Reprinted from Presynaptic Receptors and the Question of Autoregulation of Neurotransmitter Release
Volume 604 of the *Annals of the New York Academy of Sciences*
August 31, 1990

In Vivo Electrophysiology of Central Nervous System Terminal Autoreceptors[a]

JAMES M. TEPPER[b] AND PHILIP M. GROVES[c]

[b]*Center for Molecular and Behavioral Neuroscience*
Rutgers, The State University of New Jersey
195 University Avenue
Newark, New Jersey 07102

[c]*Department of Psychiatry*
University of California, San Diego
School of Medicine
La Jolla, California 92093

INTRODUCTION

Evidence both for and against the existence of presynaptic autoreceptors and the related question of, if they exist, do they play a significant role in the "normal" or physiological functioning of synaptic transmission, has been presented throughout this volume (for a recent excellent and comprehensive review, see Reference 1). Some of the controversy regarding the existence and functioning of autoreceptors derives from the techniques and conditions under which their existence and functioning have been measured. In this chapter we will review data derived from an electrophysiological technique for examining the neurophysiological consequences of autoreceptor stimulation and blockade at the terminals of central nervous system neurons *in vivo* that support the existence of such autoreceptors, and give some clues to the conditions under which they operate *in situ*. The data to be discussed concern changes in the electrical excitability of single central nervous system (CNS) axon terminals as a function of the local and systemic application of drugs, and the rate of impulses reaching the terminal fields. Thus far, the technique has been used to study apparent autoreceptor-mediated changes in terminal excitability in rat mesencephalic dopaminergic neurons projecting to neostriatum,[2–7] nucleus accumbens[8] prefrontal, cingulate, and entorhinal cortices,[9] in rat noradrenergic locus ceruleus neurons projecting to frontal cortex,[10–12] in rat serotonergic dorsal raphé neurons projecting to neostriatum,[13] in presumed glutamatergic rat corticostriatal neurons,[14] and in monkey nigral dopaminergic neurons projecting to the putamen.[15] In addition, this technique has been used to study the electrophysiological consequences of activation or blockade of presynaptic heteroreceptors in several different systems, e.g., opioid receptors on cortical noradrenergic terminal of locus ceruleus neurons,[16] dopamine receptors on hippocampal and cortical axons projecting to ventral striatum,[14,17] and dopamine receptors on the terminals of presumed GABAergic (γ-aminobutyric acid) striatonigral neurons.[18]

Despite the fact that neurotransmitter release itself is not directly measured in these studies, the nature of the changes in terminal excitability and the conditions under which these changes are observed suggest rather strongly that many different classes of CNS neurons possess at their terminal fields neurotransmitter receptors

[a]Some of the research and the writing of this manuscript were supported by the National Institute of Mental Health and the National Institute on Drug Abuse, grants MH-45286, DA-02854, and DA-00079.

sensitive to the neurotransmitter that these neurons release, and that these receptors can and do operate under more or less normal physiological conditions *in vivo* to modify the electrophysiological properties of the nerve terminals in a manner consistent with the observed modulation of impulse-dependent neurotransmitter release *in vitro* and *in vivo*.

TERMINAL EXCITABILITY TESTING PARADIGM

The details of the methods for measuring autoreceptor-mediated changes in terminal excitability resulting from local drug application and changes in the rate of impulses reaching the terminal fields have been published previously.[4,5,10] The paradigm is illustrated schematically for the nigrostriatal dopaminergic system in FIGURE 1A. Briefly, terminal excitability is indexed by the current intensity necessary to elicit antidromic responses from single units recorded extracellularly. A number of trials (50–100) at a variety of different stimulating currents are presented, ranging from the minimum current needed to evoke antidromic responses to each stimulus delivery (excluding collisions) to the maximum current that fails to evoke any antidromic responses. The entire procedure is repeated several times, in a counterbalanced fashion, until successive determinations at the same currents yield the same proportion of antidromic responding ±10%. These points are taken as baseline, and an excitability curve relating percent antidromic response to stimulus current can be plotted as shown in FIGURE 1B. Drugs or vehicle is then administered, either systemically via an intravenous catheter or locally, directly into the terminal fields or preterminal axons via infusion cannulae coupled to the stimulating electrode. Local drug administration consists of relatively low concentrations (0.1–10 μM) in small volumes (325 nL). Post drug measures of terminal excitability are performed, and the pre- and postdrug excitability curves compared. Shifts to the right of the excitability curve signify decreases in terminal excitability whereas shifts to the left indicate increased terminal excitability. In some cases, instead of administering drugs, preterminal regions of the axon are stimulated at different rates to alter the synaptic concentrations of released neurotransmitter at the terminal regions and the excitability reassessed.

AUTORECEPTOR-MEDIATED CHANGES IN TERMINAL EXCITABILITY

When appropriate autoreceptor agonists are administered systemically or applied directly to the terminal regions of dopaminergic, noradrenergic, or serotonergic neurons, the terminal excitability of each is reduced, as illustrated in FIGURE 2.[2–4,8,10,13] The decreased excitability is dose dependent and obtains only in the terminal fields; if drugs are locally infused into and excitability tested from preterminal regions of monoaminergic axons (e.g., the medial forebrain bundle or the dorsal noradrenergic pathway), no changes in excitability are observed. In addition to increasing the threshold for antidromic activation, autoreceptor agonists produce slight increases in the antidromic latency, and in the variability of the antidromic latency.[4,10] These decreases in terminal excitability can be reversed by subsequent infusions of appropriate autoreceptor antagonists, or blocked by pretreatment with these drugs.[4,8,10,19]

In addition, appropriate autoreceptor antagonists can not only reverse the effects of prior administration of agonists, but, when administered alone, produce increased terminal excitability.[4,8,10] This is an important observation indicating that *in vivo*, there is a sufficient concentration of endogenous agonist in the terminal regions to activate terminal autoreceptors, thus suggesting a physiological role for these autoreceptors.

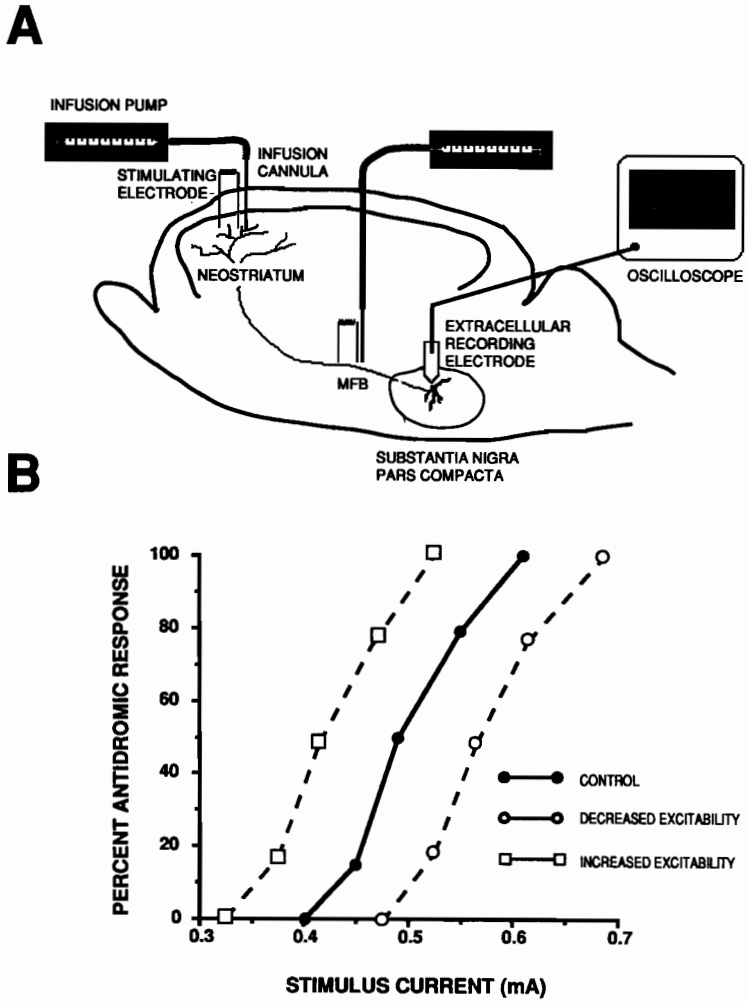

FIGURE 1. (A) Schematic illustration of method for measuring autoreceptor-mediated changes in dopaminergic terminal excitability *in vivo*. Excitability is indexed by the stimulating currents required to elicit varying proportions of antidromic responding from terminal fields in neostriatum or preterminal axons in the medial forebrain bundle (MFB). Excitability is compared before and after local infusions of drugs or spontaneous or stimulation-induced alterations in firing rate. (B) Idealized sample excitability curves resulting from application of the method in A. Shifts to the right of the excitability curve indicate decreased excitability, whereas shifts to the left indicate increased excitability.

As a general rule, the nature of the terminal autoreceptor mediating the decreases in terminal excitability seems to be of the same general class as that of the somadendritic autoreceptor which inhibits the firing rate of monoamine neurons and the axonal autoreceptor that inhibits transmitter release. Thus, for example, noradrenergic terminal excitability is decreased by clonidine, and increased by phentolamine or

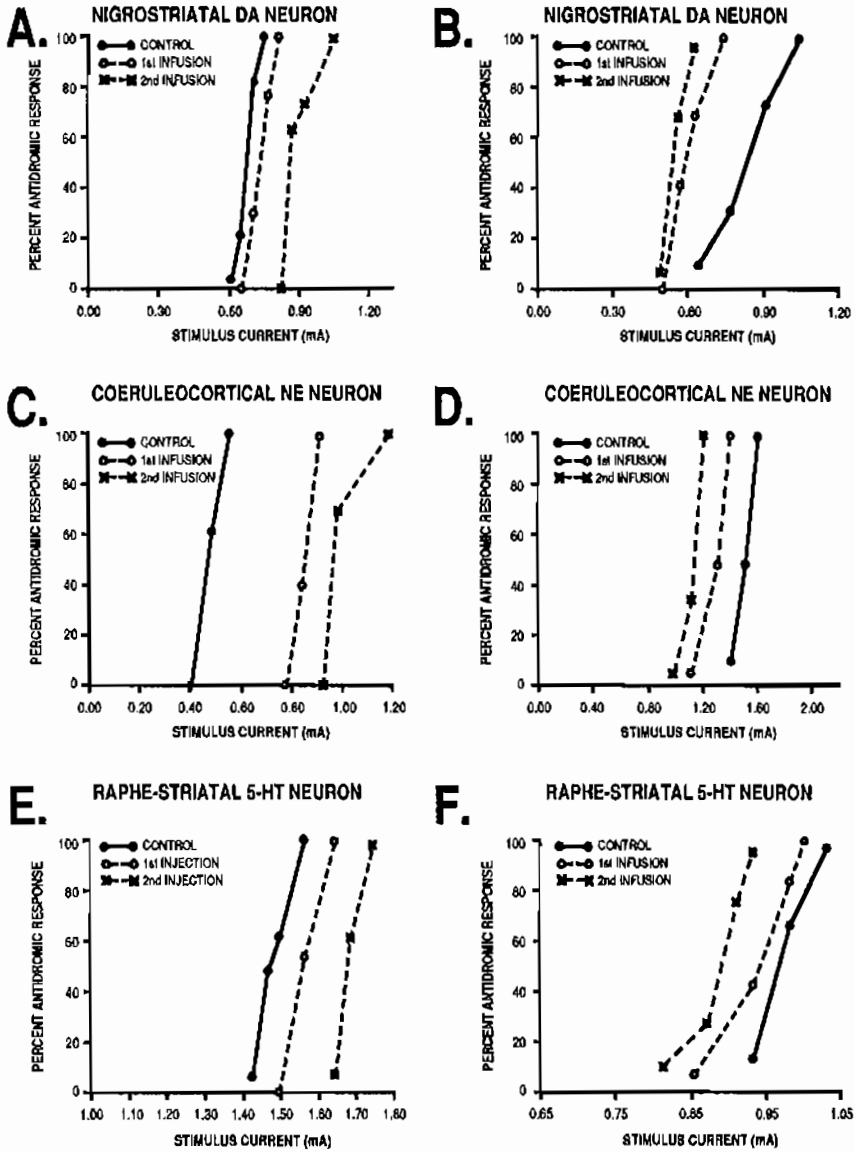

FIGURE 2. Sample excitability curves illustrating autoreceptor-mediated changes in terminal excitability *in vivo* in dopaminergic nigrostriatal (A, B), noradrenergic ceruleocortical (C, D) and serotonergic raphé-striatal neurons (E, F). In all cases, local or systemic application of the appropriate autoreceptor agonist produces a dose-dependent decrease in terminal excitability, whereas the appropriate autoreceptor antagonist produces increased terminal excitability. (A) 10 μM apomorphine. (B) 10 μM sulpiride. (C) 10 μM clonidine. (D) 10 μM phentolamine. (E) 20 μg/kg 5-methoxy dimethyl tryptamine. (F) 10 μM methiothepin. A–D, and F are local infusions of drugs into the stimulating site in the terminal fields. E is intravenous injection.

yohimbine, suggesting that the receptor may be pharmacologically defined as an α_2-receptor,[10] consistent with the receptor subtype that mediates the inhibition of firing of locus ceruleus neurons *in vivo* and *in vitro*[20–22] and the autoinhibition of norepinephrine release from cortical noradrenergic terminals *in vitro*.[1,23,24] Similarly, based on the results obtained with haloperidol and sulpiride,[2,4,8] the dopaminergic terminal autoreceptor appears to be of the D_2 variety, as does the somadendritic autoreceptor[25–27] as well as the receptor responsible for autoinhibition of dopamine release both *in vitro* and *in vivo*.[1,28,29] It should be noted however, that recent terminal excitability experiments indicate the possible existence of D_1 receptors on the terminals of nigrostriatal neurons that, like the D_2 receptors, serve to decrease terminal excitability.[30] It remains to be seen whether these receptors operate as functional autoreceptors, i.e., are sensitive to endogenously released dopamine or modify terminal excitability depending on changes in impulse flow, and whether they function to modulate dopamine release. Finally, serotonergic terminal excitability is decreased by the serotonin ($5HT_1$) agonist 5-methoxy dimethyl tryptamine.[13] These effects can be reversed by the relatively nonselective serotonergic antagonist methiothepin,[19] consistent with the identification of the serotonergic somadendritic autoreceptor modulating serotonergic neuron firing rate[31] as well as the axonal autoreceptor mediating autoinhibition of serotonin release[32] as a $5HT_1$ receptor.

It should be noted that not all terminal autoreceptors mediate feedback inhibition of terminal excitability and neurotransmitter release. Although this appears to be the case for all monoaminergic terminal autoreceptors,[33] recent data on drug and impulse dependent changes in the excitability of presumably glutamatergic corticostriatal terminals suggest that while these axons do possess terminal autoreceptors, these autoreceptors appear to mediate increases in the excitability of corticostriatal terminals,[14] as well as increases in evoked glutamate release from hippocampal slices *in vitro*.[34]

BIOPHYSICAL INTERPRETATION OF CHANGES IN TERMINAL EXCITABILITY AND THEIR RELATION TO TRANSMITTER RELEASE

The most direct way to uncover the membrane events resulting in presynaptic receptor–mediated changes in terminal excitability would be to record intracellularly from the presynaptic terminals while applying receptor agonists and antagonists. While this has been achieved in certain invertebrate preparations (e.g., Reference 35), the small size of central monoamine presynaptic axons has, so far, precluded this approach. Classically, synaptic potentials are associated with an alteration in conductance to an ion or ions that leads to a change in the membrane potential. At the nerve terminal, if the membrane were hyperpolarized, more current would be required to depolarize it to threshold for initiation of an antidromic spike. It is also true that an increase in membrane conductance alone could raise the threshold.[36] Either or both could result in the decrease in terminal excitability observed following terminal monoamine autoreceptor stimulation. However, there are several indirect lines of evidence suggesting that what is monitored by terminal excitability measurements in monoamine neurons is in fact the membrane potential or level of polarization of the terminal fields.[37] For example, when dopaminergic and noradrenergic terminal fields are depolarized by local infusion of potassium chloride (which probably also produces a slight increase in membrane conductance), their terminal excitability is increased,[4,10] suggesting that decreases in terminal excitability are associated with a terminal hyperpolarization and increases in terminal excitability are associated with a terminal depolarization. This is consistent with the results from *in vitro* intracellular recordings

from central monoaminergic neurons which have revealed that although the autoreceptor recognition site is distinct for dopaminergic, noradrenergic, and serotonergic neurons, all three autoreceptors act to hyperpolarize the neuron by increasing its conductance to potassium.[21,26,38] Further evidence that terminal excitability measurements index membrane potential rather than membrane conductance comes from studies of presynaptic inhibition in the mammalian spinal cord where the presynaptic inhibition is known to be associated with a depolarization of the primary afferent terminals mediated by an increase in chloride conductance.[39] Despite the increased conductance, terminal excitability measurements similar to those described in this chapter revealed an increase in terminal excitability rather than a decrease.[40]

Thus, it is likely that decreases in monoamine terminal excitability seen following autoreceptor stimulation reflect a hyperpolarization of the terminal membranes. Since these same agonists produce a reduction in evoked release of monoamines, it follows that autoinhibition of monoamine release occurs when these nerve terminals are hyperpolarized. It is important to note that this does not necessarily suggest that it is the hyperpolarization *per se* that produces the inhibition of release. Indeed, such a hypothesis would be inconsistent with the mechanism of presynaptic inhibition in the spinal cord which, as described above, is associated with a depolarization of the primary afferent terminals. It is more likely that it is the conductance increase underlying the change in potential that serves to shunt presynaptic action currents out through the terminal membrane that is responsible for the inhibition, as has been demonstrated in the crayfish claw opener system.[35]

It must be noted that the above arguments regarding the interpretation of autoreceptor-mediated changes in terminal excitability and their relation to modulation of transmitter release hold only for receptors and their associated ionophores that function by altering conductance to ions other than Ca^{2+}, as seems to be the case with monoamine autoreceptors.[21,26,38] Since it is well-established that the only absolute requisite for evoking neurotransmitter release from nerve terminals is an increase in the availability of intracellular calcium (e.g., Reference 41), presynaptic receptors that act to directly modify Ca^{2+} permeability may be expected to lead to altered transmitter release regardless of other concurrent biophysical changes at the terminal. Such may be the case with the *N*-methyl-D-aspartate (NMDA) autoreceptor postulated to exist on the terminals of glutamatergic corticostriatal neurons,[14] as has been shown for NMDA receptors on other central neurons.[42]

NEURONAL LOCALIZATION OF THE TERMINAL AUTORECEPTOR

Attempts to localize the neuronal site of the receptors mediating changes in dopaminergic terminal excitability were made in dopaminergic nigrostriatal neurons by using kainic acid to destroy postsynaptic neostriatal neurons in the region from which drug infusions and terminal excitability testing were performed.[4] Three to six days following kainate-induced lesions, the effects of local infusions of apomorphine and haloperidol were tested, and found to cause changes in nigrostriatal terminal excitability indistinguishable from those in intact animals. Histological examination of the neostriata from these animals indicated that the lesions extended for at least 0.5–1.0 mm beyond the site of drug infusion and terminal excitability testing, verifying that the drug infusions and excitability changes occurred at sites devoid of intrinsic neurons. A similar lack of effect of ibotenic acid lesions of the nucleus accumbens on agonist-induced changes in mesoaccumbens terminal excitability[8] and of kainate lesions of striatum on changes in corticostriatal terminal excitability[14] has also been reported. Thus, the receptors mediating drug-induced changes in dopaminergic and

corticostriatal terminal excitability would seem to be located directly on their respective axons and not on postsynaptic striatal neurons, and drug-induced changes in terminal excitability do not require the participation of postsynaptic neurons. Further, since local infusions or even intravenous administration of agonists and antagonists failed to alter excitability from preterminal regions of monoamine axons in the medial forebrain bundle, the axonal autoreceptors appear to be constrained to terminal regions of the axonal field.[2–4,10,13]

EVIDENCE FROM TERMINAL EXCITABILITY TESTING SUPPORTING A PHYSIOLOGICAL ROLE FOR TERMINAL AUTORECEPTORS

The data discussed thus far provide electrophysiological evidence that *in vivo*, the terminal excitability of central monoamine neurons can be altered by the local application of drugs that directly stimulate or block receptors located on the axon terminal regions that are sensitive to the transmitter that the neuron itself releases. However, with the exception of the observation that terminal excitability can be increased by the local infusion of antagonists by themselves, the data cannot be used to argue convincingly that these presynaptic receptors function as autoreceptors.

One piece of evidence to support the operation of these presynaptic receptors as true autoreceptors derives from the use of amphetamine as a probe. Amphetamine increases extracellular levels of catecholamines by simultaneously promoting catecholamine release and blocking catecholamine uptake.[43] When amphetamine is locally infused into neostriatal, ventral striatal, or neocortical terminal fields, the excitability of dopaminergic or noradrenergic terminals is markedly reduced, just as with infusions of exogenously applied direct-acting agonists,[4,8,11] as illustrated in FIGURE 3. In both catecholaminergic systems, the effects of amphetamine were completely eliminated by prior treatment with the catecholamine synthesis inhibitor α-methyl-*p*-tyrosine, and could be reversed by haloperidol or sulpiride in the dopaminergic system or phentolamine or yohimbine in the noradrenergic system.[4,8,11,12] Thus, the axonal presynaptic receptors of both systems can be considered autoreceptors since they are responsive to their endogenously released neurotransmitter.

In a recent review, Kalsner suggested several operational criteria that should be fulfilled before accepting the hypothesis that autoreceptors modulate impulse- or stimulus-evoked transmitter release.[44] Two of these criteria had to do with the relation between the magnitude of the effects of agonists and antagonists on evoked release of transmitters and the level of ongoing autoinhibition. If the synaptic concentration of endogenous transmitter is high, for example, due to a high rate of neuronal activity and consequent transmitter release, then antagonists would be expected to exert relatively large effects due to the occupation of many autoreceptor sites by the endogenous neurotransmitter. By similar logic, the effects of exogenously applied agonists would be expected to be relatively small at high rates of firing, since a fixed concentration (dose) of the applied agonist corresponds to a progressively smaller proportion of the total agonist available for binding to the autoreceptor. The converse should be true at low rates of impulse activity and release, when agonists would be expected to exert relatively large effects and antagonists relatively small effects. Precisely this relation was observed with dopaminergic nigrostriatal terminal excitability with a variety of autoreceptor agonists and antagonists infused into the terminal fields,[4] as illustrated in FIGURE 4. When the magnitude of the drug-induced changes in terminal excitability was correlated with the baseline firing rate of the neuron, a significant negative correlation was observed with the agonists amphetamine and apomorphine, and a significant positive correlation with the antagonists haloperidol and sulpiride. Similar

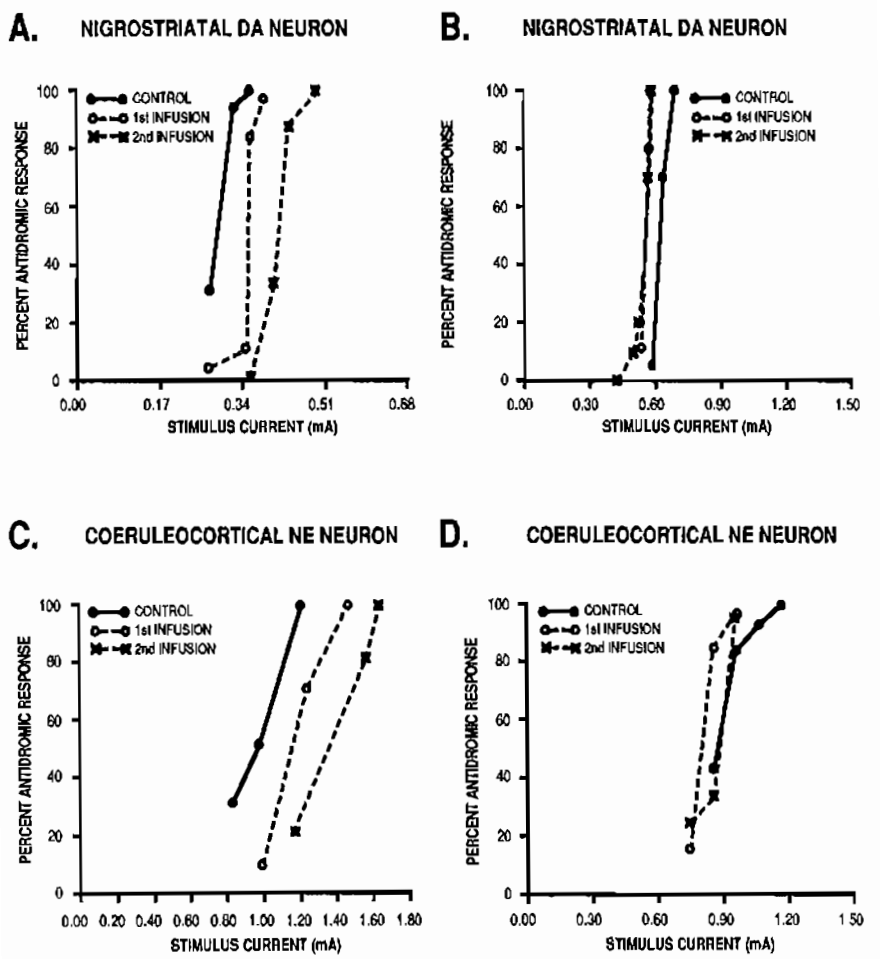

FIGURE 3. Sample excitability curves illustrating effects of local infusion of amphetamine on terminal excitability of nigrostriatal dopaminergic (A, 1 μM) and ceruleocortical noradrenergic (C, 10 μM) neurons. In both systems, increasing endogenous levels of catecholamines by amphetamine infusion leads to decreased terminal excitability. The effects of amphetamine can be prevented in both systems by pretreatment with the catecholamine synthesis inhibitor α-methyl-*p*-tyrosine. (B) 10 μM amphetamine after α-methyl-*p*-tyrosine, nigrostriatal neuron. (D) 10 μM amphetamine after α-methyl-*p*-tyrosine, ceruleocortical neuron.

effects were observed with 5-methoxy-dimethyl tryptamine induced decreases in serotonergic terminal excitability.[13] A similar relation, i.e., that between the rate of stimulation and the strength of the inhibitory and facilitatory effects of autoreceptor agonists and antagonists *in vitro* has also been described for dopamine, norepinephrine, and serotonin release.[45-50] It is interesting to note that a similar negative correlation obtains between the baseline firing rate of monoaminergic neurons and the extent to which exogenously applied autoreceptor agonists depress the firing rate of the neuron by acting on soma-dendritic autoreceptors.[51-54]

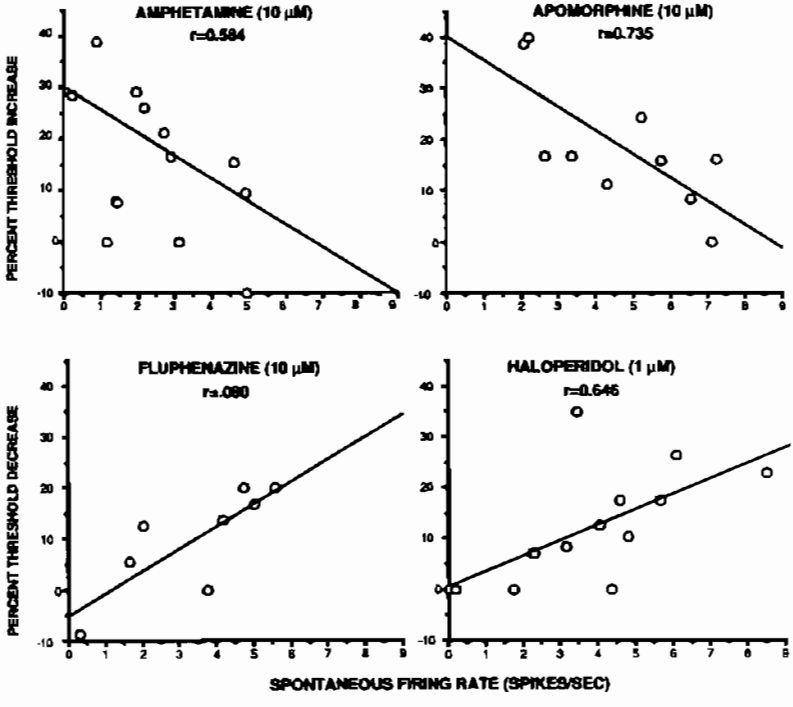

FIGURE 4. Correlations between the magnitude of the change in dopaminergic terminal excitability resulting from local neostriatal infusions of amphetamine, apomorphine, fluphenazine, and haloperidol and the predrug spontaneous firing rate. Autoreceptor agonists are most effective at decreasing terminal excitability in slowly firing neurons, whereas autoreceptor antagonists are most effective at increasing terminal excitability in rapidly firing neurons. $p < 0.05$ for all regressions. (Data replotted and modified from Tepper *et al.*)[4]

Another criterion suggested by Kalsner was that changes in the rate of neuronal activity, in the absence of exogenous drug application, should in some way reflect changes in ongoing autoinhibition.[44] Terminal excitability measurements *in vivo* in the nigrostriatal and mesolimbic dopaminergic system,[5,9] ceruleocortical noradrenergic projections,[10] and presumed glutamatergic corticostriatal system[14] have all afforded direct evidence for this phenomenon. This is illustrated for a noradrenergic ceruleocortical neuron and a dopaminergic mesoprefrontal cortical neuron in FIGURE 5. Although the firing rate of individual central monoamine neurons *in vivo* is usually relatively constant, occasionally neurons are recorded whose firing rate varies significantly over the course of a few minutes. FIGURE 5A illustrates the results of repeatedly measuring the spontaneous firing rate and the antidromic threshold from the frontal cortex of a noradrenergic locus ceruleus neuron whose firing rate is varying over the course of the measurements.[10] The antidromic threshold current is seen to exhibit a tight direct relationship to the firing rate. FIGURE 5B illustrates the same relation in a slightly different way for a dopaminergic ventral tegmental area neuron projecting to the prefrontal cortex.[37] In this case, the antidromic stimulating current was adjusted to a subthreshold value, and the percent antidromic responding and spontaneous firing rate of the neuron were measured every 30 seconds for several minutes. The terminal

excitability exhibits a significant inverse correlation to the firing rate ($r = -0.84$, $df = 22$, $p < 0.001$) which is abolished by intravenous administration of haloperidol (0.05 mg/kg).

In addition to responding to these relatively prolonged changes in firing rate (over the course of several seconds), dopaminergic terminal excitability is sensitive to considerably briefer events, such as bursts consisting of 2 or 3 spikes lasting on the order of 150–200 mseconds, or even the occurrence of single spikes just outside the antidromic collision interval. In these cases, terminal excitability is transiently depressed by increased spiking that occurs within 5–10 mseconds of the delivery of the antidromic stimulus.[3,5]

Impulse-dependent changes in terminal excitability of noradrenergic and dopaminergic systems have also been demonstrated by altering the frequency of impulses along the axon by the application of conditioning stimuli to preterminal regions of the axon. The range of effective frequencies employed (brief trains of pulses at 1–10 Hz) are well within the range of the rates of spontaneous activity for these neurons. Three important characteristics of stimulus-dependent decreases in terminal excitability are illustrated in FIGURES 6–8. FIGURE 6 illustrates decreased terminal excitability of cortical locus ceruleus terminals following suprathreshold stimulation of the preterminal axons in the dorsal noradrenergic pathway (DP).[10] It was possible to determine the threshold for the conditioning stimulation by simply observing the antidromic response to the conditioning pulses. When conditioning was applied at suprathreshold currents, terminal excitability was reduced. If however, the dorsal pathway stimulus current was slightly reduced to a value that failed to elicit antidromic responding, there was no alteration in terminal excitability. This illustrates the point that the reduced terminal excitability results from some consequence of impulse flow along the axon whose excitability is being tested. That this phenomenon occurs only at the terminal regions of the axon is

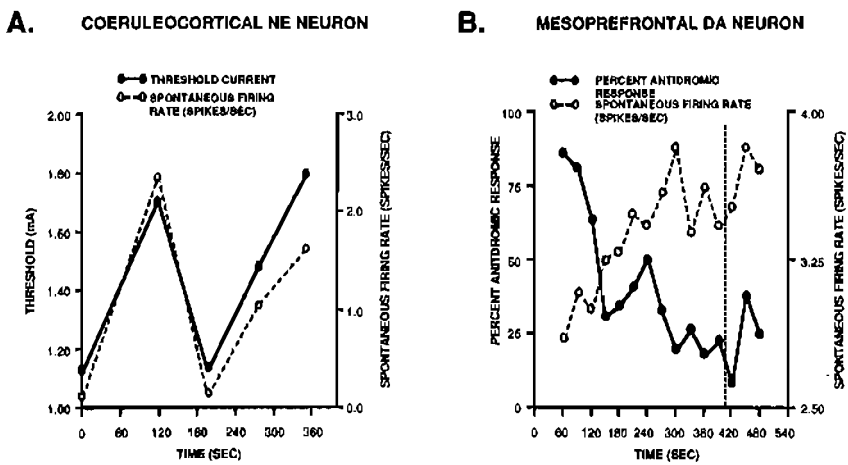

FIGURE 5. Changes in terminal excitability associated with spontaneous changes in neuronal firing rate. (**A**) Threshold current for a noradrenergic ceruleocortical neuron varies directly with spontaneous changes in firing rate (Redrawn from Nakamura *et al.*, with permission)[10] (**B**) Antidromic excitability of a dopaminergic mesocortical neuron exhibits significant negative correlation with spontaneous firing rate ($r = -0.84$, $p < 0.05$). This relation is abolished by intravenous administration of haloperidol (50 μg/kg) at the double dashed line. (Redrawn from Tepper et al., with permission.)[37]

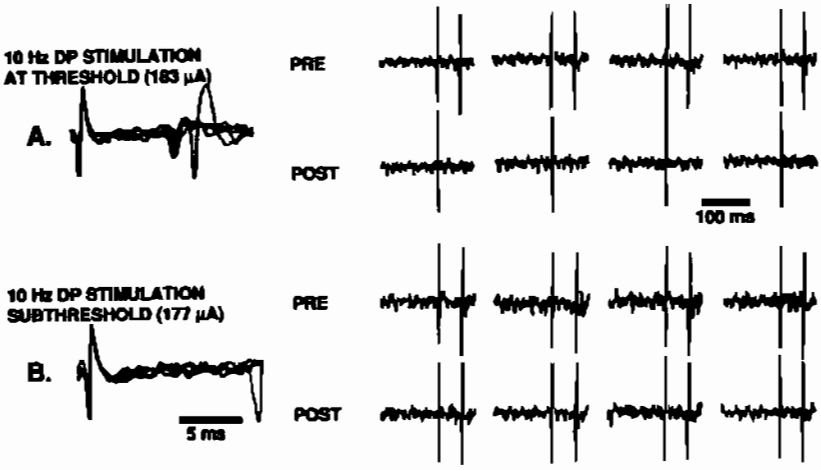

FIGURE 6. (A) High frequency conditioning stimulation applied at threshold (183 μA) to preterminal axon (DP) of a noradrenergic ceruleocortical neuron elicits antidromic responses and produces decreased terminal excitability that outlasts the conditioning stimulation (compare PRE versus POST traces at same stimulating current). (B) Similar conditioning stimulation applied to DP just below threshold (177 μA) does not evoke antidromic responses and does not affect terminal excitability. (Modified from Nakamura et al., with permission.)[10]

illustrated for a nigrostriatal dopaminergic neuron in FIGURE 7. The roles of the conditioning and testing electrodes can be reversed within a single experiment so that in one case the preterminal axon electrode (MFB electrode) can be used to condition the axon for excitability testing from the terminal regions. The terminal field electrode can then be used to send an identical train of stimuli down through the medial forebrain bundle, and the medial forebrain bundle electrode can be used to test excitability from this preterminal site.[5] The results from this experiment show that although increased impulse flow decreases terminal excitability, there is little or no effect along preterminal regions of the axon, which are also insensitive to the effects of locally or systemically administered autoreceptor agonists and antagonists. Finally, FIGURE 8 illustrates that the effects of conditioning stimulation of the medial forebrain bundle on dopaminergic terminal excitability can be blocked by local infusion of haloperidol into the neostriatal stimulating site.[5] Thus, the reduction in terminal excitability is not simply due to the increased impulse flow, but to stimulation of terminal autoreceptors by increased levels of transmitter evoked by the conditioning stimuli.

In the monoaminergic systems, as discussed above, autoreceptor stimulation results in decreased terminal excitability and decreased transmitter release. However, as mentioned previously, corticostriatal neurons also appear to possess autoreceptors, but these autoreceptors act in an opposite fashion to those on monoamine terminals. Thus the terminal excitability of (presumed) glutamatergic corticostriatal neurons is directly correlated with the firing rate of these cortical neurons, a relationship that, like that described above for mesocortical dopaminergic neurons, can be abolished by the local application of an appropriate autoreceptor antagonist, in this case, an NMDA antagonist.[14]

PRESYNAPTIC HETERORECEPTOR-MEDITATED CHANGES IN TERMINAL EXCITABILITY

In addition to the presynaptic autoreceptor systems discussed above, terminal excitability testing has also been employed to investigate the electrophysiological consequences of stimulation and blockade of presynaptic heteroreceptors. Local infusions of opioid agonists have been shown to decrease the terminal excitability of cortical terminals of noradrenergic locus ceruleus neurons in a naloxone-reversible manner.[16] The decreased excitability is consistent with *in vitro* intracellular recording studies of locus ceruleus neurons showing that opioids hyperpolarize locus ceruleus neurons by activating a potassium conductance identical to that activated by α_2-autoreceptor agonists.[55,56] The fact that naloxone alone produced increases in noradrenergic terminal excitability suggests that the presynaptic opioid receptors may have physiological relevance since they appear to be at least partially occupied by an endogenous agonist *in vivo*. It is even possible that these presynaptic opioid

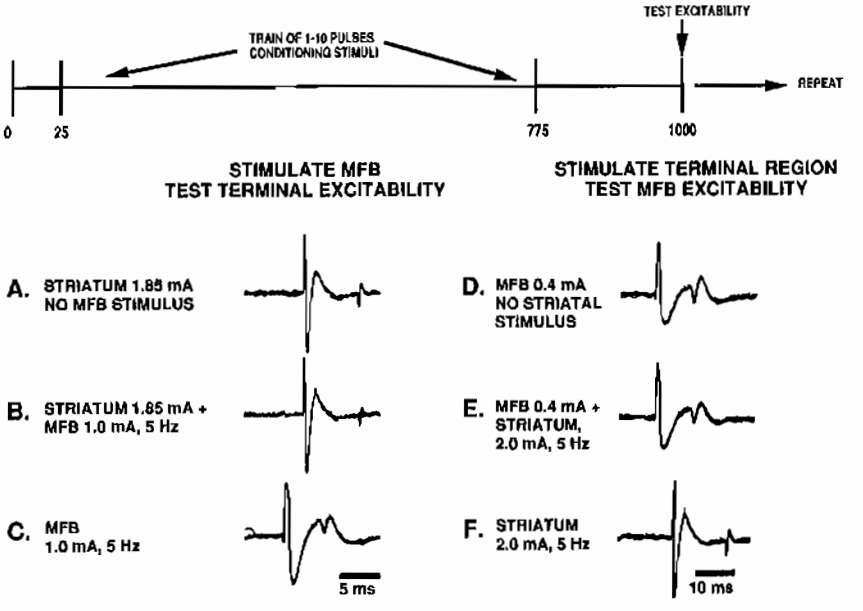

FIGURE 7. Increasing the rate of impulse flow in a dopaminergic nigrostriatal neuron decreases excitability, but only at the terminal. A train of conditioning stimuli is delivered in a 750 msecond window that terminates 225 mseconds prior to excitability testing. Entire sequence is repeated once per second. (**A**) Threshold for antidromic responding from striatum is 1.85 mA. (**B**) When preterminal axon in medial forebrain bundle (MFB) is stimulated at 5Hz, 1.85 mA stimulus to terminal field evokes antidromic spike on only one of 5 trials. (**C**) Antidromic response elicited by the MFB conditioning pulses at 5 Hz. (**D**) Threshold for antidromic responding from MFB is 0.4 mA. (**E**) When axon is antidromically conditioned by suprathreshold stimulation of terminal at 5 Hz, there is no change in antidromic responses elicited from MFB. (**F**) Antidromic responses elicited by terminal conditioning pulses at 5 Hz proving that the conditioning pulses pass through the region of the MFB from which excitability was tested, and demonstrating that increases in impulse flow only produce decreased excitability at the terminal region. Each trace comprised of superimposition of 5 consecutive sweeps. (Modified from Tepper et al., with permission).[5]

"heteroreceptors" are in fact autoreceptors (see Reference 57), since at least in some species, there is evidence for colocalization of norepinephrine and enkephalin in locus ceruleus neurons.[58] In any event, the decreased noradrenergic terminal excitability resulting from opioid receptor stimulation *in vivo* is consistent with the decreases in evoked norepinephrine release seen with presynaptic opioid receptor stimulation *in vitro*.[1,23,24]

In contrast to its apparent hyperpolarizing action at striatal dopaminergic terminals, at least one study has reported increased terminal excitability of hippocampal afferents to ventral striatum elicited by stimulation of the ventral tegmental area or by

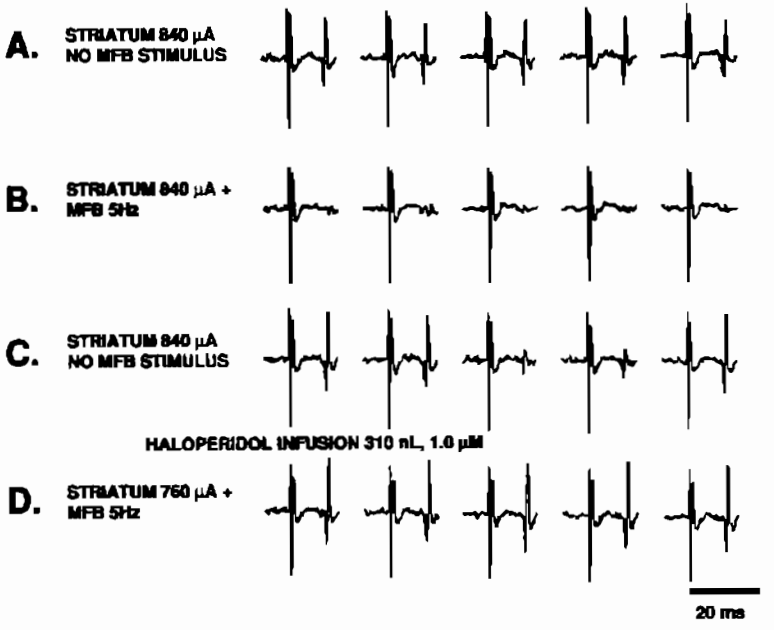

FIGURE 8. Decreased terminal excitability resulting from stimulation-induced increases in impulse flow in dopaminergic nigrostriatal neuron are blocked by local infusion of haloperidol into the terminal fields (A) Predrug, preconditioning threshold is 0.84 mA. (B) 5 Hz conditioning stimulation of MFB decreases threshold and eliminates antidromic responding. (C) Recovery baseline terminal excitability after cessation of MFB conditioning pulses. (D) After local neostriatal infusion of 310 nL haloperidol (1 μM), antidromic threshold decreases slightly and MFB conditioning no longer affects terminal excitability. (Modified from Tepper et al.)[33]

iontophoretic application of dopamine to these terminals. These changes were mimicked by iontophoresis of a D_2 agonist (quinpirole), but not by a D_1 agonist (SKF38393), suggesting that they were mediated by dopamine D_2 heteroreceptors.[17] The interpretation of these findings is complicated by a seeming lack of correspondence between the actions of dopamine at hippocampal terminals *in vivo* and dopamine's action on hippocampal cell bodies *in vitro*, where it causes a membrane hyperpolarization and decreases excitability.[59,60] In addition, some aspects of the dopamine response in the hippocampal slice preparation may be mediated by β-adrenergic receptors;[61] it is at present unclear if a similar situation may also obtain at hippocampal terminals.

Two different studies have been conducted on dopamine receptor–mediated effects on corticostriatal terminal excitability.[14,62] In one, conditioning stimulation of the substantia nigra led to prolonged increases in corticostriatal terminal excitability.[62] The increased terminal excitability could be blocked by systemic administration of sulpiride, suggesting D_2 receptor mediation. In a second study, corticostriatal terminal excitability was decreased by local infusion of amphetamine or apomorphine, or electrical stimulation of substantia nigra,[14] consistent with the ability of D_2 receptor stimulation to decrease evoked release of glutamate from corticostriatal terminals.[63] These effects could be blocked by administration of haloperidol or sulpiride, and obtained even in animals with kainate lesions of the striatal terminal fields. Perhaps even more interesting, local infusions of haloperidol or sulpiride alone produced increased terminal excitability, suggesting that *in vivo,* the presynaptic dopamine heteroreceptors on corticostriatal afferents are stimulated by endogenously released dopamine. It is difficult to reconcile the contradictory findings of these two studies. Despite the success of sulpiride at antagonizing the opposite changes in terminal excitability in both experiments, it is conceivable that transmitters other than dopamine may have been involved. For example, since cholecystokinin has been shown to be colocalized in some ascending dopaminergic pathways,[64] it is possible that the increased terminal excitability seen by Mogenson and Yang may be due to release of this tachykinin, similar to effects observed on dopaminergic mesoaccumbens terminals.[65] Cholecystokinin has also been shown to exert an excitatory effect on dopamine-sensitive cortical neurons, which, incidentally, appear to possess dopamine receptors that are not easily classified as D_1 or D_2, perhaps further contributing to the disparate results.[66] Clearly, further experiments are necessary to clarify these issues.

Finally, evidence from terminal excitability testing also reveals the presence of dopaminergic receptors on the terminals of presumed GABAergic striatonigral neurons,[18] consistent with previous observations suggesting a role for presynaptic dopamine receptors in the modulation of nigral GABA release.[67] Interestingly, in contrast to all of the autoreceptors and most of the presynaptic heteroreceptor systems described above, careful examination of the presynaptic dopamine heteroreceptor on striatonigral terminals in vivo suggests that these receptors may not have a physiological function since although they can be activated by local infusions of a D_1 agonist, they appear insensitive to systemic administration of amphetamine, even at high doses. Furthermore, neither haloperidol nor the D_1 antagonist SCH23390, administered alone either systemically or locally, produced a significant change in striatonigral terminal excitability. These last two observations suggest that the levels of endogenously released dopamine, even when augmented by amphetamine, are not sufficient to stimulate these receptors.[18] Alternatively, perhaps the appropriate physiological conditions necessary for endogenous activation of these receptors have not yet been determined.

CONCLUSIONS

The data reviewed in this chapter consist largely of electrophysiological measurements of autoreceptor-mediated changes in terminal excitability in vivo induced by direct application of drugs or by changes in the rate of impulses reaching the axon terminal fields of several neurochemically and topographically distinct neuron types. These data are consistent with the presence of autoreceptors on the nerve terminals of a variety of different CNS neurons. At least within the class of monoaminergic neurons, presynaptic autoreceptor stimulation appears to be associated with a hyperpolarization of the terminal regions. As mentioned at the outset, the actual release of neurotransmit-

ter is not measured in these experiments, but these electrophysiological techniques do offer the advantages that they are performed *in vivo* in intact brains, under more or less "normal" physiological conditions, and can be correlated with the neuronal activity of the systems under study with temporal resolution in the millisecond range. These characteristics are particularly important when trying to address questions like "Are presynaptic autoreceptors physiologically functional *in vivo?*" and "Under what conditions of neuronal activity might they operate?". The results obtained following local infusions of autoreceptor antagonists alone into various axon terminal regions suggest quite strongly that the autoreceptors are physiologically functional *in vivo*, as do the observed correlations between the magnitude of the changes in terminal excitability observed after application of both agonists and antagonists. The nature of the correlations, coupled with the observations that physiologically relevant increases in impulse flow decrease monoaminergic terminal excitability through an autoreceptor-mediated mechanism, suggests that the presynaptic autoreceptors are most likely to be activated in vivo during prolonged periods of high activity. However, these systems also have the capacity to respond to very transient changes in neuronal activity, such as during brief bursts that may last less than 100 mseconds. These data paint a picture of an autoregulatory system that may be constantly changing its tone, or level of control perhaps on a spike by spike basis, to fine tune neurotransmitter output at individual sites within an axonal arbor in response to local conditions, at least partially independently of events occurring at the cell body.

ACKNOWLEDGMENTS

Thanks to F. Trent for assistance in preparing the figures and to Dr. D. H. Schwartz for helpful comments on the manuscript.

REFERENCES

1. STARKE, K., M. GOTHERT & H. KILBINGER. 1989. Modulation of neurotransmitter release by presynaptic autoreceptors. Physiol. Rev. **69**: 864–989.
2. GROVES, P. M., G. A. FENSTER, J. M. TEPPER, S. NAKAMURA & S. J. YOUNG. 1981. Changes in dopaminergic terminal excitability induced by amphetamine and haloperidol. Brain Res. **221**: 425–431.
3. TAKEUCHI, H., S. J. YOUNG & P. M. GROVES. 1982. Dopaminergic terminal excitability following arrival of the nerve impulse: the influence of amphetamine and haloperidol. Brain Res. **245**: 47–56.
4. TEPPER, J. M., S. NAKAMURA, S. J. YOUNG & P. M. GROVES. 1984. Autoreceptor-mediated changes in dopaminergic terminal excitability: effects of striatal drug infusions. Brain Res. **309**: 317–333.
5. TEPPER, J. M., S. J. YOUNG & P. M. GROVES. 1984. Autoreceptor-mediated changes in dopaminergic terminal excitability: effects of increases in impulse flow. Brain Res. **309**: 309–316.
6. TEPPER, J. M., S. F. SAWYER, S. J. YOUNG & P. M. GROVES. 1986. Autoreceptor-mediated changes in dopaminergic terminal excitability: effects of potassium channel blockers. Brain Res. **367**: 230–237.
7. CHAVEZ-NORIEGA, L., P. PATINO & M. GARCIA-MUNOZ. 1986. Excitability changes induced in the striatal dopamine-containing terminals following frontal cortex stimulation. Brain Res. **379**: 300–306.
8. MEREU, G., T. C. WESTFALL & R. Y. WANG. 1985. Modulation of terminal excitability of mesolimbic dopaminergic neurons by D-amphetamine and haloperidol. Brain Res. **359**: 88–96.

9. GARIANO, R. F., S. F. SAWYER, J. M. TEPPER, S. J. YOUNG & P. M. GROVES. 1989. Mesocortical dopaminergic neurons. 2. Consequences of terminal autoreceptor activation. Brain Res. Bull. **22:** 517–523.
10. NAKAMURA, S., J. M. TEPPER, S. J. YOUNG & P. M. GROVES. 1981. Neurophysiological consequences of presynaptic receptor activation: changes in noradrenergic terminal excitability. Brain Res. **226:** 155–170.
11. NAKAMURA, S., J. M. TEPPER, S. J. YOUNG & P. M. GROVES. 1982. Changes in noradrenergic terminal excitability induced by amphetamine and their relation to impulse traffic. Neuroscience **7:** 2217–2224.
12. RYAN, L. J., J. M. TEPPER, S. J. YOUNG & P. M. GROVES. 1985. Amphetamine's effect on terminal excitability of noradrenergic locus coeruleus neurons are impulse dependent at low but not high doses. Brain Res. **341:** 155–163.
13. SAWYER, S. F., J. M. TEPPER, S. J. YOUNG & P. M. GROVES. 1985. Antidromic activation of dorsal raphé neurons from neostriatum: physiological characterization and effects of autoreceptor activation. Brain Res. **332:** 15–28.
14. GARCIA-MUNOZ, M., S. J. YOUNG, & P. M. GROVES. 1989. Modification of excitability of corticostriatal afferents: evidence for auto and heteroreceptor regulation. Soc. Neurosci. Abstr. **15:** 584.
15. ROMO, R. & W. SCHULTZ. 1985. Prolonged changes in dopaminergic terminal excitability and short changes in dopaminergic neuron discharge rate after short peripheral stimulation in monkey. Neurosci. Lett. **62:** 335–340.
16. NAKAMURA, S., J. M. TEPPER, S. J. YOUNG, N. LING & P. M. GROVES. 1982. Noradrenergic terminal excitability: effects of opioids. Neurosci. Lett. **30:** 57–62.
17. YANG, C. R. & G. J. MOGENSON. 1986. Dopamine enhances terminal excitability of hippocampal-accumbens in presynaptic inhibition. J. Neurosci. **6:** 2470–2478.
18. RYAN, L. J., M. DIANA, S. J. YOUNG & P. M. GROVES. 1989. Dopamine D1 heteroreceptors on striatonigral axons are not stimulated by endogenous dopamine either tonically or after amphetamine: evidence from terminal excitability. Exp. Brain Res. **77:** 161–165.
19. TEPPER, J. M., S. F. SAWYER, S. NAKAMURA & P. M. GROVES. Dopaminergic and serotonergic terminal excitability: effects of autoreceptor stimulation and blockade. *In* The Presynaptic Regulation of Neurotransmitter Release. J. J. Feigenbaum & M. Hanai, Eds. Freund Publishing Co. Tel Aviv, Israel. (In press.)
20. CEDARBAUM, J. M. & G. K. AGHAJANIAN. 1977. Catecholamine receptors on locus coeruleus neurons: pharmacological characterization. Eur. J. Pharmacol. **44:** 375–385.
21. AGHAJANIAN, G. K. & C. P. VANDERMAELEN. 1982. Alpha2-adrenoceptor-mediated hyperpolarization of locus coeruleus neurons: intracellular studies in vivo. Science **215:** 1394–1396.
22. WILLIAMS, J. T., G. HENDERSON & R. A. NORTH. 1985. Characterization of alpha2-adrenoceptors which increase potassium conductance in rat locus coeruleus neurones. Neuroscience **14:** 95–101.
23. TAUBE, H. D., K. STARKE & E. BOROWSKI. 1977. Presynaptic receptor systems on the noradrenergic neurones of rat brain. Naunyn Schmiedebergs Arch. Pharmacol. **299:** 123–141.
24. LANGER, S. Z. 1977. Presynaptic receptors and their role in the regulation of transmitter release. Br. J. Pharmacol. **60:** 481–497.
25. AGHAJANIAN, G. K. & B. S. BUNNEY. 1977. Dopamine 'autoreceptors': pharmacological characterization by microiontophoretic single cell recording studies. Naunyn Schmiedebergs Arch. Pharmacol. **297:** 1–7.
26. LACEY, M. G., N. B. MERCURI & R. A. NORTH. 1987. Dopamine acts on D2 receptors to increase potassium conductance in neurons of the rat substantia nigra zona compacta. J. Physiol. London **392:** 497–516.
27. MORELLI, M., T. MENNINI & G. DI CHIARA. 1988. Nigral dopamine autoreceptors are exclusively of the D2 type: quantitative autoradiography of [^{125}I]iodosulpiride and [^{125}I]SCH 23982 in adjacent brain sections. Neuroscience **27:** 865–870.
28. HELMREICH, I., W. REIMANN, G. HERTTING & K. STARKE. 1982. Are presynaptic dopamine autoreceptors and postsynaptic dopamine receptors in the rabbit caudate nucleus pharamcologically different? Neuroscience **7:** 1559–1566.

29. BOYAR, W. C. & C. A. ALTAR. 1987. Modulation of in vivo dopamine release by D2 but not D1 receptor agonists and antagonists. J. Neurochem. **48:** 824–831.
30. DIANA, M., S. J. YOUNG & P. M. GROVES. 1989. Modulation of dopaminergic terminal excitability by D1 selective agents. Neuropharmacology **28:** 99–101.
31. VANDERMAELEN, C. P. & G. K. AGHAJANIAN. 1983. Electrophysiological and pharmacological characterization of serotonergic dorsal raphé neurons recorded extracellularly and intracellularly in rat brain slices. Brain Res. **289:** 109–119.
32. MARTIN, L. L. & E. SANDERS-BUSCH. 1982. Comparison of the pharmacological characteristics of 5HT1 and 5HT2 binding sites with those of serotonin autoreceptors which modulate serotonin release. Naunyn Schmiedebergs Arch. Pharmacol. **321:** 165–170.
33. TEPPER, J. M., P. M. GROVES & S. J. YOUNG. 1985. The neuropharmacology of the autoinhibition of monoamine release. Trends Pharmacol. Sci. **6:** 251–256.
34. CONNICK, J. H. & T. W. STONE. 1988. Excitatory amino acid antagonists and endogenous aspartate and glutamate release from rat hippocampal slices. Br. J. Pharmacol. **93:** 863–867.
35. BAXTER, D. A. & G. D. BITTNER. 1981. Intracellular recordings from crustacean motor axons during presynaptic inhibition. Brain Res. **223:** 422–428.
36. HUBBARD, J. I., R. LLINAS & D. M. J. QUASTAL. 1969. Electrophysiological Basis of Synaptic Transmission: 15–84, 112–173.50. Williams & Wilkins Co. Baltimore, Md.
37. TEPPER, J. M., R. F. GARIANO & P. M. GROVES. 1987. The neurophysiology of dopamine nerve terminal autoreceptors. Neurophysiology of Dopaminergic Systems: Current Status and Clinical Perspectives. *In* L. A. Chiodo & A. S. Freeman, Eds.: 93–127. Lakeshore Press. Grosse Pt., Mich.
38. AGHAJANIAN, G. K. & J. M. LAKOSKI. 1984. Hyperpolarization of serotonergic neurons by serotonin and LSD: studies in brain slices showing increased K+ conductance. Brain Res. **305:** 181–185.
39. ECCLES, J. C., R. F. SCHMIDT & W. D. WILLIS. 1963. The mode of operation of the synaptic mechanism producing presynaptic inhibition. J. Neurophysiol. **26:** 523–536.
40. WALL, P. D. 1958. Excitability changes in afferent fibre terminations and their relation to slow potentials. J. Physiol. London **142:** 1–21.
41. ZUCKER, R. S. & L. LANDO. 1986. Mechanism of transmitter release: voltage hypothesis and calcium hypothesis. Science **231:** 574–579.
42. DINGLEDINE, R. 1983. *N*-Methyl aspartate activates voltage-dependent calcium conductance in rat hippocampal pyramidal cells. J. Physiol. London **343:** 385–405.
43. KUCZENSKI, R. 1983. Biochemical actions of amphetamine and other stimulants. *In* Stimulants: Neurochemical, Behavioral and Clinical Perspectives. I. Creese, Ed.: 31–61. Raven Press. New York, N.Y.
44. KALSNER, S. 1985. Is there feedback regulation of neurotransmitter release by autoreceptors? Biochem. Pharmacol. **34:** 4085–4097.
45. CUBEDDU, L. X. & I. S. HOFFMANN. 1982. Operational characteristics of the inhibitory feedback mechanism for regulation of dopamine release via presynaptic receptors. J. Pharmacol. Exp. Ther. **223:** 497–501.
46. HOFFMANN, I. S. & L. X. CUBEDDU. 1982. Rate and duration of stimulation determine presynaptic effects of haloperidol on dopaminergic neurons. J. neurochem. **39:** 585–588.
47. LEHMANN, J. & S. Z. LANGER. 1982. The pharmacological distinction between central pre- and postsynaptic dopamine receptors: implications for the pathology and therapy of schizophrenia. Adv. Dopamine Res. Adv. Biosci. **37:** 25–39.
48. DUBOCOVICH, M. L. & J. G. HENSLER. 1986. Modulation of [^{3}H]-dopamine release by different frequencies of stimulation from rabbit retina. Br. J. Pharmacol. **88:** 51–61.
49. MAYER, A., N. LIMBERGER & K. STARKE. 1988. Transmitter release patters of noradrenergic, dopaminergic and cholinergic axons in rabbit brain slices during short pulse trains and the operation of presynaptic autoreceptors. Naunyn Schmiedebergs Arch. Pharmacol. **338:** 632–643.
50. BLIER, P., R. RAMDINE, A.-M. GALZIN & S. Z. LANGER. 1989. Frequency-dependence of serotonin autoreceptor but not α_2-adrenoceptor inhibition of [^{3}H]-serotonin release in rat hypothalamic slices. Naunyn Schmiedebergs Arch. Pharmacol. **339:** 60–64.
51. STAUNTON, D. A., S. J. YOUNG & P. M. GROVES. 1980. The effect of long-term amphet-

amine administration on the activity of dopaminergic neurons of the substantia nigra. Brain Res. **188:** 107–117.

52. TEPPER, J. M., S. NAKAMURA, S. J. YOUNG & P. M. GROVES. 1982. Subsensitivity of catecholaminergic neurons to direct acting agonists after single or repeated electroconvulsive shock. Biol. Psychiatry –17: 1059–1070.
53. JACOBS, B. L., J. HEYM & K. RASMUSSEN. 1983. Raphé neurons: firing rate correlates with size of drug response. Eur. J. Pharmacol. **90:** 275–278.
54. WHITE, F. J. & R. Y. WANG. 1984. A10 dopamine neurons: role of autoreceptors in determining firing rate and sensitivity to dopamine agonists. Life Sci. **34:** 1161–1170.
55. PEPPER, C. M. & G. HENDERSON. 1980. Opiates and opioid peptides hyperpolarize locus coeruleus neurons in vitro. Science **209:** 394–396.
56. NORTH, R. A., & J. T. WILLIAMS. 1985. On the potassium conductance increased by opioids in rat locus coeruleus neurones. J. Physiol. London **364:** 265–280.
57. NAKAMURA, S., J. M. TEPPER & P. M. GROVES. Noradrenergic terminal excitability: effects of presynaptic receptor stimulation and blockade. *In* The Presynaptic Regulation of Neurotransmitter Release. J. J. Feigenbaum & M. Hanani, Eds. Freund. London & Tel Aviv. (In press.)
58. CHARNAY, Y., L. LÉGER, F. DRAY, A. BEROD, M. JOUVET, J. F. PUJOL & P. M. DUBOIS. 1982. Evidence for the presence of enkephalin in catecholaminergic neurones of cat locus coeruleus. Neurosci. Lett. **30:** 147–151.
59. BERNARDO, L. S. & D. A. PRINCE. 1982. Dopamine action on hippocampal pyramidal cells. J. Neurosci. **2:** 415–423.
60. STANZIONE, P., P. CALABRESI, N. MERCURI & G. BERNARDI. 1984. Dopamine modulates CA1 hippocampal neurons by elevating the threshold for spike generation: an in vitro study. Neuroscience **13:** 1105–1116.
61. MALENKA, R. C. & R. A. NICOLL. 1986. Dopamine decreases the calcium-activated hyperpolarization in hippocampal CA1 pyramidal cells. Brain Res. **379:** 210–215.
62. MOGENSON, G. J. & C. R. YANG. 1987. Dopamine modulation of limbic and cortical input to striatal neurons. *In* Neurophysiology of Dopaminergic Systems: Current Status and Clinical Perspectives. L. A. Chiodo & A. S. Freeman, Eds.: 237–251. Lakeshore Press. Grosse Pt., Mich.
63. MAURA, G., A. GIARDI & M. RAITERI. 1988. Release-regulating D-2 dopamine receptors are located on striatal glutamatergic nerve terminals. J. Pharmacol. Exp. Ther. **247:** 680–684.
64. HOKFELT, T., J. F. REHFELD, L. SKIRBOLL, B. IREMARK, M. GOLDSTIEN & K. MARKEY. 1980. Evidence for coexistence of dopamine and CCK in mesolimbic neurons. Nature London **285:** 476–478.
65. GARCIA-MUNOZ, M. & P. PATINO. 1987. Excitability changes of the dopamine containing terminals in the nucleus accumbens induced by cholecystokinin. Neurosci. Lett. *Suppl.* **29:** 24.
66. BUNNEY, B. S. & S. R. SEESACK. 1987. Electrophysiological identification and pharmacological characterization of dopamine sensitive neurons in the rat prefrontal cortex. *In* Neurophysiology of Dopaminergic Systems: Current Status and Clinical Perspectives. L. A. Chiodo & A. S. Freeman, Eds.: 129–140. Lakeshore Press. Grosse Pt., Mich.
67. REUBI, J.-C., L. L. IVERSEN & T. M. JESSELL. 1977. Dopamine selectively increases ^{3}H-GABA release from slices of rat substantia nigra in vitro. Nature London **268:** 652–654.