activates short-lasting Ca^{2+} conductance ("High Threshold Spike; HTSg Ca^{2+}) which is not transmitted to the soma but causes local release of DA (see Section III). (2) DA diffuses indiscriminately to the soma and dendrites of the neuron from which it was released and also to neighbouring cells (Section IV), where it hyperpolarizes the proximal dendritic membranes (Section V). (3) DA gradually diffuses to neurons further from its site of release, which also become hyperpolarized (Section IV and V). As a result of this hyperpolarization, a long lasting Ca^{2+} conductance ("Low Threshold Spike: LTSg Ca^{2+}) is de-inactivated (Section VI). (4) A subsequent synaptic signal arrives at a distal dendrite of one of the neurons now hyperpolarized, and again activates the local HTSg Ca^{2+}. (5) The depolarization caused by the HTSg Ca^{2+} can now activate the LTSg Ca^{2+} (Section VI). The LTSg Ca^{2+} leads to generation of somatic action potentials (Fig. 6) i.e. has served to relay the synaptic signal along the dendrite. As a consequence of the HTSg Ca^{2+}, further dendritic release ensues to maintain hyperpolarization until tachyphylaxis occurs. It is possible that AChE is also released (Section III) and acts to protect released DA or in some way amplify the DA "signal" (Sections IV and V).

and Woolf, 1982). However, according to the "low pass filter" theory, DA-induced hyperpolarization would need to be for a prolonged period and occur at multiple sites remote from that of release. A large protein such as AChE would be well suited to either increase the stability of the transmitter or in some other way to "amplify" the DA signal (see sections II and V).

(4) The non-specificity of diffusion of dendritically released material has been indicated but not explained (Hefti and Lichtensteiger, 1978). However the fact that a group of neurons could be affected by DA and AChE actually suggests an elegant means of neuronal processing. According to the "low pass filter" theory, a group of nigrostriatal neurons would be *potentially* more sensitive to incoming information: however the specificity of any particular signal arriving at a particular neuron within that group, would be preserved. This feature would probably be very important considering the convergence of inputs that occurs even to a single neuron, from all over the striatum (Somogyi *et al.*, 1981). It is conceivable that a series of such signals could constitute a more global message for example mediation of "complex" movements i.e. sequence of individual movements (Evarts and Wise, 1984). This complex movement could then be preferentially transmitted via the DA-sensitized group of nigrostriatal neurons, with the individual components intact. Another argument in favour of functional compartmentalisation of nigrostriatal neurons concerns their possible target sites. Within the striatum, we already know that neurons are organized into neurochemically distinct clusters or "striosomes" (Graybiel and Ragsdale, 1983). It would be very appropriate if neurons projecting to target striosomes were also functionally organized into sub-populations.

However, we have now come to questions beyond the scope of the "low pass filter" theory. Why should the substantia nigra need to possess a mechanism for selectively filtering sustained messages? What type of information would be relayed in this way? Where in the striatum would such signals be transmitted, and why? These issues are more the domain of organizational or "black box" physiology: nonetheless it seems that dendritic release of transmitter and protein could be a cog in the black box.

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