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Effects of inhibition of membrane-bound metalloendopeptidase (EC 3.4.24.11) on the firing of neurones in the substantia nigra of the rat

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Effects of Inhibition of Membrane-Bound Metalloendopeptidase (EC 3.4.24.11) on the Firing of Neurones in the Substantia Nigra of the Rat

By

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ABSTRACT

Effects of Inhibition of Membrane-Bound Metalloendopeptidase (EC 3.4.24.11) on the Firing of Neurones in the Substantia Nigra of the Rat

By

MICHAEL J. BIER

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The effect of inhibitors of the membrane-bound metalloendopeptidase-24.11 ('enkephalinase') on the activity of electrophysiologically identifiable dopaminergic and non-dopaminergic neurones in the substantia nigra of the rat is described. Only those cells in which the stimulation evoked excitation (alone or mixed with inhibition) responded to the inhibitors. Infusion of 1 umol of N-[1-(R,S)-carboxy- 2-phenylethyl] Phe-pAB (CPAB), 1 or 2 umol of N-[1-(R,S)- carboxy-3-phenylpropyl] Phe-pAB (CPPAB) into the lateral ventricle produced significant increases in the spontaneous activity and short latency evoked responses of non-dopaminergic and dopaminergic cells. The increased firing frequency results from inhibition of the enzyme as infusion of N-[1-(R,S)-carboxy-2- phenyl-ethyl] Leu-pAB (CPLAB), an inhibitor structurally related to CPAB and CPPAB yet two

orders of magnitude less potent, was without effect on the spontaneous activity of nigral neurones. Enzyme inhibition was verified through in vitro assay.

Inhibitors were administered directly into the nigra and striatum in order to localize the possible site of action of the CPAB i.c.v. effects. Perfusion of approximately 4% of the striatum with 1 uL of a 40 millimolar solution of CPAB produces 60% of the response on spontaneous activity of non-dopaminergic and 30% of the response of dopaminergic cells obtained following i.c.v. administration of CPAB. Treatment of approximately 4% of the striatum with CPAB produces 25% of the evoked response of dopaminergic cells obtained following CPAB i.c.v. It is concluded that the striatum may be an important site of action in the generation of the increase in the spontaneous activity of non-dopaminergic and dopaminergic cells following CPAB i.c.v. and may also contribute to, but can not fully account for, the increase in the magnitude of the evoked response of dopaminergic cells.

The increase in the spontaneous activity and magnitude of the evoked response of non-dopaminergic or dopaminergic cells following 1 umol CPAB i.c.v. can not be accounted for by inhibition of the activity of endopeptidase-24.11 in the substantia nigra alone. Application of 0.04 umol CPAB (a dose which was determined to yield inhibition of enzyme

activity nearly equivalent to CPAB i.c.v.) into the substantia nigra had no significant effect on the spontaneous activity of nigral neurons. Significant increases in the magnitude of the evoked response of non-dopaminergic (average increase 35%) but not dopaminergic cells was observed following inhibition of the activity of 24.11 in the substantia nigra. But, the increase in the magnitude of the evoked response of the non-dopaminergic cells could not account for the increases observed following CPAB i.c.v.

Increases in spontaneous and evoked activity of dopaminergic and non-dopaminergic cells, following application of CPAB i.c.v., are dependent, in part, on enhanced opioid peptide (enkephalin and/or dynorphin) activity in the striatum and substantia nigra. In each circumstance naloxone (1 mg/kg, i.v. or local infusion of 0.002 nmol into the striatum or substantia nigra) significantly reduced CPAB i.c.v. induced increases in activity with two exceptions. Increases in the spontaneous activity of dopaminergic cells following CPAB i.c.v. was not antagonized by application of naloxone i.v. or local application into the substantia nigra.

Application of 0.002 nmol naloxone into the striatum is able to antagonize the CPAB i.c.v. induced increase in the spontaneous firing of dopaminergic cells while 0.002

nmol naloxone applied to the nigra or 1 mg/kg, i.v. is unable to antagonize the effect of CPAB i.c.v. The effect of CPAB i.c.v. on the spontaneous activity of dopaminergic cells is therefore dependent on enhanced opioid peptide activity in the striatum.

Application of 1 mg/kg, i.v. naloxone is able to antagonize the effect of CPAB i.c.v. on the magnitude of the evoked response of non-dopaminergic cells. Sustained opioid peptide activity in either the substantia nigra or striatum is sufficient for expression of this effect in non-dopaminergic cells. This antagonism (following naloxone i.v.) can be accounted for by the action of naloxone in the substantia nigra or striatum. Application of 1 mg/kg, i.v. naloxone is able to reverse the effect of CPAB i.c.v. on the magnitude of the evoked response of dopaminergic cells. This effect can not be accounted for by the action of naloxone in the substantia nigra or striatum alone. Enhanced opioid peptide activity in the striatum and nigra are necessary for full expression of the CPAB i.c.v. effect.

DEDICATION

To Lola - Who knew I would finish when I thought I would not. Who's love sustained me through what was a difficult time. And my father - who thought I might finish in 1985.

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ABBREVIATIONS

AchE.....Acetylcholine Esterase

ACE.....Angiotensin Converting Enzyme

CPAB.....N-[1-(R,S)-carboxy-2-phenylethyl]-Phe-pAB

CPPAB.....N-[1-(R,S)-carboxy-3-phenylpropyl]-Phe-pAB

CPLAB.....N-[1-(R,S)-carboxy-2-phenylethyl]-Leu-pAB

DOPAC.....Dihydroxyphenylacetic Acid

epsp.....Excitatory Post Synaptic Potential

GABA.....Gamma-Aminobutyric Acid

HVA.....Homovanillic Acid

i.c.v.....Intracerebroventricular

i.p.....Intraperotioneal

ipsp.....Inhibitory Post Synaptic Potential

i.v.....Intraveneous

s.c.....Subcutaneous

SNc.....Substantia Nigra Compacta

SNr.....Substantia Nigra Reticulata

INTRODUCTION

A. The Hydrolysis of the Enkephalins

In nervous tissue two processes act to regulate the duration of non-peptide transmitter action; uptake and inactivation via enzymatic degradation. Since there is no evidence for specific uptake systems for the peptide transmitters, attention has focused on peptide degradation as the primary regulatory mechanism of peptide activity following release. Much of the work which has been performed on the metabolism of peptides has focused on the isolation and characterization of a group of enzymes that metabolize the enkephalins.

Soon after the publication of the isolation and characterization of the pentapeptides Met- and Leu-enkephalin by Hughes and Kosterlitz in 1975 it was observed that these peptides are rapidly enzymatically degraded in vivo following intrajugular injection (Dupont et al., 1977), perfusion of Met-enkephalin into the cerebral ventricle and in vitro in tissue homogenates (Craves et al., 1978). This rapid metabolism of the enkephalins engendered the hypothesis that enkephalin-specific enzymes might account for the rapid specific hydrolysis of these peptides.

As of 1988 the study of the enzymes responsible for the synthesis and degradation of biological peptides remains in its infancy. Confusion remains concerning both the identity of the enzyme or enzymes terminating the action of specific peptides as well as the substrate specificity of well characterized enzymes. For example, there is much contention concerning the function of endopeptidase-24.11 in-vivo (Schwartz et al., 1985; Turner et al., 1986). The controversy over the in-vivo function of this important enzyme stems from the initial finding both in-vivo and in-vitro that the enzyme degrades enkephalins while later studies have clearly shown that the enzyme preferentially cleaves substance P (Mauborgne et al., 1987; Matsas et al., 1984; Hooper et al., 1985) and its distribution in the CNS more closely parallels that of substance P than enkephalin (Matsas et al., 1986). Early studies on endopeptidase-24.11 "enkephalinase" ascribed a Km of 41 nM (Malfroy et al., 1978). Subsequently it was ascribed Km values of 0.1 uM (Swerts et al., 1979b) and 33 uM (Patey et al., 1981). Further, various forms of the same enzyme (with the same activity) have been given different names (Gorenstein and Snyder 1980). One of the more vexing problems has been the classification of the activity of "enkephalinase" (which is in most studies properly attributable to the action of 24.11) as a dipeptidyl carboxypeptidase.

In preparations of synaptosomal membranes (Malfroy et al., 1978), CNS homogenates (Vogel and Alstein 1980), and striatal slices (De La Baume et al., 1983) enkephalin is cleaved primarily at two sites: the Tyr^1-Gly^2 and Gly^3-Phe^4 bonds. Hydrolysis of the Tyr^1-Gly^2 bond, releasing the amino terminal Tyr is attributed to the action of aminopeptidases (Hersh, 1981; Gorenstein and Snyder,1980; Vogel and Alstein, 1980), while hydrolysis at the Gly^3-Phe^4 bond has been variously attributed to dipeptidyl carboxypeptidase action (Malfroy et al., 1978; Sullivan et al., 1978; Gorenstein and Snyder 1980; Schwartz et al., 1981), originally believed to be anglotensin converting enzyme (EC 3.4.15.1, peptidyl dipeptidase) (Swerts et al., 1979; Benuck and Marks, 1979). This bond is also cleaved by endopeptidase action; specifically a neutral metalloendopeptidase (EC 3.4.24.11) "enkephalinase" (Sullivan et al 1978, 1980; Malfroy et al., 1978; Almenoff et al., 1981; Blumberg et al., 1981; Fulcher et al., 1982). This preoccupation with the hydrolysis of enkephalin has misled some investigators from considering a less restricted range of activities for the enzymes they have isolated. This is especially true of the enzyme commonly refered to as "enkephalinase"; metalloendopeptidase (EC 3.4.24.11).

A.1. Aminopeptidases

In 1981 Hersh identified two different aminopeptidases which have both membrane bound and soluble components which he designated as M1 and M2. The two enzymes were primarily distinguished on the basis of their activity against synthetic substrates and enkephalins. The fraction called M1 is relatively insensitive to inhibition by puromycin (Ki 1.0 mM) and exhibits low affinity for enkephalin. The M2 fraction is potently inhibited by puromycin (Ki 1.0 uM) and hydrolyzes enkephalins with a Km of 20-40 uM. There is, however, conflicting data concerning the action of these enzymes as De La Baume et al (1983) studied an aminopeptidase which was sensitive to bestatin inhibition but not puromycin. De La Baume suggested that the inability of puromycin to inhibit the enzyme might be the result of inaccessibility of the inhibitor to the enzyme. A report by Hersh (1985) suggests that an aminopeptidase activity called M2, released by thiol treatment, is highly localized in the striatum, hypothalamus and cortex of the rat. These enzymes are largely soluble but have some membrane-bound components (Hersh 1981,1985; Hortsthemke et al., 1983; Gorenstein and Snyder, 1979). On the whole, as their distributions do not correlate well with localization of specific peptides the aminopeptidases are thought to act non-specifically (Gorenstein and Snyder 1980; Sullivan et al 1978).

A.2. Dipeptidyl Carboxypeptidase and Angiotensin

Converting Enzyme

Much research attention has centered on the characterization of the enzyme which acts at the Gly^2-Phe^4 bond. This activity was first suggested to be a dipeptidyl carboxypeptidase (Schwartz et al 1981, Schwartz 1983, Malfroy et al 1979). The possibility that the dipeptidyl carboxypeptidase activity observed in vitro could be attributed to angiotensin converting enzyme was suggested (Erdos et al., 1978, Benuck and Marks 1979). Gorenstein and Snyder 1980 showed that the enzymes they characterized as enkephalinase A and B were separable from angiotensin converting enzyme via DEAE column chromatography. Largely through the use of specific inhibitors the differential activity of "enkephalinase" and angiotensin converting enzyme has been established. Sullivan et al (1980) demonstrated the independence of these enzymes on the basis of different sensitivities of the enzymes to inhibition by a snake venom nonapeptide, chloride dependence and different activities in phosphate buffers. The enkephalin degrading activity was shown to be insensitive to Teprotide (SQ 20,881) and the potent antihypertensive drug Captopril (SQ 14,225); both are inhibitors of angiotensin converting enzyme (Swerts et al., 1979). Thus, the term "enkephalinase" has come to be associated with the membrane-bound metalloendopeptidase.

A.3. The use of enzyme inhibitors in the characterization of peptidases

In 1980 Roques et al. reported the synthesis of an inhibitor of "enkephalinase" called Thiorphan (N-(DL-2-benzyl-3-mercapto- propionyl)-glycine). Thiorphan has been reported to have no inhibitory action against the activity of ACE (Roques et al., 1983, 1982). But thiorphan is non-selective as its inhibitory potency against ACE is within two log units of endopeptidase-24.11 and could therefore concelvably have inhibitory action against ACE. The enzyme which is primarily inhibited by thiorphan is proposed to be a Zn-metalloenzyme since its inhibition by 1,10-phenanthroline is reversed by Zn^{+2} (Swerts et al., 1979). This enzyme is strongly inhibited by phosphoramidon, metal chelating agents, and by thiols (Gorenstein and Snyder 1979; Fournie-Zaluski et al., 1979).

Thiorphan administered i.v. or i.c.v. was shown to potentiate the analgesic activity of D-Ala^2-Met-enkephalin, an aminopeptidase resistant enkephalin analogue, when administered i.c.v. by prolonging the the tail withdrawal latency of mice (Roques et al., 1980; Chipkin et al., 1982; Yaksh and Harty 1982). The antinociceptive action of thiorphan and bestatin have been shown to be separable. Zhang et al. (1982) performed in-vivo inhibition studies utilizing bestatin [(2S, 3R)]-3-amino-2-hydroxy-4-phenylbutanoyl- L-leucine], an aminopeptidase inhibitor

which is 1,000 times more potent than puromycin in inhibiting the hydrolysis of Leu-enkephalin in striatal slices (Chaillet et al., 1983). Intracerebroventricular administration of bestatin failed to change striatal Met-enkephalin levels (as determined by radioimmunoassay) or to produce analgesia when measured by latency to jump off a hot plate, while thiorphan increased the striatal Met-enkephalin content 30% in 30 minutes. Zhang noted that bestatin could potentiate the effects of thiorphan and he concluded that the aminopeptidase had a higher Km than the enkephalin degrading enzyme and became operative when the extracellular content of the enkephalins reached an appropriate level. Carenzi et al. (1983) showed that only concomitant i.c.v. administration of both inhibitors increased the threshold of pain as measured by hot plate withdrawal in the presence and absence of exogenously applied enkephalin. Only thiorphan was active in potentiating the effect of exogenously applied enkephalins when the inhibitors were given i.v. Constentin et al. (1986) showed that the antinociceptive action of the inhibitors bestatin and thiorphan were specific to the tests being performed. When administered together the inhibitors produced naloxone sensitive analgesia in vocalization, hot plate, and writhing tests but not to tail withdrawal, hot plate-licking or tail flick tests. These results suggest aminopeptidases and "enkephalinase" are involved in different aspects of pain processing; neither

having a universal physiological role. Further, nociception is a multipathway phenomena and any simple correlation with a single peptide or a single enzyme in the expression of this behavioral event would not be expected.

Thiorphan was shown in-vitro to enhance the recovery of potassium stimulated Met-enkephalin released in rat striatal slices while puromycin (inhibitor of aminopeptidases) and captopril (inhibitor of ACE) had little effect on the recovery of the peptide (Patey et al., 1981). Other experiments have shown, using the same preparation, that complete recovery of enkephalin is achieved only following the application of both thiorphan and bestatin, indicating that enkephalin released synaptically is inactivated by both enkephalinase and aminopeptidase action (Schwartz et al., 1983).

Kayser et al. (1984) studied the effects of a systemically administered derivative of thiorphan, ES52, on the electrical activity of neurons in the ventrobasal thalamus in response to noxious stimuli. The rationale for the study being that the ventrobasal thalamus functions in mediation of pain processes. They showed decreases in tail pinch "noxious" stimulus response of 12/13 neurons studied following 5 mg/kg i.v., ES52. The time course of action of the drug was 5 minutes to onset with maximal effect 15 to 20 minutes following administration. They observed a reversal of the effect at 60 minutes. It is of interest

that naloxone reversed the effect of this enkephalinase inhibitor in only one half of the cells they examined.

A.4. Metalloendopeptidase-24.11

Blumberg et al. (1981) first suggested that the cleavage at the Gly^3-Phe^4 bond was due to an endopeptidase attack rather than a dipeptidyl carboxypeptidase action. Benzyloxycarbonylamino acid hydroxamate inhibitors were prepared and the K_i values determined for "enkephalinase" were compared with a known bacterial metalloendopeptidase thermolysin. Benzyloxycarbonyl derivatives of leucine, phenylalanine and D-phenylalanine with a free carboxylic group are weak inhibitors of thermolysin (1-2 mM) whereas the hydroxamates are 1000 times more potent inhibitors (3-23 uM). This also turned out to be true of the "enkephalinase" suggesting that "enkephalinase" may act like an endopeptidase.

The identity of this "enkephalinase" with the kidney neutral endopeptidase (EC 3.4.24.11), an enzyme first isolated by Kerr and Kenny in 1974 from the brush border of the rabbit kidney, has recently been established. This enzyme identity was established through the use of specific enzyme inhibitors (Almenoff and Orlowski 1983; Matsas 1984), immunohistochemical identification (Almenoff and Orlowski 1984; Matsas et al., 1986), and most importantly

through careful consideration of the substrate specificity of the enzyme (Almenoff et al., 1981; Almenoff and Orlowski 1983; Matsas et al., 1984; Mumford et al., 1981).

In 1981 Orlowski and Wilk isolated and purified a membrane bound metalloendopeptidase from the bovine pituitary. This enzyme directs its specificity toward the amino side of hydrophobic amino acid residues in a manner resembling thermolysin. They reported that this enzyme is also similar to the neutral endopeptidase EC 3.4.24.11. In 1981 Almenoff, Wilk and Orlowski reported that the purified pituitary enzyme cleaved the enkephalins, oxytocin, bradykinin and neurotensin. They concluded that the enzyme they had been working with was most likely the enzyme identified as the "enkephalinase" and that this enzyme did not act as a dipeptidyl carboxypeptidase but instead as an endopeptidase, which preferentially cleaves bonds on the amino side of hydrophobic residues. The endopeptidase acts like a dipeptidyl carboxypeptidase if the hydrophobic residue being attacked is in the penultimate position. Studies using different substrates confirmed the endopeptidase activity of the enzyme (Matsas et al., 1984; Hooper et al., 1985). The specificity of the metalloendopeptidase isolated from rabbit kidney, as determined from the study of napthylamide substrates, is similar to the specificity of the metalloendopeptidase isolated from bovine pituitary and rabbit brain. (Almenoff

and Orlowski 1983; Matsas et al., 1983; Almenoff and Orlowski 1984).

Many biologically active peptides contain internal hydrophobic residues and are therefore substrates for the endopeptidase-24.11. Studies using natural peptide substrates demonstrate that the specificity of the enzyme is not directed primarily at the enkephalins. The lowest reported Km values are for substance P and the specificity of the enzyme is 3 fold higher for the tachykinins than the enkephalins (Mumford et al., 1981; Matsas et al., 1984).

In 1983 Almenoff and Orlowski reported the synthesis of N-carboxymethyl derivatives of amino acids containing p-aminobenzoate which act as inhibitors of the membrane-bound kidney neutral metalloendopeptidase. These inhibitors are quite specific and are superior to the other inhibitors of EC 24.11. for in vivo use (Matsas et al. 1984). Unlike the other commonly used inhibitors of endopeptidase-24.11, thiorphan and phosphoramidon, the inhibitors used in this study selectively inhibit only endopeptidase-24.11 with no effect on peptidyl dipeptidase activity (Fulcher et al., 1982; Matsas et al., 1984). Thiorphan, which has been the most widely used inhibitor of endopeptidase-24.11, is not as selective as its inhibitory potency against angiotensin-converting enzyme (ACE; peptidyldipeptide hydrolase, EC 3.4.15.1) is only two log units from that of endopeptidase-24.11 (Roques et al.,

1983). Angiotensin-converting enzyme is present in high concentration in the substantia nigra and is able to hydrolyse substance P and enkephalin (Marks et al., 1980; Strittmatter et al., 1984; Thiele et al., 1985). Inhibition of ACE would therefore be expected to affect both peptide metabolism and electrical activity in the nigra. The inhibitors used in this study are also more suitable for in-vivo use as they are more biologically stable; phosphoramidon is susceptible to enzymatic degradation while thiorphan is susceptible to oxidation at its sulfhydryl group (Fulcher et al., 1982; Matsas et al., 1984). Thus, inhibitors used in this study avoid effects secondary to degradative products of the inhibitors and spurious enzyme inhibition.

A.5. Anatomic localization of 24.11

Early studies on the location of "enkephalinase" in the CNS tested the idea that there may be a correlation between the location of enkephalins and opiate receptors and the enzyme. The initial findings showed that "enkephalinase" was indeed found to be roughly localized to brain regions which are rich in opiate receptors and peptides: the striatum and hypothalamus (Malfroy et al., 1979; Sullivan et al., 1978). This enzyme was shown to increase its activity in the striatum of mice chronically treated with morphine (Malfroy et al., 1979).

The distribution of endopeptidase-24.11 in the CNS has been examined using enzyme assays on dissected areas (Almenoff et al., 1981), autoradiography (Pollard et al., 1978, 1987a, 1987b; Waksman et al., 1986, 1987) and immunohistochemistry (Matsas et al., 1986). The enzyme was shown to be an ectoenzyme (Kenny et al. 1983) which is highly heterogeneously distributed in the CNS with its highest activity and staining in the striatum and substantia nigra (Almenoff et al, 1981; Matsas et al.1986). Matsas et al. (1986) also showed in the pig striatum that the immunohistochemical staining of 24.11 was highly localized to striosomes. Further, this staining was more often associated with patches of substance P staining than with patches of enkephalin. In the striatum the staining of 24.11 overlaps mu opioid binding sites but is more closely related to the distribution of the more diffusely distributed delta binding sites. Alternatively in the substantia nigra the distribution of 24.11 was more highly correlated with mu rather than delta sites (Waksman et al., 1986). Matsas also note that they observed very scant labeling in the globus pallidus. This is in contrast with other studies in the rat which show very high staining in the globus pallidus (Waksman et al., 1986, 1987; Pollard et al., 1987). Differences in staining may be accounted for by species variation or differences in methodology. There is agreement among the studies in the rat showing a highly heterogeneously distributed staining of the enzyme with the

most intense staining in the striatum, substantia nigra and olfactory tubercle and scant staining of the nucleus accumbens, nucleus interpeduncularis, superior colliculus, hippocampus and entopeduncular nucleus. A recent study by Pollard et al (1987) in the rat showed a continuous band of 24.11 immunoreactivity along the striatonigral pathway.

B. Basal Ganglia: Anatomy

Early studies of the basal ganglia suggested that the neurochemical circuitry was organized in a relatively simple manner. The striatum receives dopaminergic projections from the substantia nigra which affect cholinergic and GABAergic transmission (interneurons) within the striatum. Cholinergic, GABAergic and dopaminergic influnces shape the GABAergic efferent transmission of the striatum. The GABA fibers project from the striatum, globus pallidus inner and outer segments, to the substantia nigra; completing the "loop". One important recent change in this scheme has been the identification of densely staining, highly heterogeneously localized neuropeptides in the striatum, nigra and globus pallidus (Graybiel 1984). Special interest has been focused on the striosomal staining pattern of many of the transmitters in the striatum (Graybiel 1984). There has also recently been the recognition, using advanced neuroanatomic tracing methods, of the complexity and large number of

interconnections that exist between the basal ganglia and areas such as the limbic system (Nauta and Domeslck, 1984). More recently the entire notion of how information might be processed within the nigro-striatal loop has come into question with a new emphasis on GABA's role in permissive, feed forward, disinhibitory processes (which will be discussed below) through the nigra (Chevalier et al., 1985; Gale and Casu 1981; Deniau and Chevalier 1985; Scheel-Kruger 1986). This is opposed to the focus on the nigro-striatal dopaminergic feedback on which many of the models concerning the neuropharmacological functioning of the basal ganglia have been based (Groves 1983).

B.1.a. Striatum : General Organizational Features

The striatum is now recognized to be functionally split into ventral and dorsal sections. The ventral portion includes ventral caudate-putamen, the nuclei accumbens septi and the adjoining olfactory tubercle (Heimer and Wilson 1975). This ventral striatum provides a link between the limbic system and basal ganglia. These structures receive afferents from subicular cortex and amygdala and project to a part of the substantia innominata that is now recognized to be an extension of the pallidum; the ventral pallidum (Nauta and Domeslck 1984). Efferents of the ventral pallidum project to medial dorsal nucleus of the thalamus which in turn projects to prefrontal cortex.

The existence of histochemically differentiated zones within the striatum has, in recent years, changed the simple view of the organization of the striatum. In 1972 Olson et al. observed clustering of dopamine histofluorescence in the rat which he called dopamine islands. Pert et al. (1976) showed islands of opiate binding in the striatum. Graybiel and Ragsdale (1978) showed the dorsal striatum of cat, monkey and man is highly compartmentalized into zones of acetylcholinesterase-poor staining 300 to 600 um wide which they called striosomes. The striosomes have subsequently been shown to correspond to areas of high enkephalin immunoreactivity and are also high in substance P, glutamic acid decarboxylase, neurotensin, and dynorphin staining (Graybiel 1984; Graybiel and Chesselet 1984; Goedert et al., 1983) and have come to be called patches which stand out against the areas rich in acetylcholinesterase staining called the matrix. Opiate and muscarinic binding sites have also been demonstrated to be organized around the striosomes (Herkenham and Pert 1981). Cortical and thalamic projections are organized to either avoid (intralaminar nuclei of the thalamus) or project directly to the striosomes (Graybiel 1983). Recent studies in the rat (Donoghue and Herkenham 1985; Gerfin 1984) show patches which receive frontal cortical efferents and project to SNc and matrix receiving motor and sensory cortical input projecting to the SNr.

Many of the studies on the striosomal organization of the striatum as well as the neurochemical characterization of the SN have been performed in the cat and monkey. Often there are important differences in the transmitter staining between cat, rat and monkey. These differences are noted below.

B.i.b. Striatum : Cytology

The cells of the striatum have been classified according to size of the soma and appearance of neuronal processes with special emphasis on the dendritic spines. The most useful classification scheme is that of Pasik et al. (1979). His study is based on examination of the monkey striatum but can be generalized to the rat (Heimer et al., 1985).

Pasik distinguishes six major cell types; medium sized (10 - 20 um) spiny type I, medium sized spiny type II, medium to small (10 - 20 um) aspiny type I , large (30-60 um) aspiny type II, small (10 um) aspiny type III and a very small neuron which may be neuroglia. The medium spiny type I comprises the bulk (up to 96%) of the neurons in the striatum. These cells are long axoned with 4 to 8 dendrites which extend 200 - 300 um from the soma. The primary dendrites and initial part of the secondary dendrites are spine free with dense spines occurring more distally giving these cells a bottle brush appearance

(Dimova et al., 1980). These cells have been retrogradely labeled from the SN and globus pallidus (Grofova 1975; Preston et al., 1980). Immunocytochemical studies have demonstrated these cells to be the principal GABAergic projection neurons to the SN. The spiny type II comprise 1% of the striatal population. This cell has a long axon and has less numerous dendritic spines with spines on the soma (a feature absent on spiny type I neurons). The transmitter of this cell has not been established with certainty but it may be tachykinin (Groves 1983). The aspiny type I cell is short axoned and comprises perhaps 1% of the striatal population. This cell gives rise to dendrites 150 um in length. Immunocytochemical evidence indicates this cell may be the intrinsic GABAergic neuron of the striatum (Ribak et al., 1979). The aspiny type II also represents around 1% of the striatal population. The dendrites extend up to 250 um with short axon. The neurotransmitter associated with this neuron is thought to be acetylcholine (Bolam et al., 1984).

B.1.c. Striatum : Afferent Connections

Striatal afferents arise from at least five well characterized sources: cerebral cortex, substantia nigra (compacta and reticulata), thalamus (intralaminar nuclei), raphe and locus coeruleus. The anatomy of the corticostriate and nigrostriatal projections will be

discussed here in some detail while other pathways will be discussed as necessary in the text.

B.1.c.i. Corticostriatal

The striatum of the rat receives afferent input from the cerebral cortex (layer V). The projection in the rat appears to have a general topographic organization with practically all parts of the cortex projecting to immediately adjacent parts of the striatum (Donoghue and Herkenham 1985). There are of course many features of the projection which complicate any notion of a simple organization. For example prefrontal cortex projects throughout the entire extent of the striatum and association cortex has a complex specific projection pattern (Donoghue and Herkenham 1985; Veening et al., 1980). The corticostriatal projection fibers apparently terminate on the spines and most distal aspect of the dendrites of the medium spiny type I neurons and on the spines of spiny type II neurons (Kital 1981; Somogyi et al., 1981). There is evidence that this input is excitatory and uses glutamate as its transmitter (Fonnum et al., 1981; Kital 1981). Different histochemical stainings have further revealed that the cortical projections terminate in specific patch like patterns (Gerfin 1984).

B.1.c.ii. Nigrostriatal

This pathway arises largely from the medium sized dopaminergic neurons of the ipsilateral SNc (Hokfelt and Ungerstedt 1969; Lindvall and Bjorklund 1974; Loughlin and Fallon 1982). There is a small contralateral projection from the substantia nigra (Loughlin and Fallon 1982). The ipsilateral pathway is 95% dopaminergic and arises from two types of neurons. One type supplies collaterals to the SNc and SNr while the other does not (Preston et al., 1981). Not all nigrostriatal fibers are dopaminergic. Retrograde HRP and histofluorescence studies have revealed a population of cells in the ventromedial caudal SNr which project to the striatum but do not stain for tyrosine hydroxylase (Faull and Mehler 1978). The nigrostriatal pathway terminates in the striatum in an orderly topographic fashion. Medially placed nigral neurons project to the medial, ventral and anterior striatum. Laterally placed cells project lateral and posterior. There is also an anterior-posterior topography as the ventrally placed pyramidal shaped dopaminergic cells project dorsally and the more dorsally placed cells project ventrally in the striatum (Lindvall and Bjorklund 1974).

The fine unmylenated dopaminergic axons make either diffuse, collateralized endings in the striatum or highly fluorescent terminals known as dopaminergic islands (Olson et al., 1972). The terminals formed in the striatum are of two basic types. First there are large asymmetrical synapses made on the spines of striatal cells and the

second type are small en-passant symmetric terminals with several postsynaptic elements (Pickel et al., 1979; Freund et al., 1984). Both of these types of synapses appear on both GABAergic and cholinergic cells in the striatum. The pre-synaptic action of dopamine on cells in the SNc is apparently inhibitory (AghaJanian and Bunney 1973) while the post synaptic action in the striatum has been shown, in various preparations in a wide variety of experimental conditions to be both inhibitory and excitatory. For example evidence for the excitatory action of dopamine stems largely from electrophysiologic studies which show epsp and ipsp sequences in the striatum following stimulation of the SNc (Frigyesi and Purpura 1967; Hull et al., 1970; Kitai et al., 1976). Evidence for the inhibitory nature of dopamine in the striatum stems largely from iontophoretic application of dopamine which is almost universally inhibitory (Connor 1970; Groves et al., 1975).

B.1.d. Striatum : Efferents

The efferents of the striatum will be discussed below under afferents of the substantia nigra.

B.2.a. Substantia Nigra : Cytology

The substantia nigra, a large well defined nucleus in the mesencephalon, lies dorsal and parallel to the crus

cerebri and extends the length of the midbrain. Three zones of the SN are commonly recognized: The pars compacta SNc, the pars reticulata SNr and the pars lateralis SNl. Three general types of neurons have been identified in the substantia nigra and classified according to size and location (Gulley and Wood 1971; Juraska et al., 1977; Hanaway et al., 1970; Grofova et al., 1982). Large to medium sized oval or elongated neurons 25 to 40 um in diameter are found scattered in the SNr (and lateralis). These neurons give rise to the efferents of the SNr; the majority are nigrothalamic and nigrotectal. These neurons have thick, sparsely branched dendrites and extensive dendritic fields. Medium sized oval or multipolar neurons 15 to 20 um in diameter are found largely in the SNc and are thought to be the dopaminergic efferents. These cells may be further characterized by their relatively sparse dendritic arborization radiating laterally, medially and dorsally which remain within the compacta. These cells also emit one or two dendrites ventrally or ventrolaterally into the SNr. These thick dendrites are highly branched with secondary and tertiary dendrites (Tepper et al., 1987). Medium to large sized dopaminergic cells are also found scattered in the caudal and ventrolateral part of the SNr (Bjorklund and Lindvall 1975; Moore and Bloom 1978). Small cells 8 to 12 um in diameter with short axons are scattered through the SNc and SNr. These neurons have thin

short dendrites which have no preferential orientation (Tepper et al., 1987).

The substantia nigra of the rat may be roughly said to be organized into three dorsoventral layers based on dendritic organization. The superior layer is the cellular pars compacta which is one to ten cells thick. Much of the dendritic arborization of these neurons remains within the compacta except for a few large dendrites that are directed ventrally into the SNr (Tepper et al., 1987). Early studies showed that the dendrites which extend into the SNr were highly varicose and it was suggested that these varicosities may reflect the morphological substrate for dendritic release of dopamine which has been demonstrated in various neurochemical studies (Bjorklund and Lindvall 1975; Groves et al., 1975). Whether this extensive varicosity revealed using histofluorescence represents actual morphological structure or regions of high accumulation of dopamine or of histofluorescence reaction product has come into question as recent HRP studies have revealed much less varicosity than previously shown (Tepper et al., 1987). The second layer is the dorsomedial area or the SNr where both SNc and SNr dendrites run rostrocaudally and dorsoventrally. The third layer is the peripeduncular region where dendrites from all areas run parallel to the crus cerebri. One important feature of the SNr is the extensive axon collateralization of the medium sized and

large neurons. The branching of these cells extends well beyond the dendritic field of the parent cell. These axon collaterals of the SNr cells have numerous dilations or varicosities suggestive of presynaptic terminals (Grofova et al., 1982). This organization suggests that they are involved in complex integration processes. Recent intracellular electrophysiologic study shows that activation of the thalamus or superior colliculus yields ipsp's in the SNr which are consistent with monosynaptic inhibitory responses generated by antidromic activation of the collaterals of the SNr; these responses are found in cells other than those in which antidromic spikes are recorded (Deniau et al., 1982). In 1980 Karabelas and Purpura demonstrated using intracellular recording and HRP staining, the existence of inhibitory autapic synapses in the SNr of the cat.

Electron microscopic studies have revealed several different types of synaptic contacts within the substantia nigra. The most common type, thought to be striatonigral afferents of the striatum, are symmetrical axosomatic or proximal axodendritic synapses (Bak et al., 1975). Pallidonigral afferents also make symmetrical axodendritic and axosomatic synapses (Hattori et al., 1975). Dopaminergic cells of the SNc are thought to be in dendrodendritic contact (Wilson et al., 1977) with each other and with terminals of striatonigral or pallidonigral

neurons (dendroaxonic) (McGeer et al., 1979). There is evidence that these cells may be electrotonically coupled (Grace and Bunney 1983 a,b,c). Lucifer yellow injected into a single dopaminergic cell has been demonstrated to cross junctions and label multiple cells; somatic and dendritic coupling of 2 to 5 cells was seen by Grace and Bunney (1983c). They suggest that such coupling may result in a regenerative re-excitatory loop of excitation within select groups of dopaminergic cells which could lead to a large amount of dopamine released within select projection areas. The appearance of some of the dopaminergic input into the striatum as "islands of fluorescence" could represent a localized input from these coupled cells. The majority of striatal afferents to the substantia nigra terminate ventrally within the SNr (Hattori et al., 1975; Gerfen 1985), and afferents from the globus pallidus terminate preferentially in the SNc (Hattori et al., 1975). A recent report showed that the majority of synapses onto dopaminergic neurons are located on the proximal dendrites with long stretches of distal dendrite extending ventrally into the SNr relatively devoid of synaptic contact (Grofova et al., 1986).

B.2.b. Substantia Nigra : Afferent Connections

B.2.b.i. Striatonigral

The most extensively documented afferent of the substantia nigra is the striatonigral projection. That the striatum projects ipsilaterally to the substantia nigra from medium sized spiny neurons has been demonstrated by retrograde transport of horseradish peroxidase (Preston et al., 1980; Bunney and Aghajanian 1976), degeneration following lesions of the striatonigral pathway and anterograde transport of ^{3}H-Leucine (Nagy et al., 1978). These striatal projection neurons also receive direct projections from the cortex, and make local synaptic contacts within the striatum itself (Somogyi et al., 1981). These afferents make symmetric axosomatic and axodendritic synapses as well as asymmetric axospinous synapses predominantly on the nondopaminergic cells in the SNr (Somogyi and Smith 1979). This projecton will be discussed below in more detail.

B.2.b.ii. Pallidonigral

There is much experimental evidence in support of a pallidonigral projection in the rat (Gerfen et al., 1982). This projection arises from the medium sized aspinous neurons of the globus pallidus and terminatees in the dorasl part of the SNr and through the SNc. The transmitter used in this projection has not been established although GABA is thought to be the most likely candidate (Ribak et al., 1980) and substance P has as well been suggested (Kanazawa et al., 1977).

B.2.b.iii. Corticonigral

The corticonigral projection has been demonstrated following retrograde horseradish peroxidase transport from the SNr to the ipsilateral prefrontal cortex (Bunney and Aghajanian 1976). Many other areas of the cortex may pass through the nigra on the way to other terminations (Dray 1980). This pathway has not been characterized electrophysiologically or pharmacologically.

The subthalamic and pedunculopontine pathway will be discussed below.

C. Strionigral Transmitters

C.1.a. Substance P and GABA : Anatomy

The highest density of substance P-like immunoreactivity (SPLI) in the CNS is found in a network of terminals in the zona reticulata of the nigra; these terminals are most likely of striatal origin (Hong et al., 1977). It is of interest that this SPLI is tightly associated with endopeptidase-24.11. On the macroscopic level SPLI and endopeptidase-24.11 staining in the striatum are striosomal. On the microscopic level SPLI and 24.11 co-localize in neuronal cell bodies and terminal boutons (Matsas et al., 1986).

The SNr contains the highest concentration of GABA in the rat brain. GABA in the SNr is located both on terminals and cell bodies (Brownstein et al., 1977; Ribak et al., 1980). Both substance P (Brownstein et al., 1977; Cuello and Kanazawa 1978; Hong et al., 1977; Kohno et al., 1984; McLean et al., 1985) and GABAergic (Bolam et al., 1981; Grofova 1975; Jessel 1978; Stalnes et al., 1980) projections arise from medium sized spiny neurons in the ipsilateral striatum. Substance P has been shown to be synthesized in the striatum (Brownstein et al., 1977; Sperk and Singer 1982) and transported to substantia nigra via axonal transport (Krause et al., 1984). GABAergic projection is thought to arise from the spiny type I neurons while the substance P pathway is thought to arise from the spiny type II population (Groves 1982)

There is a poor correlation between substance P immunoreactive terminals and substance P receptor density in the substantia nigra (Rothman et al., 1984). Recently the tachykinin substance K was shown to project to the substantia nigra in a fashion analogous to substance P (Lee et al., 1986). Substance K has a high density of binding sites in the SNc of the rat (Mantyh et al., 1984).

C.1.b. Enkephalin : Anatomy

There is a well characterized enkephalinergic projection to the substantia nigra from the striatum in the

cat and monkey. In the rat this projection is controversial. In 1977 Hong et al. showed, using radioimmunoassay against Met-enkephalin, that the striatum of the rat was very heavily labeled. Using immunohistochemical methods Sar et al. showed in 1978 that cell bodies in the striatum and terminals in the substantia nigra of the rat stained for both Met- and Leu-enkephalin. In 1980 Pickel et al., using PAP-immunohistochemical methods, showed uneven distribution of Met- and Leu-enkephalin in the striatum of the rat; greatest concentration in the ventro- and caudo-lateral portions. They also showed staining was localized on medium spiny projection neurons. In 1981 Finley et al. showed, using immunohistochemistry, that the densest terminal staining for Leu- or Met-enkephalin was in the globus pallidus and SNc with very little staining in the SNr of the rat. This is in contrast to the pattern of staining in the cat and monkey which have moderate enkephalin staining in the SNc and SNr. These differences may be important when considering interpetation of experiments on the physiologic effects of the peptides and the consequences of the inhibition of 24.11 in the rat. Many of the important models of the function of the strionigral loop are based on experiments performed in the cat and thus are not directly applicable without modification to the rat. Certain differences or variance with the models established (expected results) may be attributed to the differences in

the neurotransmitter profile between the species. In 1982 Difiglia et al., using PAP-immunohistochemistry, examined Met-enkephalin in the striatum of the cat on the electron microscopic level. She found staining in some of the medium sized spiny type I projection neurons. Electron microscopic study by Pasik et al. (1976) revealed that the majority of all synaptic contacts examined in the caudate of the monkey are axospinous and most of the presynaptic elements belong to afferent axons. Difiglia showed boutons which were enkephalin positive which synapsed with soma, proximal dendrites and shafts of distal dendrites. It is possible that the enkephalin positive neurons are modulating the afferent (possibly cortical) input into the spines. There is some electrophysiologic evidence to support this as Met-enkephalin inhibits cortically evoked responses in the striatum (Fry and Zieglgansberger 1979).

As up to 96% of the striatal projection to the substantia nigra is thought to be GABAergic (Oertel and Mugnaini 1983) the possibility of the co-localization of enkephalins and GABA has been raised (Zahm et al., 1985; Aronin et al., 1984; Morelli et al., 1983; Oertel et al., 1983). This suggestion has been supported by a recent study which showed co-localization of staining for glutamic acid decarboxylase and enkephalin on the light microscope level in the rat striatum (Aronin et al., 1984). A more recent study by Pasik et al. (1987) on the electron microscopic level in the monkey showed co-localization

using double labeling with a polyclonal rabbit anti-GABA and monoclonal mouse anti enkephalin. This study suggests that GABA and enkephalin may coexist in striatal spiny type I neurons. In the same paper they showed another purely GABA positive neuron in the striatum which resembles the a-spiny I neuron. They suggest this purely GABAergic cell functions as an inhibitory interneuron in the striatum.

C.1.c. Dynorphin : Anatomy

More recently a third pathway, arising from the medium sized spiny neurons, has been identified. This pathway contains proenkephalin B (prodynorphin) products; dynorphin A and B, alpha-neoendorphin and beta-neoendorphin (Vincent et al., 1982; Palkovits et al., 1984; Zamir et al., 1984). The peptides dynorphin A and B, alpha- and beta-neoendorphin as well as lower molecular weight members of the dynorphin family, dynorphin 1-8 and leu-enkephalin are found in high concentration in the substantia nigra of the rat (Zamir et al., 1984; Christensson-Nylander et al., 1986; Vincent et al., 1982). Recently it has been suggested that the strionigral dynorphin pathway may be the source of the Leu-enkephalin staining seen in the substantia nigra (Zamir et al., 1984). Dynorphin staining has been demonstrated in the soma of striatal neurons and terminals in the SNr (Fallon et al., 1985; Vincent et al., 1982). Lesions of the internal capsule reduce the immunoreactivity of alpha neoendorphin dynorphin B and

Leu-enkephalin but not Met-enkephalin in the substantia nigra (Palkovitz et al., 1984; Zamir et al., 1984). Various experiments including lesioning (Zamir et al., 1984; McLean et al., 1985), ibotenic acid injections into the striatum (Vincent et al., 1982; Christensson-Nylander 1986) suggest that the majority of the striatonigral dynorphin projection arises from the rostral part of the striatum. Processing of proenkephalin A should, in theory, yield a ratio of 4 Met- to 1 Leu-enkephalin. This is in contrast to the 1:2 ratio found in the substantia nigra (Zamir et al., 1984). Both Zamir and Christensson-Nylander have demonstrated that lesions which decrease the immunoreactivity of dynorphin in the SNc also decrease the staining for Leu-enkephalin. The source of the Met-enkephalin fiber staining in the SNc remains uncertain.

C.2.a. Neurotensin : Anatomy and Physiology

Immunohistochemical analysis of neurotensin in the striatum of the rat reveals a patchy distribution of fiber staining (Jennes et al., 1982). These patches of neurotensin staining coincide with patches of opiate receptor binding (Herkenham and Pert 1981). But, neurotensin receptors in the rat are not localized in the patches and are found in the acetylcholinesterase rich neurotensin poor matrix (Goedert et al., 1984a). Goedert et al. also show a large number of neurotensin binding sites in the striatum are on intrinsic neurons as kainic

acid lesioning of the striatum decreases neurotensin binding 50 to 60%. Some neurotensin receptors in the striatum are concentrated on presynaptic dopaminergic terminals (Quirion et al., 1985).

Neurotensin receptors are concentrated in the SNc of the rat (Quirion et al., 1985). Infusion of 6-hydroxydopamine into the SNc of the rat decreases neurotensin binding in the SNc (Palacios and Kuhar 1981) and striatum (Goedert et al., 1984b).

Neurotensin may act to facilitate the action of dopamine in the striatum and nigra. Infusion of neurotensin i.c.v. increases the turnover of dopamine as assessed by the increase in HVA in the striatum of the rat (Nemeroff et al., 1983). Infusion of 2 to 5 ug directly into the SNc of the rat increased the recovery of striatal dopamine, HVA and DOPAC.

Neurotensin has also been shown to depolarize dopaminergic cells in the rat slice (Pinnock 1985). Neurotensin depolarized 10/19 cells tested in a dose dependent manner with a threshold dose of 0.01 to 0.03 u and maximal responses at 1.0 to 3.0 uM. The responses were slow to develop, 0.5 to 1.5 minutes, and sustained in duration 5 to 20 minutes. Pinnock (1985) also examined changes in the spike frequency of dopaminergic cells in response to local infusion of neurotensin and found increased spike rates. Cells which were silent could also be induced to fire.

C.2.b. Somatostatin : Anatomy and Physiology

Somatostatin levels are relatively high in the striatum, and low in the substantia nigra of the rat with labeling restricted to the lateralis of the nigra (Reubi and Maurier 1985; Beal et al., 1983). Lesion studies indicate that much of the striatal somatostatin is present in intrinsic neurons (Beal et al., 1985). Immunohistochemical staining of the rat striatum reveals labeling on the medium sized aspiny neurons (Difiglia et al.,1982). These neurons which stain for somatostatin are not the same cells which stain for acetylcholinesterase (Vincent et al., 1983) and are present in the matrix (Graybiel 1984). Kainic acid lesioning of the striatum of the rat reduces somatostatin staining by 40% (Araki et al., 1985).

Intrastriatal infusion of somatostatin increases the turnover of dopamine (Beal and Martin 1984). In the rat striatal slice somatostatin increases the spontaneous and potassium evoked release of dopamine (Starr 1982).

C.2.c. Cholecystokinin : Anatomy and Physiology

In the cat the SNc contains the highest proportions of dopamine-CCK coexistence (Hokfelt et al., 1980). In the rat most dopamine cells in the rostral part of the SNc contain CCK-like immunoreactivity, but the peptide is

present in fewer cells in the caudal part of this zone (Vanderhagen 1981).

Cholecystokinin may act to facilitate the action of dopamine in those cells in which dopamine and CCK are co-localized (Hommer et al., 1986). There is evidence to support the idea that CCK may have two different actions on target cells. The sulfated form of CCK appears to have direct excitatory action on dopaminergic cells while both sulfated and non-sulfated forms facilitate the inhibitory actions of dopamine in the nigra of the rat (Hommer and Skirboll 1983).

D. Opiate Receptors

The neostriatum of the rat is one of the brain regions which contains the highest density of opiate binding sites. Delta receptors are apparently uniformly distributed through the striatum (Goodman et al., 1983; Moskowitz and Goodman 1984) with mu and kappa receptors more heterogeneously distributed and concentrated into patches; area outside AchE striosome (Quirion et al., 1982). Most of the mu labeling is on membranes and is apparently extrajunctional (Hamel and Beaudet 1987). Enkephalin binds with high affinity to delta and at least one subtype of mu receptor (Pasternak et al., 1983). Mansour et al. (1986) using autoradiographic analysis of tritiated agonist binding showed low levels of mu binding in the caudal part

of the striatum with higher levels of binding in the rostral striatum. Relatively dense kappa binding was also found in the striatum. Lesioning of the rat nigrostriatal pathway results in a significant decrease in the labeling of opiate receptors in the striatum (Resine et al., 1979). Pollard et al. (1978) examined the effect of lesioning the substantia nigra with 6-hydroxydopamine. They found such lesions caused a 30% decrease in tritiated Leu-enkephalin binding in the caudate-putamen and 33% decrease in naloxone binding sites in the SN. Further, intrastriatal kainic acid lesioning lead to a 45% decrease in tritiated Leu-enkephalin. These authors concluded that 1/3 of the opiate receptors are on dopaminergic terminals in the caudate-putamen and the remaining 2/3 are intrinsic. These experiments support the idea that there may be pre-synaptic opiate control of dopaminergic activity at the dopaminergic terminals in the striatum. There is also evidence for opiate receptors on the cortical afferents of the striatum (Childers et al., 1978) and on local neurons (Antkiewicz-Michaluk et al., 1984; Pollard et al., 1978). Lewis et al. (1985) analyzed adjacent striatal sections in the rat and processed them for tritiated naloxone binding and Leu-enkephalin immunoreactivity. They found no correspondence between receptor rich patches and small enkephalin rich patches. In fact they demonstrated a negative correlation between the distribution of the naloxone binding sites and peptide staining.

Although there is a very dense enkephalinergic projection to the globus pallidus (in the rat) the striatal enkephalinergic projection to the substantia nigra shows scant enkephalin labeling (Pickel et al., 1980; Somogyi et al., 1982; Del Fiacco et al., 1982). Transection of the internal capsule depletes substance P, glutamic acid decarboxylase and dynorphin staining in the substantia nigra with little measurable effect on enkephalin immunoreactivity (Paxinos et al., 1984). This suggests that there is no direct strionigral enkephalinergic pathway in the rat. The majority of the enkephalin immunoreactivity extant in the substantia nigra of the rat is in the form of fiber staining in the compacta. A few enkephalinergic immunoreactive cell bodies have been seen in the SNc (Paxinos et al., 1984). The SNc of the rat is rich in naloxone binding sites with the SNr showing a much lower low density of these receptors (Lewis et al., 1985). These results raise questions concerning the function of the opiate receptors in the nigra of the rat and the apparent absence of enkephalinergic afferents.

E. Electrophysiology of the Striatonigral Projection

Early studies on the responses evoked in the substantia nigra following striatal stimulation were equivocal but it was argued that the short latency (monosynaptic) responses were only inhibitory. But, in a

study by Frigyesi and Purpura in 1967 both epsps and ipsps were recorded at short latency in the cat. The epsps recorded were of longer latency than the ipsp's and failed to follow low frequencies of stimulation. Goswell and Sedgwick (1971) argued that such epsps were most likely evoked not from direct activation of the strionigral pathway but from corticofugal stimulation due to stimulus spread. Yoshida and Precht (1971) working with pentobarbital anesthetized cats observed only inhibitory responses intra- and extracellularly which occurred at latency of 14.6 to 20 msec. They concluded that these responses were monosynaptic because the ipsps recorded were of constant shape and latency, double stimuli applied at various intervals gave no temporal facilitation in response to the second stimulus and regression analysis of the distance between recording and stimulating sites versus latency of the ipsps produced a value which was in agreement with values obtained in other monosynaptic preparations. They observed that there was often a period of increased activity following the inhibition which was unexplained but may be the result of a disinhibitory process; such processes will be discussed in detail later in the text. McNair et al. (1972) working with pentobarbital anesthesized cats observed inhibition in 95% of the nigral units they recorded. They observed that most often ipsps were preceded by small depolarizing potentials especially following lateral caudate stimulation.

Latencies reported were; 3 to 6 msec for the early positive potential, 8 to 15 msec for the negative. McNair proposed that the barbiturates used as anesthetics might facilitate the appearance of ipsps recorded in the substantia nigra following stimulation of the striatum. It was found that inhibitory responses could be produced in spontaneously active substantia nigra units by ionophoresing GABA (Crossman et al, 1973). Further support for the notion of the strionigral projection as a purely inhibitory GABAergic pathway was the observation that striatally evoked inhibition in the substantia nigra could be blocked by application of GABA antagonists. (Crossman et al., 1973; Dray et al., 1976; Collingridge and Davies 1981)

Work on encephale isole cats was performed by Frigyesi and Szabo (1975) in order to address the problem of barbiturate anesthesia. They found both epsp and ipsp sequences at short latency of 3 to 4 msec. Epsps evoked from the head of the caudate nucleus were found only in rostral portions of the nigra, caudal nigra was unresponsive. They also observed short latency (3 to 5 msec) and long latency (15 to 20 msec) ipsps. The suggestion was made, based on the appearance of epsps and ipsps at short latency, that there may be direct excitatory and inhibitory pathways from the striatum to the nigra (Frigyesi and Purpura 1967; Frigyesi and Szabo 1975). In 1975 Feger and Ohye observed both excitatory and inhibitory responses to striatal stimulation in the unanesthesized

monkey using chronically implanted electrodes. They found no correlation between the site of striatal stimulation or the location of the substantia nigra cell and the type of response (excitation or inhibition) they recorded. But, they noted that the kind of response seemed to depend on the firing rate of the cell recorded; cells with higher firing frequencies (70 Hz) responded with inhibition while cells which fired more slowly (20 Hz) responded with excitation.

Dray et al. (1976) studied the responses of 320 cells in the substantia nigra of the rat using urethane and pentobarbital anesthesia. The most common response following stimulation of the striatum was inhibition of spontaneous activity which is in agreement with the previous studies in the cat and monkey. Dray found short latency (5.4 +/- 0.2 msec) inhibition which was of variable duration (8 to 380 msec). In other units there was an excitation preceding or during the inhibitory period. Sometimes the inhibitory period was followed by an excitation which often showed reverberation. These rhythmically recurring nigral discharges have been observed by other groups (Frigyesi and Purpura 1967; Collingridge and Davies 1981). Dray hypothesized that this reverberatory response may be due to a local interaction within the nigra itself. A small number of units showed only excitation with a latency of 5.2 +/- 0.8 msec and duration of 21.6 +/- 1.7 msec following 20 uA stimulation.

GABA, glycine, acetylcholine, and glutamate were iontophoretically applied directly onto nigral cells (undefined) in the urethane treated animals. GABA and glycine depressed or were ineffective in altering the firing frequency of units tested, acetylcholine and glutamate excited or were ineffective on the cells tested. He noted a regional sensitivity only to GABA with the SNr being more responsive than the SNc. Dray concluded that the short latency excitation and inhibition he got could have been monosynaptic as he was using such small stimulus; reducing the stimulus spread from the striatum. But, since he could prolong the inhibitory latency using pentobarbital, he concluded that he could not rule out a polysynaptic ipsp event. Finally he states that his data support the idea that GABA is the inhibitory transmitter in the nigra; a weak assertion as no antagonists were used. Certainly his results are not in conflict with this hypothesis but the identity of the excitatory transmitter was not resolved.

Collingridge and Davies (1981) studied the effect of striatal stimulation and iontophoretic application of drugs on the extracellular response of SNc and SNr neurons in the urethane anesthesized rat. They found both excitation and inhibition of SNc cells following stimulation (40 to 1000 uA) of the striatum. The predominant response was inhibition. Both short latency (less than 10 msec) and long latency inhibition (20 to 100 msec), with a mean

duration for both short and long latency of 128.2 +/- 12.5 msec. The longer latency inhibition usually occurred following near-threshold stimuli. They also observed weak excitation at short latency 14.6 +/- 2.7 msec with a duration of 20.8 +/- 2.7 msec and a more robust excitation following inhibition at a latency of 177.9 +/- 21.4 msec with a duration of 80.0 +/- 14.2 msec; this was often followed by inhibition and was sometimes followed by sequences of excitation and inhibition (reverberation). The SNr neurons could be stimulated (both inhibitory and excitatory responses) at lower intensity than the SNc neurons (20 to 300 uA). The responses of the SNr cells were of four types; early inhibition, late inhibition, early excitation and late excitation. They observed that at low intensity (just above threshold) stimulation they usually evoked short latency inhibition or excitation in SNr neurons. It is interesting that changing the stimulus intensity changed the nature of the response they observed in both the SNc and SNr with more complex responses (mixtures of short and long latency excitation-inhibition) at higher stimulus intensity. For example they show the response of a cell in the SNr which at 300 uA gives a mixture of excitation and inhibition but its near-threshold response is reduced to short latency excitation. This obviously raises questions concerning justification of the stimulus intensity used in all experiments discussed thus far. They also showed that they were able to abolish the

inhibitory response in SNr and SNc following striatal stimulation by ionophoresing bicuculline methachloride directly onto the cells. In addition to reducing or abolishing inhibition in SNr cells bicuculline treatment increased or revealed excitations not present prior to treatment. This result may support an additional role for GABA in the SNr; in addition to being the primary inhibitory transmitter GABA may also play a role in regulating excitatory transmission.

The dependence of dopaminergic neurons on afferent input is indicated, in part, by the differences in spontaneous activity observed between in vivo and in vitro preparations. In vivo extracellular recordings typically show dopaminergic neurons firing spontaneously at a relatively slow rate (0.5 to 10 Hz), with an irregular pattern that is punctuated by short duration bursts of activity (Bunney et al., 1973; Wilson et al., 1977; Tepper et al., 1984; Grace and Bunney 1984a). Identical parameters of spontaneous activity are also observed in vivo using intracellular recording (Grace and Bunney 1983b). In contrast, intracellular recordings of SNc neurons in slice preparations reveal that these neurons are more hyperpolarized, exhibit less spontaneous activity, and possess greater input resistances (Llinas et al., 1984; Kita et al., 1986). They also lack the bursting pattern found in in vivo recordings (Grace and Bunney 1984b).

These differences suggest that the afferent input to these cells plays an important role in the modulation of dopaminergic neuronal activity.

One complication in the analysis of striatally evoked potentials in the nigra is that such stimuli can antidromically activate substantia nigra fibers which terminate in the striatum. Antidromic activation has been used to characterize and identify units in the SN. Guyenet and Aghajanian (1978) found that they could antidromically activate two classes of cells in the SN. The first, type I neurons, were slowly firing (0.5 to 8 Hz) had unusually wide action potentials (4 to 7 msec) and could be antidromically activated from the striatum and globus pallidus. These cells were found, by location, 6-hydroxydopamine lesioning, response to neuroleptics (Guyenet and Aghajanian 1978) and by retrograde horseradish peroxidase transport (Preston et al., 1981) to be SNc neurons. Type II cells were generally firing at a higher frequency (0.1 to 60 Hz) and had narrow action potentials (2 to 3 msec). These cells could be antidromically activated from the thalamus whereas the type I units could not be. These cells were found predominantly in the SNr.

In summary, four general classes of synaptically mediated (orthodromic) striatally evoked events have been observed in the nigra. 1. Simple inhibition occurring at short and long latency. 2. Excitation occurring at short

latency preceding inhibition. 3. Post-inhibitory excitation which may be reverbatory. 4. Short latency excitation. Many of the short latency events are thought to be monosynaptic. The source of the inhibitory input is probably the medium sized spiny GABAergic neurons residing in the striatum. The nature of the excitatory responses remains equivocal and theories concerning the mechanism of generating such responses will be addressed below.

F. Excitatory Responses Evoked in Nigral Cells

In a recent report (Albe-Fessard and Sanderson 1987) the technique of spreading depression was used to stimulate the striatum while recording from the nigra of the rat. Striatal spreading depression was provoked by perfusing the head of the striatum with 0.06 M KCL for 2-5 seconds via a push-pull cannula. This treatment generates an initial excitation (2-5 seconds) followed by an arrest of cell activity (depolarization block 30 seconds in duration). Most of the responsive cells in the SNr (42/54; 78%) exhibited a short duration increase in firing followed by an arrest in the firing (27/54; 64%) an increase in activity (9/54; 22%) or biphasic changes in firing (6/54; 14%). All changes in the firing of SNr neurons were correlated in time with the spreading depression in the striatum. None of the SNc cells studied responded to the treatment. They conclude that 64% of the cells tested in

the SNr are under facilitatory influence while 22% are under inhibitory influence. The remaining cells in the SNr are under a mixed influence of inhibition and excitation. Their results are important as they indicate that the majority of cells in the SNr (at least in their sample) may be under striatal facilitatory influence in contrast to studies in which the striatum is electrically stimulated which yields inhibition in the majority of nigral cells. It is interesting that the SNc cells were not responsive and may indicate that under the conditions of this spreading depression stimulus, only those SNr cells which are not influencing the firing of SNc cells are activated (selective stimulation).

F.i.a. Transmitters: Amino Acids and Acetylcholine

Several reports have shown that acetylcholine has an excitatory action when applied to SNc (Collingridge and Davies 1981; Dray et al 1976) or SNr (Dray et al., 1976). Recent immunohistochemical (Deutch et al., 1987) and autoradiographic (Clark et al., 1984) studies demonstrate nicotinic receptors localized in the soma and dendrites of the SNc. Clark also showed that iontophoretic application of nicotine is able to excite SNc neurons. Calcium dependent release of acetylcholinesterase (which is consistent with a physiological role for acetylcholine) has been demonstrated in the guinea pig mesencephalic slice (Llinas and Greenfield 1987). It has been proposed that

this release of AChE is dendritic from dopaminergic cells in the SNc which also release dopamine (Greenfield 1984). In 1986 Weston and Greenfield studied the electrophysiologic effects of acetylcholine perfused into the substantia nigra. They found acetylcholine increased the firing of dopaminergic cells and increased the release of acetylcholinesterase (AChE) in the striatum. Further, dendritic release of AChE in the substantia nigra itself is independent of the firing of the dopaminergic cells and is instead dependent on a calcium conductance which is TTX resistant (Cheramy et al., 1981). Lesion studies suggest that cholinergic innervation of the substantia nigra is extrinsic (Nagy et al., 1978). The major source of acetylcholine afferents to the substantia nigra are thought to arise from the pontine mesencephalon. This has been demonstrated by combined cholinergic immunocytochemistry and fluorescence tracing (Woolf and Butcher 1986) and combined HRP retrograde tracing and choline acetyltransferase immunohistochemistry (Beninato and Spencer 1987). The best characterized projection is from the pedunculopontine tegmental nucleus (PPn) to the SNc (Jackson and Crossman 1983).

Electrophysiologic studies have shown that stimulation of the PPn evokes short latency excitation in the SNc (Scarnati et al., 1984). Microperfusion of low doses of kainic acid into the PPN revealed a dose dependent increase in firing in dopaminergic neurons (Clarke et al., 1987)

which was antagonized by mecamylamine. Systemic administration of mecamylaine (Clarke et al.; 1985) or direct application of AChE (Greenfield et al., 1981) was able to decrease the spontaneous firing frequency of SNc dopaminergic cells.

Another possible source of excitatory afferents of the substantia nigra is the subthalamic nucleus. Subthalamic projections to the SNr have been demonstrated by anatomical (Kita et al., 1983; Kita and Kitai 1987) and electrophysiologic (Deniau et al., 1978; Nakanishi et al., 1987; Kita and Kitai 1987) studies. Recent electron microscopic studies show subthalamic axon terminals making asymmetric synaptic contact with dendritic shafts of substantia nigra neurons (Kita and Kitai 1987). In a recent electrophysiologic study Nakanishi et al. (1987) showed both short latency epsp and ipsp responses recorded in SNr neurons following stimulation of the subthalamic nucleus. The ipsp's could be eliminated by transection of the internal capsule. This suggests the ipsp's may have been evoked by stimulation of striatonigral fibers. Small ipsp's which persisted following transection were suggested to be due to local inhibitory circuit action rather than direct subthalamic projection.

In 1979 Collingridge and Davies studied the effect of ionophoretic application of d-alpha-aminoadipate and d-alpha-aminosuberate (antagonists of L-glutamate and

L-aspartate) on the striatally evoked excitatory responses in the SN. They found that these antagonists did not abolish the excitatory responses in the substantia nigra and concluded that excitatory amino acids were probably not responsible for mediation of these responses in the SN. Acetylcholine is also unlikely to be the primary transmitter released following striatal stimulation as ablation of this pathway as well as striatal lesioning have no effect on CAT levels in the substantia nigra (Kataoka et al., 1974).

F.1.b. Substance P

Application of substance P to the SN affects the electrophysiology of neurons in the SNc and SNr, the release and turnover of dopamine and the behavior of the animal. Infusion of 1 to 10 ug of substance P directly into the SNr of the rat over a period of five minutes causes contralateral turning and a 18% increase in the dopamine, assayed post-mortem, in the ipsilateral striatum (James and Starr 1979). Intranigral infusion of 70.4 ug into the SNr of the rat (60 minute infusion) caused an increase in HVA and DOPAC recovered postmortem from the striatum (Waldmeier et al., 1978). These authors state that they infused what they considered to be a rather large dose of substance P and this was necessary to obtain measurable results as the peptide was subject to rapid enzymatic degradation. A 40 minute superfusion of 10^{-8}M substance P

directly into the substantia nigra decreased ipsilateral dendritic release of dopamine but increased ipsilateral release at the nerve terminals in the striatum (Michelot et al., 1979). Antisera directed against substance P infused into the SNc decreased ipsilateral release of dopamine in the striatum and increased dendritic release of dopamine in the ipsilateral SNc of the cat (Cheramy et al., 1978; Michelot et al., 1979).

Studies on the effect of the iontophoresis of substance P in vivo on nigral cell electrical activity have demonstrated that approximately one half of the cells examined in the SNr and very few cells in the SNc are responsive. Davies and Dray 1976 ionophoresed (25 to 300 nA) substance P into the nigra (in this study no clear distinction was made between compacta and reticulata) and found 26 of 34 cells increased their spontaneous firing frequency 20 to 50% above baseline firing; they note that some cells responded with increases of greater than 100%. The responses were characterized by slow onset (10 to 20 seconds following the onset of ejection) followed by a sustained (0.5 to 3 minutes) period of excitation following termination of drug ejection. All cells tested were excited by acetylcholine and glutamate and depressed by GABA. The effects of substance P were not always reproducible and they suggest that the cells may become desensitized to the effect of the peptide. In 1982 Pinnock

and Dray examined the response of cells in the SNr and SNc to the application of substance P in the rat. They found the same slow onset and sustained response with the same timecourse as Davies and Dray. Iontophoretic application of substance P (50 to 150 nA) excited 3 of 10 cells in the SNc with insignificant increases of 0.68% +/- 0.9 and significantly raised the firing above background (22.4% +/- 1.2) in 6 of 15 SNr cells examined. All cells in the SNc were inhibited by the application of dopamine or GABA and acetylcholine produced no effect on the firing of these cells. In the reticulata 7 of 11 cells were excited by acetylcholine while GABA inhibited all of the cells examined. In 1983 Pinnock et al. got similar results ionophoresing substance P (50 to 100 nA) with only 2 out of 10 cells in the SNc weakly excited.

Substance P, substance P 1-9 amide and substance P 1-9 methylester excited approximately one half of the cells tested in the SNr while substance P 1-2, 4-9, 5-9 methyl ester fragments had no effect in the SNr. These fragments were not tested in the SNc due to lack of responsiveness in the SNr (Pinnock et al., 1983). This result was important as endopeptidase-24.11 is able to cleave substance P into substance P 1-9, 1-7 and 1-6. Therefore substance P 1-9 may retain activity but the activity of the other smaller fragments is unknown. Stewart et al. 1982 tested the substance P 1-7 fragment in vivo, i.v. and i.p. injections

into mice and found a naloxone reversible antinociceptive effect (hot plate escape latency). They attribute this to an amino terminal substance P activity not previously recognized. They failed to consider the possibility that this substance P 1-7 fragment could inhibit 24.11 and thus prolong the action of endogenous enkephalin (naloxone reversible). Substance P itself has been reported to produce antinociception when infused i.c.v. or i.p. (Starr et al., 1978; Stewart et al., 1976). All these findings may, in part, be a function of the inhibition of 24.11 although direct interaction with an endogenous opiate system via receptor action can not be ruled out. Substance P has been demonstrated to stimulate the release of met-enkephalin in the spinal cord of the rat in vivo (Tang et al., 1983).

Effects similar to those seen following iontophoresis of substance P into the nigra have been found elsewhere in the CNS. G. Le Gal Sa Salle and Ben-Ari 1977 showed slow onset sustained excitation in select cells of the medial amygdala and caudate-putamen of the rat. Henry 1976 studied the effect of substance P on spinal dorsal horn neurons in the cat and found slow onset excitation in 50% of the spontaneously active neurons he recorded. He concluded that the slow time course of action was inconsistent with the idea that substance P was acting as the primary transmitter of the spinal afferents mediating

the short latency evoked responses. He found there was a correlation between the units responsive to substance P and those which were responsive to noxious radiant heat. Several units were recorded which were not responsive to heat before the application of substance P but became responsive following application. He concluded that substance P may be able to facilitate postsynaptic activation by other transmitters; 5-HT, acetylcholine.

These experiments support the idea that there may be a select group of cells in the substantia nigra which may be identifiable by some physiologic criteria (response to noxious stimulus or other evoked response) which which might be consistently responsive to the iontophoretic application of substance P. Iontophoretic application of substance P to SNc neurons has been reported to have no electrophysiological effect (Collingridge and Davies 1982) or an excitatory effect on few of the SNc neurons selected (Pinnock and Dray 1982). It is of interest that so few cells in the SNc have been found to be responsive to the iontophoretic application of substance P as the application of substance P to the substantia nigra produces rotational behavioral and biochemical effects which are associated with the activation of dopaminergic cells located in the SNc. Clearly the effect on the release of dopamine in the striatum may be through indirect activation of SNc

dopaminergic neurons or those few cells activated might account for the pharmacological effects seen.

F.1.c. Substance K

It has recently been postulated that the excitatory transmission in the nigra, formerly attributed only to substance P, may more correctly be ascribed to substance K or substance K acting in concert with substance P (Innis et al., 1985). Iontophoresis of substance K (80 nA) has been reported to activate 14 of 30 SNr and 25 of 45 SNc units tested (Innis et al., 1985). A positive response was defined as an increase above baseline firing of greater than 20%. But, Lanthorn et al 1984 found substance K excited a population of cells near or in the SNc which were not dopaminergic cells but were in very close proximity. Substance K is a good substrate of endopeptidase-24.11 with a specificity constant of 1.2 relative to substance P (Hooper et al., 1985).

F.2. Possible Mechanism of Action

F.2.a. Tachykinins

The mechanism of tachykinin action remains uncertain. Depolarization evoked by substance P has been associated with an increase (Nicoll 1978; frog spinal motorneurons), decrease (Katayama et al., 1979; myenteric plexus of the guinea pig small intestine) or no change (Zieglgansberger et al., 1979; dorsal horn of the cat) in membrane

conductance. The increase in membrane conductance has been attributed to an increase in sodium conductance (Nicoll 1978) while membrane conductance decreases have been attributed to a decrease in potassium conductance (Katayama et al., 1979) or chloride conductance. Nowak and McDonald (1981, 1982) studied conductance changes in cultured spinal cord neurons and found that substance P depolarized these cells by decreasing the membrane potassium conductance in a voltage sensitive manner which most resembles the muscarinic sensitive potassium conductance. This conductance was found to be activated following membrane depolarization, absent at very negative potentials and present at resting membrane potential. They account for variation in the effects they observed and those in other preparations, in which the membrane conductance was unchanged or increased, by noting that they applied substance P from a large tipped micropipet which bathed a larger neuronal area than is possible using iontophoresis making it more likely that small decreases in conductance could be observed. They also speculate that in those preparations in which conductance increases were observed the membrane depolarization may have activated voltage-dependent conductances which could mask coincident substance P induced decreases in conductance. They concluded that substance P induced excitability would be strongly modified by (dependent on) concurrent synaptic

input onto the post synaptic cell. This supports a neuromodulatory role for this transmitter.

This result is also of interest as it was postulated in a recent report using a slice of rat nigral tissue that the electrical activity of cells in the SNr may be sensitive to such neuromodulation (Nakanishi et al., 1987). Nakanishi et al. report that SNr neurons could be classified into two types based on their electrical membrane properties (Nakanishi et al., 1987). Type 1 neurons have strong voltage dependent potassium conductances and exhibit spontaneous activity of high frequency. These cells are thought to be GABAergic as they were encountered most frequently in the SNr, they had short duration action potentials and were able to fire at high frequency. Type 2 neurons have strong calcium dependent potassium conductances with less voltage dependence. These cells are thought to be dopaminergic as they had no spontaneous activity, exhibited long duration action potentials and had membrane properties similar to SNc neurons observed in the slice preparation (Kita et al., 1986). From studies on the voltage dependence of these cells, their responses to changes in their membrane potentials, the authors conclude that responses recorded in the SNr to phasic synaptic input may differ depending on their level of tonic input; with the activity of the SNr cells more sensitive to the level of the membrane potential than the SNc cells.

F.2.b. Mechanisms of generating short latency excitatory responses in the substantia nigra

The identity of excitatory transmitters and the mechanisms of generating excitatory responses following stimulation of the strionigral pathway remains uncertain. Electrophysiologic and neuroanatomic studies support the hypothesis that there are at least two different mechanisms (to be discussed in detail below) which may be responsible for the generation of orthodromic short latency excitatory responses in the substantia nigra following striatal stimulation. As discussed above the tachykinin afferents to the SNr may account for slow onset sustained excitatory drive (tonic input) on these cells and may act to modulate the action of other transmitters with faster onset and short duration of action (phasic input). Both types of transmitter actions mmay contribute to the short latency excitatory responses evoked following striatal stimulaton.

One postulated mechanism of generating short latency excitatory responses in the SNr following striatal stimulation is through a process of disinhibition (Chevalier et al., 1985). Striatonigral GABAergic input to the SNr may act to inhibit GABAergic interneurons in the SNr resulting in disinhibition of a third neuron (SNc or SNr) or auto disinhibition of the secondary GABAergic neuron. The latter is supported by neuroanatomic evidence of autapses in the SNr (Karabelas and Purpura 1980).

Another possible mechanism for generating short latency excitatory responses in the substantia nigra following striatal stimulation of GABAergic efferents involves the idea that excitatory input to the substantia nigra may be under inhibitory control which is released when the activity of the inhibitory interneuron is decreased (disinhibition of an excitatory nigral afferent).

These possibilities will be discussed below with special emphasis on the participation of the opioid peptides in these mechanisms.

F.2.c. Pharmacological Actions of Opiates and Opioid Peptides

The actions of opiates (morphine, enkephalin and dynorphin) in the basal ganglia have been examined with special emphasis on the activity (biochemical and electrophysiological) of the nigrostriatal dopaminergic projection. It could be inferred, based on examination of the anatomy alone (enkephalin and dynorphin staining in the striatum and substantia nigra with numerous opiate receptors located on dopaminergic nerve terminals, striatal interneurons and projection neurons as well as dopaminergic cell bodies in the SN), that the opiates would have a role in transmission here.

Initial studies on the effect of opiates on the nigrostriatal dopaminergic projection engendered the idea that the opiates exerted their action primarily in the

striatum. This conclusion was based on studies which demonstrated that the increases in striatal dopamine synthesis and release in vivo (Smith et al., 1974; Clouet and Ratner 1970) and in vitro (Gauchy et al., 1973) following systemic morphine or D-ala-met-enkephalinamide in vivo (Biggio et al., 1978) could be replicated by local application of these opiates into the striatum (Biggio et al., 1978; Chesselet et al., 1983a). This conclusion was also supported by the observation that enhanced dopamine turnover was sustained following application of alpha-methyl-tyrosine directly into the nigra (Moleman and Bruinvels 1979). All these effects appear to be specific as they are blocked or reversed by naloxone. It is important to note that increases in dopamine release in the striatum following local or systemic application of morphine has been most documented in mice and cats, less in rats (Slater and Bundell 1979; Wood et al., 1980; Chesselet et al., 1981, 1983a) which suggests that the regulation of dopaminergic action in the striatum is different in different species. The paucity of opiate effects in the rat may be a function of the apparent lack of a striatonigral enkephalinergic projection (Pickel et al., 1980; Somogyi et al., 1982; Del Fiacco et al., 1982).

Subsequent experiments have addressed two general questions concerning these observed opiate effects in the striatum. First, are the observed effects mediated pre- (dopaminergic terminals) or post-synaptically (striatal

interneurons or projection neurons). Secondly, are the opiates acting directly, blocking the action of dopamine at the receptor or indirectly, acting on an interneuron which in turn is modulating the activity of dopamine.

As the release of dopamine is assumed to be correlated with the activity of SNc dopaminergic cells (increased turnover reflects increased electrical activity) experiments have been performed examining the effects of opiates on the electrical activity of these cells. Iwatsubo and Clouet (1977) examined the effect of systemic and local striatal application of morphine and haloperidol on the activity of SNc neurons in unanesthesized rats. Intravenous administration of 5 mg/kg of morphine increases the firing frequency 52-300% above baseline. Haloperidol 0.05 mg/kg, i.v. produced increases of 30-400%. Local striatal infusion of 25 ug of morphine produced a naloxone reversible increase in the firing frequency of these cells 50-300% above baseline with infusion of 0.5 ug haloperidol causing a 50-400% increase. Application of 0.1 mg/kg, i.v. of apomorphine was able to decrease the spontaneous firing of SNc cells and antagonize the effect of haloperidol. L-Dopa 200 mg/kg, i.v. was able to decrease the spontaneous firing of SNc cells and antagonize the effect of morphine and haloperidol. The effect of opiates on the activity of the nigrostriatal dopaminergic projection is not thought to be due to a neuroleptic-like action as morphine is unable to antagonize the decrease in dopaminergic cell firing

induced by systemic application of apomorphine or l-DOPA; also naloxone is able to block the action of systemically or locally applied morphine on dopaminergic firing but not that of haloperidol. Antipsychotics such as haloperidol stimulate the synthesis and metabolism of dopamine in the striatum (Carlsson and Lindqvist 1963) and increase the firing rates of nigral dopaminergic cells (Bunney et al., 1973). The effects of antipsychotics are attributed to the blockade of dopamine receptors, while morphine affects dopaminergic transmission without affecting receptor blockade. Iwatsubo and Clouet (1977) also conclude that morphine has no direct effect on post-synaptic dopamine receptors in the striatum. They propose that the effect of morphine they observed in the striatum induces excitation of striatal projection neurons which in turn lead to an increase in firing of dopaminergic neurons in the substantia nigra.

Both D-Ala-D-Leu-enkephalin, a delta receptor agonist and FK 33-824, a mu agonist, can elevate striatal dopamine synthesis but not release in the rat striatum (Wood et al., 1980). The suggestion that the actions of these mu and delta agonists are dependent upon opiate receptors on striatal dopaminergic nerve endings (Pollard et al., 1978) is supported by experiments which show that changes in dopamine metabolism which occur following intrastriatal (local) infusion of opiates are not blocked by kainic acid lesioning of the striatum (Biggio et al., 1978; Moroni et

al., 1978). In 1980 Kondo and Iwatsubo reported that kainic acid lesioning of the rat striatum abolished the stimulation of dopaminergic cell firing induced by systemic administration of haloperidol or morphine. They concluded that this supported the notion that systemic application of haloperidol or morphine induced increases in dopaminergic cell firing in the substantia nigra via a post-synaptic dopamine receptor activity on striatal neurons.

Herrera-Marschitz et al. (1986) found that dynorphin A infused into the substantia nigra of rats induces a dose-dependent weak contralateral rotational response which is similar to the response produced the by kappa agonist U50,488H, delta agonist D-Ala-Leu-enkephalin (DALE), substance P or substance K; with the tachykinins and DALE producing a much stronger rotational response for an equivalent dose. Striatal ibotenic acid lesioning reduced substance P and dynorphin like immunoreactivity in the SNr. Following lesioning, application of dynorphin caused a more potent rotational response when compared to non-lesioned rats with a diminished response to substance P. But, following 6-hydroxydopamine lesioning of the nigra only dynorphin retained potency to induce rotation. They conclude that the rotational response to dynorphin does not require an intact nigrostriatal dopaminergic system and may be due to stimulation of receptors located on nigro-thalamic and nigro-tectal pathways. This has been

verified in similar studies on rotational behavior (Matsumoto et al., 1988). They also propose that these results suggest that the response to dynorphin may be mediated in part by kappa receptors which is consistent with the results of other studies (Christensson-Nylander et al., 1986). But, as the peptide receptor selectivity is not absolute it is possible that dynorphin is working partially through delta receptor stimulation and/or the DALE is exerting its effect partly through kappa receptor activation. Autoradiographic studies of kappa binding in the substantia nigra of the rat showed labeling restricted to the SNr with scant labeling in the SNc (Mansour et al., 1986). Electrophysiologic and behavioral studies suggest that the endogenous ligands with the highest affinity for the kappa receptor are prodynorphin products (Matsumoto et al., 1988). Pressure infusion of dynorphin (Lavin and Garcia-Munoz 1985) directly into the SNr or kappa agonist U50,488H (Walker et al., 1987) produce a naloxone reversible slow onset long duration decrease in the activity of the majority of cells recorded extracellularly in the SNr. The electrophysiological response of SNc cells is equivocal with one study showing an increase in the firing frequency in 2/10 cells (Lavin and Garcia-Munoz 1985) following application of dynorphin directly into the nigra while the application of the kappa agonist U50,488H 2 mg/kg, i.v. produced a decrease in the activity of all cells recorded in the SNc. This difference could be due to

activation of kappa receptors in the striatum following i.v. application of U50, 488H as striatal application of this agonist has been demonstrated to decrease the activity of the majority of SNc cell recorded (Walker et al., 1987)

F.2.d. Interaction of Opiates and GABA

Several recent publications have suggested the existence of a relationship between GABA and the opiates (enkephalins and morphine) within the basal ganglia. Moroni et al. (1978) observed a dose dependent decrease in the turnover of GABA in the striatum with a corresponding increase in turnover in the substantia nigra following systemic morphine or beta-endorphin. They suggest that the short axoned GABAergic neurons in the striatum may exert a tonic inhibitory influence on the striatal GABAergic projection neurons. It appeared that both mu and delta receptor agonists could activate striatal opiate receptors to elicit an increase in GABA turnover. Duka et al. (1980) found that systemic application of muscimol or diazepam provokes a rapid decrease of Met-enkephalin levels in the rat striatum. In the rat striatal slice, GABA agonists have been demonstrated to both stimulate (Sawynok and Labella 1981) and inhibit (Osborne and Herz 1980) the release of Met-enkephalin. These contradictory results may be the result of differences in the experimental procedures used. Bourgoin et al. (1985) showed, using a push pull cannula implanted in the rat striatum that 10 uM GABA

infused into the striatum caused a transient increase in Met-enkephalin release followed by a sustained decrease in release. At a concentration of 0.5 mM GABA caused a decrease in enkephalin release with no transient increase. Burgoin et al. (1985) also demonstrated that both spontaneous and potassium evoked release of Met-enkephalin in a striatal slice preparation were enhanced by the addition of 10 uM GABA but inhibited by 0.5 mM GABA. They attribute these effects to differential GABA-A or GABA-B receptor stimulation.

Similar effects (transient increase in release followed by decrease in release) using low concentrations of GABA have been observed for dopamine in vivo (Cheramy et al., 1978). Cheramy proposed that the initial increase in dopamine release involves an intrastriatal interneuronal GABAergic process while the subsequent decrease is mediated by striatonigral feedback.

G. Model for the Disinhibitory Control of Dopaminergic Cells

Although the dopaminergic neurons in the SNc are known to be inhibited following direct application of GABA (Aghajanian and Bunney 1973), when GABA agonists are administered into the SNr or applied systemically an increase in the firing of dopaminergic cells is found (Grace and Bunney 1979). Additionally, introduction of GABA or GABA agonists into the substantia nigra results in

an increase in striatal HVA and DOPAC (Biggo et al., 1978). Grace and Bunney 1979 examined the effects of GABA in the nigra by recording extracellularly simultaneously in the SNc and SNr while ionophoresing GABA into the SNr or administering GABA iv. They found an inverse relationship in the SNc and SNr firing following application of GABA into the SNr with SNr firing decreasing and SNc firing increasing. The dose of the GABA agonist muscimol which induced the maximal decrease in firing in the SNr corresponded to the the dose producing the largest increase in firing in the SNc. Low i.v. doses of the GABA antagonist picrotoxin reversed the increase in the SNc firing rate induced by GABA ionophoresed directly onto SNr cells. Higher doses of picrotoxin increased the firing of SNc cells. The SNr cells which displayed this inverse firing relationship with the SNc cells were found to also be responsive to noxious (foot pinch) stimulus. In fact this physiologic stimulus also induced the inverse response in SNc and SNr cells with an immediate short term increase in the firing rate followed by a longer decrease in SNc with the inverse in SNr. The dose response relationship, following iontophoretic application of GABA, was determined for SNc and SNr neurons. The SNr cells were apparently 20 times more sensitive to the effects of GABA than the SNc cells. They conclude that a GABAergic neuron in the SNr exerts an inhibitory influence on SNc cells. As this SNr cell is more sensitive to the inhibitory effects of GABA

than the SNc cells this may serve as a basis for the paradoxical effects of GABA on these cells.

In 1979 Finnerty and Chan found that 50 ug/kg dopamine administered locally into the striatum of the rat resulted in an increase in the firing of nigral cells (unspecified location although SNr recording is most likely) 120-200% above control levels. Systemically administered morphine exerted a naloxone reversible suppression of this effect as well as decreasing the spontaneous activity 25-57% below control values of cells in the in the SN (again most likely SNr cells). Subsequent injection of dopamine into the striatum had no effect on the suppressed discharges of these units. They proposed that the morphine was acting to disinhibit dopamine containing SNc cells by depressing the activity of inhibitory SNr neurons.

In 1982 Collingridge and Davies found that iontophoretic application of Met-enkephalin (50-100 nA) had no effect on the firing of SNc cells in the rat while decreasing the firing of 4 of 8 SNr cells. Morphine (30-100 nA) was tested on 7 compacta neurons and was without effect in contrast to an increase in firing 25-120% induced in 16/18 SNr cells. Responses evoked by striatal stimulation were also examined in SNr cells. Initial excitation (in cells with mixed excitation/inhibition) was increased in 3 of 4 cells and inhibition reduced in 2 of 6 cells by morphine. They conclude, in support of Finnerty

and Chan (1979), that the increases in SNc cell firing in response to systemic morphine are mediated indirectly; possibly through SNr cells. They cannot explain the differences in responses of morphine and enkephalin but suggest that this may be the result of different receptor activation.

In 1983 Hommer and Pert extended the study of the action of opiates on the dopaminergic system by direct infusion of opiates into the substantia nigra while monitoring SNr and SNc cell firing. They confirmed the results of Kondo and Iwatsubo (1980) by demonstrating that systemically administered morphine was able to increase the spontaneous activity of cells recorded in the SNc in a naloxone reversible manner while decreasing the firing of foot pinch sensitive cells in the SNr. Morphine applied directly into the substantia nigra via pressure injection had no effect on 6 of 8 cells in the SNc while 2 out of the 8 cells showed increased firing which could not be reversed by naloxone. In the SNr 3 of 6 cells recorded increased their firing frequency. This effect could not be reversed by naloxone. DADLE applied directly into the substantia nigra showed 0 of 9 cells in the SNc responding with 8 out of 8 cells in the SNr decreasing firing in a naloxone reversible manner. There was no response to the DADLE in the SNr neurons which were not responsive to foot pinch. These results support the suggestion first made by Finnerty

and Chan 1979 that the opiates act in the substantia nigra to disinhibit SNc dopaminergic cells. Further, there is a special population in the SNr (apparently sensitive to foot pinch stimulus) which mediates this effect. Hommer and Pert speculate that the opiates act to decrease GABA release by these SNr cells. The non-specific effects of the morphine could not be explained but may be due to activation of opiate receptors other than mu.

Further support for the key role of the SNr GABAergic interneuron was demonstrated by Grace and Bunney in 1985a,b. They proposed a model for the relation between the striatonigral GABAergic pathway and SNc dopaminergic cell activity. Using intracellular recording of SNc and SNr cells they examined the effect of GABA agonists and antagonists on the striatally evoked responses of these cells. Single pulse stimulus (500uA) delivered in trains evoked short latency ipsp's in both SNc cells (1.8-2.2 msec) and SNr cells (1.5-2.5 msec). These latencies correspond to the fastest reported conduction velocities of antidromically activated striatonigral pathway (Richardson et al., 1977). Rebound depolarizations were observed in SNc but not SNr cells. They state that despite recording of ipsp's following stimulation they had difficulty clearly demonstrating inhibition of spontaneous activity in SNc cells. As lower levels of stimulus were used (20-50 uA)

they observed an increase in SNc cell firing. This low intensity stimulus easily inhibited SNr cell activity.

They stress that the SNr cells they studied are of a specific class. They are located typically within 100 um of SNc cells (ventral). They respond to foot pinch with a burst of activity and respond to striatal stimuli and GABA iontophoresis with a pronounced inhibition of spontaneous activity. This SNr cell is thought to be an interneuron as it could not be activated antidromically from the striatum, superior colliculus or thalamus.

The responses evoked in SNc and SNr cells support their hypothesis that the specific SNr cells are selectively inhibited at low striatal stimulus intensity by activating strionigral GABAergic pathway. The increase in SNc cell firing recorded is the result of a process of disinhibition of this cell. They test this idea by administering muscimol (7 mg/kg, i.v.) a dose sufficient to totally inhibit the firing of these SNr cells. This treatment causes a reversal of the evoked response of SNc cells at the same stimulus intensity which initially lead to an increase in firing due to activation of direct GABAergic striatal afferents. Examination of the reversal potential of striatally evoked ipsp's recorded in SNc and SNr cells lies within the range expected for GABAergic effects.

Support for this model also comes from experiments using neuroleptics. Dopamine blockade, with haloperidol

(Frey et al., 1987) results in GABAergic supersensitivity in SNr cells but not in the dopaminergic cells in the compacta. Also, infusion of GABA directly into the SNr induces a dose dependent increase in dopamine release as determined by voltammetry in the striatum (Kamata et al., 1986).

The studies described in this thesis were initiated following the isolation, substrate characterization and anatomical localization of endopeptidase-24.11 (with highest concentration in the substantia nigra and striatum) and most importantly the synthesis of inhibitors which acted with great specificity toward the enzyme. A physiological role for the enzyme was suggested in striatonigral transmission by those studies. Electrophysiological characterization of nigral cells following striatal stimulation made it an attractive site to study the in vivo consequences of enzyme inhibition.

Initial studies infusing CPAB i.c.v. indicated 24.11 was active in regulating transmission in a specific identifiable subset of cells in the substantia nigra. The bulk of studies described here were performed in order to;

1. Localize the anatomical site of action.
2. Characterize the spontaneous and evoked responses following inhibition of 24.11.
3. Characterize the pharmacological basis of the observed effects.

Materials and Methods

A Surgery

Experiments were performed on male Sprague-Dawley rats weighing 230-400 g. anesthesized by intraperitoneal (i.p.) injections of urethane (1.3g/Kg) (Sigma Chemical Co., St. Louis, MO.). The onset of anesthesia was judged by loss of response to tail pinch and the depth of anesthesia was judged by carefully monitoring the blood pressure. The temperature of the animal was maintained at 37 degrees centigrade via a heat lamp servo-feedback mechanism which continuously monitored the core temperature of the animal via rectal probe. Supplementary doses of urethane were administered i.p. as needed based on the animal's reflex responsiveness and blood pressure. The iliac artery was cannulated (via the left femoral artery) with heparinized polyethylene tubing (PE 50) fc measurement of blood pressure.

The rats were placed in a Kopf stereotaxic apparatus using the coordinate system of De Groot (1959) with the upper incisor bar 5mm above ear bar zero; the interaural line. After the skull was exposed and cleaned of all connective tissue, holes 1 to 2 mm in diameter were drilled to allow for the entry of stimulating and recording electrodes and i.c.v. injection cannula. The monopolar stimulating electrode array (described below) required

removal of a larger area of bone (3 mm by 1.5 mm) overlying the striatum. The exposed tissue was constantly moistened with 0.9% saline through the duration of the experiments.

The stereotaxic coordinates used for stimulation, recording and drug delivery were obtained from the rat stereotaxic atlas of Pellegrino (Pellegrino et al., 1979). Electrode and cannula positions were all referenced to bregma; using the intersection of bregma and the sagittal suture as zero. Striatal stimulation and nigral recordings were made on the left side of the animal with i.c.v. infusions into the right lateral ventricle. Coordinates referenced to bregma are:

1. Striatal stimulation with bipolar electrode, Anterior ((+) anterior and (-) posterior to bregma): + 4.2 to + 2.0 mm, Lateral (to the midsagittal line): 2 to 4 mm, Horizontal (ventral placement of the electrode with the brain surface as zero): 3 to 7 mm;

2. Striatal stimulation with monopolar electrode, Anterior: + 2.2 to -0.2 mm, Lateral: 2 to 4 mm, Horizontal: 3 to 7 mm;

3. Nigral recordings, Anterior: -2.2 to -4.8 mm ; Lateral: 2 to 3.5 mm; Horizontal: 7 to 9 mm;

4. Intracerebroventricular cannula, Anterior: -0.7 mm; Lateral: 1.5 mm; Horizontal: 2.0 to 4.0 mm.

B Electrodes and Cannulae

Two different stimulating electrodes were used in these experiments. In the first set of experiments a single bipolar coaxial electrode (Rhodes Instruments, Woodland Hills, CA), constructed of stainless steel was used (tip separation of 0.5 mm insulated to the tip with 0.5 mm exposed). In the later experiments an array of monopolar electrodes (0.5 mm in diameter with 0.2 mm tip exposed) were used. When bipolar stimulation was used a single bipolar electrode was positioned. When monopolar stimulation was performed five electrodes were placed in a specially machined plexiglass block with slots which held the electrodes in a flat array 2.5 mm in length with the tips separated by 0.5 mm. This apparatus was attached to the David Kopf holder which allowed for stereotaxic placement. These electrodes were lowered together with each lying in the same plane and flush at the tips. Each electrode could be be activated independently or driven in sequence. The five monopolar electrodes were placed in the anterior-posterior plane in the striatum.

The infusion cannulae used were constructed according to Crane and Glick (1979). The system includes a 26 guage stainless steel guide cannula which remained in place through the experiment while the injection cannula was removable. The guide was beveled to a sharp point (15 degree bevel) to allow for penetration of the brain. The injection cannula was 33 guage stainless steel tubing which extended, when placed into the guide, 0.5 mm below the

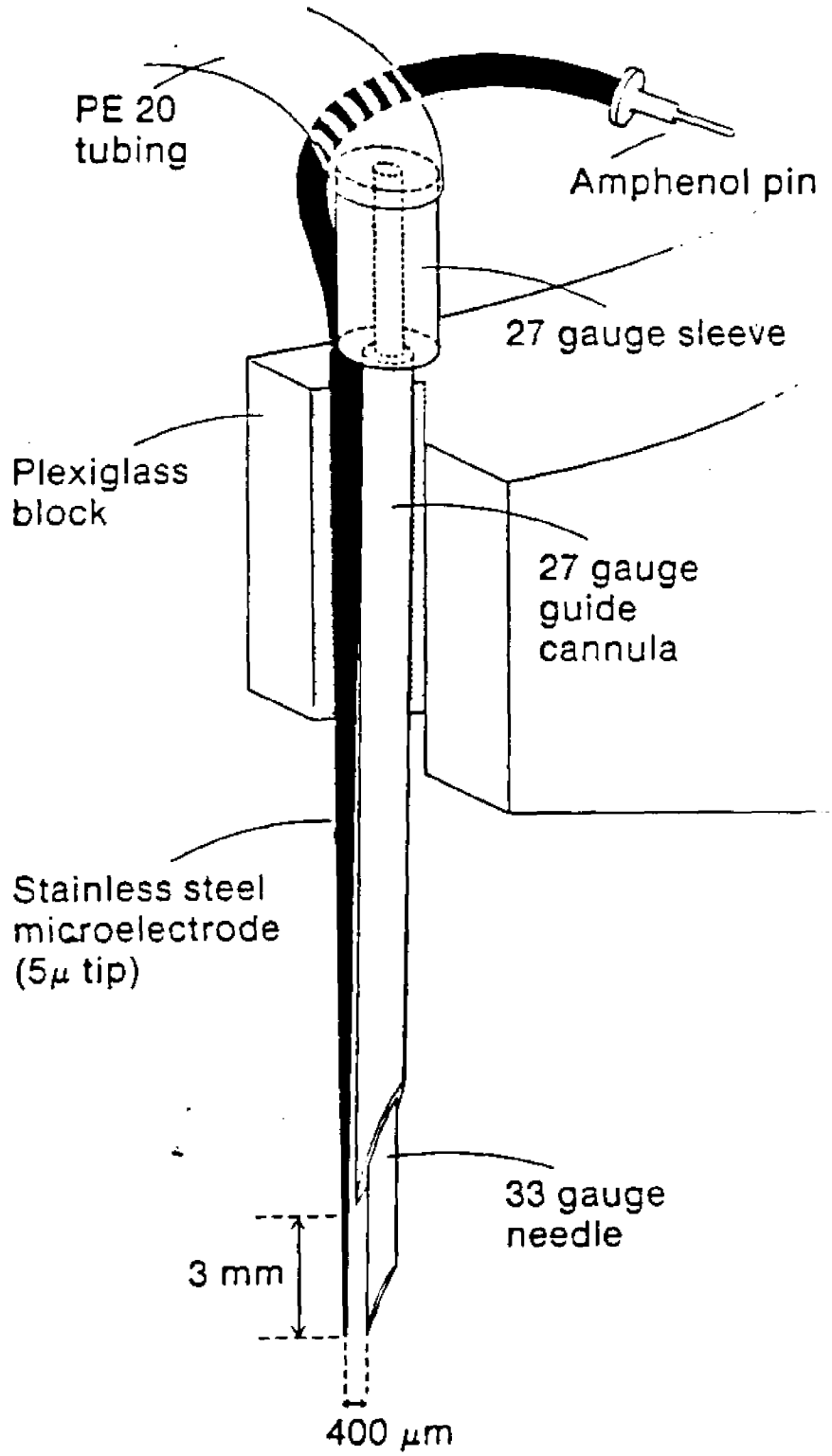
guide tip. The injection cannula was attached with epoxy, via a cuff of 26 guage stainless steel tubing, to polyethylene tubing (PE 10) which was connected to a 10 ul Hamilton syringe. The syringe was in turn driven by a syringe pump (Sage 341). Intracerebroventricular infusions were delivered at a nominal rate of 1 uL/min. The patency of the ventricular contact was checked by drawing CSF into the tubing. The cannula used was flushed with heparinized saline prior to contact with the ventricular fluid. The tubing was calibrated and marked at 1 uL intervals. As the fluid was advanced the progress of the movement of the fluid was monitored by tracking an air bubble which was introduced into the tubing. The rate of infusion into the striatum or substantia nigra was dependent on the condition of the recording; 1 to 3 minutes for delivery of 1 uL.

For those experiments in which there was infusion of drugs in close proximity to the recording electrode a machined plexiglass block was designed to hold both infusion cannula and recording electrode. The apparatus used was a modification of the apparatus described by Adams (1985) and is shown in figure 1M. The assembly used in the experiments was different from that described by Adams in that glass microelectrodes were used and the injection cannulae/electrode extended only 0.5 mm from the guide. To minimize disturbance of the tissue in the recording site the assembly was lowered with the recording electrode and

Figure 1M Apparatus used for simultaneous drug infusion and electrophysiological recording in the substantia nigra

The assembly shown was taken from Adams (1985). The assembly used in the experiments described in the text was different from the apparatus shown in that glass microelectrodes were used and the injection canulae/electrode extended only 0.5 mm from the guide.

Figure 11


PE 20
tubing
Amphenol pin
27 gauge sleeve
Plexiglass
block
27 gauge
guide
cannula
Stainless steel
microelectrode
(5μ tip)
33 gauge
needle
3 mm
400 μm

injection cannula in place. The bevel of the needle was also placed facing 45 degrees away from the recording electrode tip to minimize pressure on the cell being recorded during drug infusion. Tip separation was 400 um.

Electrodes used in recording extracellularly from the substantia nigra were pulled on a Kopf 700 C vertical electrode puller (David Kopf Instruments, Tujunga, CA). Omega dot glass tubing (O.D. 2.0 mm, I.D. 1.16 mm) was used. Tip resistances were 4-8 M Ohm with tip diameter 1 um. Electrodes were filled with either 2 M sodium acetate or 2 M NaCl. Both 2% Pontamine Sky Blue or a saturating solution of Fast Green were used as a dye markers. There was no observable difference in any combination of filling or dye solutions used. The position of the recording electrode was marked at the end of each experiment by passing 10 uA of cathodal current for 3 min.

The recording electrodes were connected directly to the headstage via silver/silver chloride junction. The ground to the headstage was connected to the stereotaxic apparatus which was in turn grounded to a master ground.

C Electrophysiological Apparatus

Extracellular activity was recorded using a high impedence Ortec 4661 dc headstage preamplifier which was located inside the faraday cage. Capacitive currents from the microelectrode were nulled using the negative capacity at the headstage: X 10 gain was used at this stage. The

signal was further amplified by an Ortec 4660 Bandpass Amplifier (Ortec, Oak Ridge, TN). Depending on the gain of the signal and the condition of the electrode, signals were filtered at 100 to 200 Hz for the low frequency and 20 K Hz for the high frequency. Single unit activity was displayed on a Tektronix D-11 dual beam storage oscilloscope during each experiment (Tektronix, Beaverton, OR). All experiments were stored on FM tape (Honeywell 5600, Denver, CO). Data stored for analysis included blood pressure, command pulse, voice, and recording electrode trace. Units were gated through an amplitude discriminator (Fredrick Haer 74-45-1, Ann Arbor, MI) which converted the positive on-going portion of the signal crossing the chosen threshold into single pulses of constant voltage. Only spikes which were at least twice the noise level were considered for analysis. If two units were recorded simultaneously the larger of the two was used. The output of the window discriminator was fed into a rate analyzer (Fredrick Haer Rate/Interval Analyzer 74-40-1). The rate analyzer is able to count the number of events per unit time using a clock which is able to sample 1 to 10 k Hz (adjustable). The dc voltage generated for each bin of time (t = 0 is triggered by the input pulse from a schmitt trigger and is reset to zero by a pulse from the clock) depends on the final count for that time period. The output of the rate meter was displayed in analogue fashion on a Gould 220 chart recorder (Gould Inc, Cleveland, OH).

This display gives a continuous record of the cell firing frequency.

D Timing and Stimulus

Timing pulses were generated by Ortec 4610 pulse generators and Ortec 4661 delay controls. These triggered a self contained, isolated, battery driven (constant voltage) stimulators (Devices Model 2533, Devices Instruments, Welwyn Garden City, Hertfordshire, England).

In experiments using bipolar stimulating electrodes constant voltage 25 V, 0.5 ms pulses were delivered in trains 110 ms in duration with a pulse frequency of 250 Hz. Currents were determined to fall within the range 500-1200 uA. In experiments using monopolar electrodes, stimuli were 50-200 uA in amplitude delivered in 1.0 ms pulses at 1 or 2 Hz. Stimulation current was monitored by following the voltage drop across a 100 Ohm resistor in series with the preparation.

The command pulse also triggered a 200 uV calibration pulse (Stoelting, Chicago, IL). The calibration pulse was used to guage the condition of the recording electrode and it serves as a measure of the amplitude of the extracellular potentials.

E Classification of the cells

Cells recorded extracellularly in the substantia nigra were classified according to anatomic location, zona

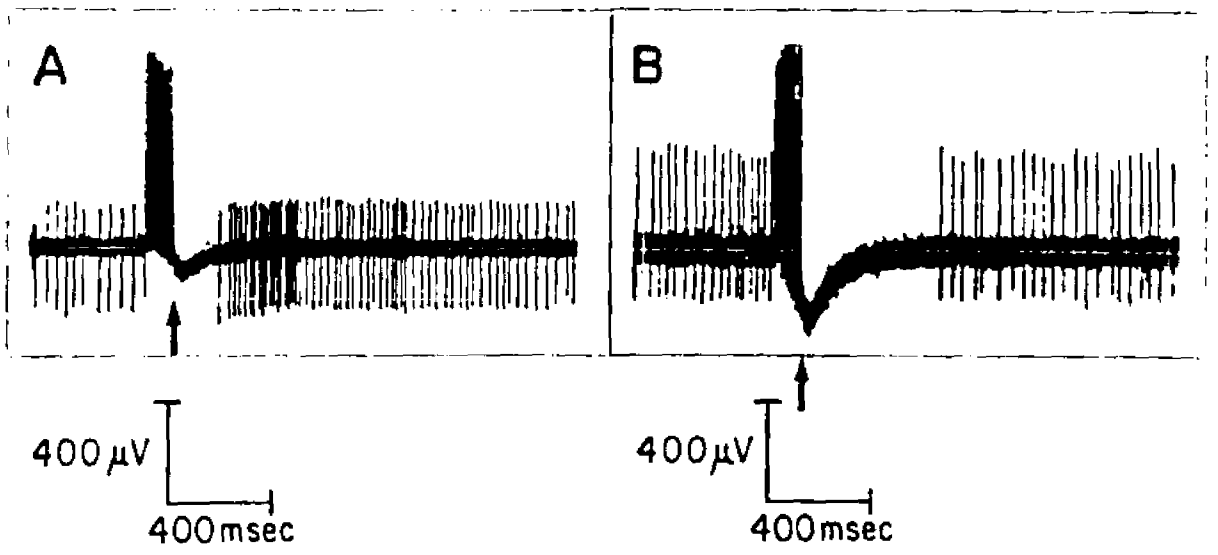
compacta or zona reticulata of the substantia nigra (Figure 1), waveform, spontaneous firing rate and firing pattern (Grace and Bunney 1983a,b; Dray et al., 1976).

The cells recorded in these experiments were also classified according to their responses to striatal stimulation. Cells exhibiting only inhibitory evoked responses are designated as displaying (-) responses. Cells exhibiting excitatory evoked responses are designated as displaying (+) responses (table 1; figure 2M). The cells exhibiting (+) responses displayed either a complex mixture of excitation and inhibition or purely excitation. The evoked response was judged excitatory if the firing frequency showed at least 100% increase over the baseline spontaneous rate.

F Experimental Procedure

Only spikes which were at least twice the noise level were considered for analysis. Data were obtained from only one neuron per animal. After a cell had been isolated and characterized it was recorded for a period of 30 to 70 minutes before administration of the inhibitors. Cells in which the spontaneous firing was not stable and/or there were major changes in the spike shape or amplitude were rejected. There were two periods, preceding and following drug infusion, in which evoked responses were collected. After infusion of the drugs, the animals were recorded for as long as it was possible to hold the cell. In the first

Figure 2M



set of experiments shown in table 1 the spontaneous rates were determined by averaging discrete one minute blocks of time taken from the continuous rate recording. In the second set of experiments the spontaneous firing rates and the magnitude of the evoked responses were obtained by computer analysis of the output of the amplitude discriminator. The data used to determine the spontaneous firing rates excludes the stimulus and infusion periods.

Cells subjected to low intensity stimulation responded with either excitation at short latency (5-10 msec) or longer latency excitation which is preceeded by inhibition. Responses selected gave consistent evoked responses prior to application of the drugs. The stimulus intensity (amplitude) was selected to be the lowest stimulus which yielded a consistent excitatory response. This was generally less than 150 uA, typically 100 uA and rarely 20-50 uA. Consonant with other studies higher intensity stimului were required to activate the dopaminergic cells than the non-dopaminergic cells. The typical experimental procedure involved searching for excitatory responsive cells at a relatively high current (150 uA) then working back; finding the threshold for the response and then setting the level of stimulus to give the invarient response. Generally as the stimulus was increased above threshold, a plateau was reached in which increases in stimulus intensity failed to recruit more spikes. Below this range of stimuli spikes were lost and the response

would become inconsistent and variable. Above this range the response grew more complex with alternating periods of inhibition and excitation. A similar relationship between stimulus amplitude and response pattern was reported by Collingridge and Davies (1981). They observed that when they used a low intensity (just supra-threshold) stimulus they evoked short latency excitation or excitation in the SNr. Changing the stimulus intensity changed the nature of the response in both SNc and SNr cells with more complex responses (mixtures of short and long latency excitation and inhibition) at higher intensity.

G Data Analysis

The data stored on the FM recorder were analyzed by playing the tapes back into (1) the Tektronix storage oscilloscope, (2) the Gould chart recorder and, (3) a Tracor signal averager (Model TN-1505, Tracor Northern, Middleton, WI). The tracor apparatus, used to generate peristimulus histograms, provided frequency analysis coded as intensity vs time. Pulses were counted for pre-selected dwell times and stored in memory sequentially. The number of addresses, the dwell time for each address and the number of sweeps were all pre-selected. The number of addresses was fixed at 1023 with a dwell time of 0.4 ms/address with 500 sweeps.

The TTL output of the amplitude discriminator was input into an Apple IIe for further analysis. The digitized

spike train information was also analyzed using a modified version of a program developed by R.C. Electronics (Santa Barbara, C.A.). This program clocked the arrival time of spikes and stored this information on disk. Frequency was determined by referencing the arrival time of events to the timing of the internal clock of the computer (Serial Pro, Applied Engineering, Carrollton TX). Information was collected for determination of the spontaneous firing frequency in the 20 to 30 minutes prior to the beginning of the first sequence of 100 stimuli and between each period of stimulation (of which there were five before and after administration of drug). Information collected during the peristimulus interval (evoked responses) was handled in the following manner. First, the spontaneous firing frequency in the interval bounded by the command pulse, which initiated a sweep, and the stimulus pulse (Figure 2M), was determined for each sequence of 100 stimuli. This interval was either 40 or 100 milliseconds in length. The number of spikes following the stimulus (evoked response) to the beginning of the next sweep (command pulse) was also counted. The evoked response magnitude computed was corrected by subtracting the expected number of spikes contributed by the spontaneous firing. This number was determined in the pre-stimulus interval (40 or 100 ms). This subtraction was performed in order to avoid skewing the number reported for the evoked response due to variations in the spontaneous firing frequency within the

sequence of stimulation. Further, to avoid introducing a negative bias in the number reported for the evoked response by subtracting the spontaneous firing during the delay between stimulus and the beginning of the response, in which there may be no firing at all, the dwell time between the stimulus, and the time when the computer began counting the evoked response, was adjustable. Therefore, the delay on the evoked response was determined prior to subtraction of the baseline firing frequency; the spontaneous firing was only subtracted from the evoked response itself. The computer stopped counting the evoked response when the response returned to the spontaneous rate determined in the pre-stimulus interval. Thus, the number reported as the evoked response represents the actual number of spikes caused by the stimuli above the background.

The numbers reported in the tables for the evoked responses, are reported as counts (following 500 stimuli), which are defined as:

(A) Total number of spikes collected in the interval between the stimulus and command pulses (adjusted for delay) for a sequence of 100 stimuli.

(B) Spontaneous firing frequency (X duration of the interval analyzed) calculated for the interval between the

command pulse and stimulus pulse for each sequence of 100 stimuli.

$$Counts = ((A) - (B))/50$$

H Inhibitors and Infusion of Drugs

The inhibitors used in this study are N-carboxymethyl derivatives of amino acid amides of p-aminobenzoate (pAB). The inhibitor CPAB (N-[1-(R,S)-carboxy-2-phenylethyl]-Phe-pAB) $K_i=7.1x10^{-8}M$, was synthesized by following the procedure described by Almenoff and Orlowski (1983). The inhibitors CPPAB (N-[1-(R,S)-carboxy-3-phenylpropyl]-Phe-pAB) $K_i=3.8x10^{-8}M$, and CPLAB (N-[1-(R,S)-carboxy-2- phenylethyl]-Leu-pAB) $K_i = 2.6 \times 10^{-4}$ M, were synthesized by following the procedure described by Pozsgay et al (1986). CPPAB, CPAB and CPLAB were all dissolved in H_2O to a final concentration of 100 mM. The pH of each solution was adjusted to 7.4. Stock solutions (prepared weekly) were assayed for their ability to inhibit the enzyme and checked for purity and identity by HPLC.

I Histology

At the end of each experiment the brain was removed and quickly frozen in freon (Ucon 12 Union Carbide Danbury CT). In the experiments in which monopolar stimulation was

used, the animals were perfused via aortic cannulation first with normal saline then with a solution of 10% formalin acetate containing potassium ferricyanide (2.5%). The ferricyanide oxidized deposits left in the tissue at the site of stimulation, leaving a black deposit which served to localize the position of the stimulating electrode. For bipolar experiments the frozen brain was sectioned without prior treatment. The brain was then blocked so that microtome sections would be in the plane of the Pelligrino atlas. The brain was then cut into serial 20um slices on a freezing microtome (SLEE London). The sections were dried onto glass slides and fixed with a standard Hematoxalin and Eosin stain.

J Enzyme Assay

Slices were made in the frozen brain through the full rostral-caudal extent of the substantia nigra and caudate-putamen. All cuts were made by hand using surface features to estimate the position of both nuclei. The sliced tissue was placed on glass slides and kept frozen on dry ice. Punches (1 mm) of substantia nigra and striatum were made with stainless steel tubing. All punches were made in the center of the nuclei assuring that no surrounding tissue was sampled. The fragments, blown free of the tubing into preweighed centrifuge tubes containing 100 ul of cold 50 mM Tris-HCL buffer pH 7.8, were

homogenized and assayed for enzyme activity . The assay conditions were as follows: 10 ul of substrate (0.4 mM glutaryl-Ala-Ala-Phe-2 -naphthylamide), 50 ul of homogenized tissue, 10 ul aminopeptidase M (10 ug), 180 ul of 50 mM Tris-HCL buffer pH 7.8. in a final volume of 250 ul. The tubes were incubated for 90 min at 37 C. The reaction was stopped by addition of 250 ul of a 10% solution of trichloroacetic acid. The enzymatic assay is based on the following reaction sequence:

$$\text{Glutaryl-Ala-Ala-Phe-2NA} \xrightarrow{\text{endopeptidase-24.11}} \text{Glut-Ala-Ala} + \text{Phe-2NA}$$

$$\text{Phe-2NA} \xrightarrow{\text{aminopeptidase M}} \text{Phe} + \text{2NA}$$

The 2-napthylamine released was determined in two different ways. In the experiments in which 1 umol CPAB was administered i.c.v. (table 2) the napthylamine was determined colorometrically by a modification (Goldbarg and Rutenburg, 1958) of the diazotization procedure of Bratton and Marshall (1939). For the experiments in which CPAB was administered directly into the substantia nigra (table 13) the presence of 2-napthylamine was determined fluorometrically using a Perkin-Elmer LS-5 luminescence spectrophotometer (Perkin-Elmer Corp, Oak Brook, Ill) with excitation and emission wavelengths of 280 nm and 410 nm respectively.

Specific activity is expressed in units/mg. of tissue. One unit is defined as the amount of enzyme catalyzing the

release of 1 umol of Phe-2NA from Glutaryl-Ala-Ala-Phe 2-NA /hour.

RESULTS

A. Bipolar Study: Spontaneous Activity

A.1. Firing characteristics

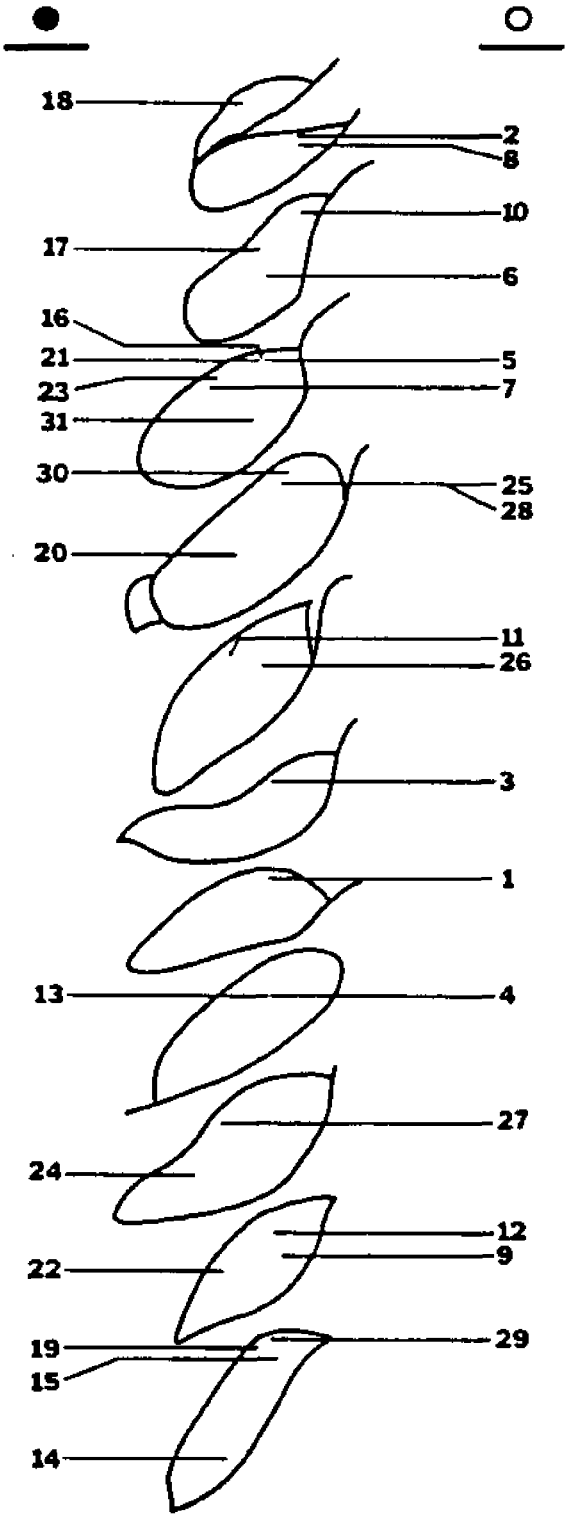
Recordings in the initial experiments in which bipolar stimulation was used to characterize the cells were made through the entire rostral-caudal extent of the substantia nigra (Figure 1). Of the 31 cells reported, 9 (29%) were classified as dopaminergic and 22 (71%) as non-dopaminergic. The pre-treatment dopamine cell firing was slow (4.4 +/- 0.74 Hz; range: 1.8 to 9.6 Hz), irregular and often appeared in bursts of spikes (decreasing in amplitude); spikes were biphasic, 3 to 5 milliseconds in duration with prominent negative phase. Non-dopaminergic cells had faster firing frequencies (32.7 +/- 12.4 Hz; range: 10.9 to 70.8 Hz) with a more regular firing pattern than the dopamine cells; spikes were biphasic with durations of no more than one millisecond. All of the reported cells were spontaneously active.

A.2. Infusion of Inhibitors: Excitatory and Inhibitory Responses

Initial experiments were performed infusing 1 umol CPAB i.c.v. It was noted that only certain cells responded to the inhibitor with an increase in firing frequency.

Figure 1. Recording sites in the substantia nigra. Coronal sections shown are re-drawn from the atlas of Pellegrino. The sections begin (top figure) 2.2 mm and extend to 4.4 mm caudal to bregma. Open circle column shows the position of (+) responses. Closed circle column shows the position of (-) responses. Numbers correspond to the experiments in table 1.

FIGURE 1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Analysis of this data permitted identification of the cells responding to drug treatment as those in which striatal stimulation evoked an excitatory (+) response. This "functional identification" allowed us, in subsequent experiments, to predict which cells encountered in the substantia nigra would be expected to respond to inhibitors.

In order to test the predictive value of the evoked response CPPAB was administered (1 and 2 umol i.c.v.). Intracerebroventricular (i.c.v.) infusion of either 1 umol CPAB, 1 or 2 umol CPPAB increased the spontaneous firing frequency of cells in all experiments in which the cells exhibited (+) responses but not in experiments in which the cells exhibited (-) responses. It can be seen, by examining table 1, that the predictive value of the evoked responses is supported by the data. The increases in spontaneous firing frequency (pre- to post-drug) are statistically significant for values pooled under each dose (paired t-test). If the post-infusion spontaneous firing frequency is expressed as a percentage of the pre-drug firing frequency, the range of increase for cells exhibiting (+) evoked responses, treated with either 1 umol CPAB, 1 umol CPPAB or 2 umol CPPAB, was 7% to 69%. For cells exhibiting (-) responses that were treated with either 1 umol CPAB, 1 umol CPPAB or 2 umol CPPAB , the response range was -10.7% to 6.7% (Table 1). In

TABLE 1

Effect of drug infusion on the firing frequency of cells in the substantia nigra exhibiting (+) and (-) responses.

DRUG	DOSE (μM)	RESPONSE TYPE AND EXPT NUMBER	CELL TYPE	PRE-DRUG FIRING FREQUENCY (HZ)	POST-DRUG FIRING FREQUENCY (HZ)	% CHANGE	
CPAB	1[a]	(+) 1	DA	5.6 ± 1.5(5)	7.6 ± 0.7(4)	+36.4	p<0.05[b]
		2	nDA	36.7 ± 4.2(3)	42.6 ± 4.7(5)	+16.3	
		3	nDA	10.9 ± 0.7(3)	13.0 ± 2.5(4)	+19.8	
		4	DA	3.4 ± 0.9(4)	5.7 ± 0.6(4)	+67.8	
CPPAB	1[a]	(+) 5	nDA	34.6 ± 1.9(7)	39.8 ± 1.5(9)	+15.1	p<0.05[b]
		6	nDA	38.1 ± 2.1(3)	50.0 ± 6.8(12)	+31.2	
		7	DA	8.5 ± 1.1(4)	12.1 ± 0.2(4)	+42.4	
		8	DA	6.2 ± 0.8(6)	7.9 ± 0.5(4)	+26.8	
		9	nDA	70.8 ± 6.5(5)	75.8 ± 7.4(3)	+7.1	
CPPAB	2[a]	(+)10	nDA	32.8 ± 2.3(6)	41.3 ± 3.3(3)	+25.8	p<0.05[b]
		11	nDA	27.8 ± 5.2(6)	39.7 ± 1.5(2)	+42.5	
		12	DA	5.0 ± 0.2(6)	8.5 ± 0.2(2)	+68.7	
CPAB	1	(-)13	nDA	33.6 ± 1.4(6)	35.4 ± 0.3(4)	+5.4	NSD[b]
		14	nDA	23.0 ± 0.6(6)	20.6 ± 0.7(5)	-10.7	
		15	nDA	32.0 ± 1.9(6)	31.7 ± 0.2(5)	-0.9	
		16	nDA	20.7 ± 0.3(6)	19.8 ± 0.3(4)	-4.6	
		17	nDA	32.6	32.5 ± 7.6(5)	-0.5	

CPPAB	1	(-)18	nDA	22.1 ± 1.0(3)	21.1 ± 1.8(2)	-4.7	NSD[b]
		19	nDA	34.4 ± 3.2(5)	35.4 ± 2.2(4)	+2.9	
		20	nDA	27.6 ± 1.7(3)	28.4 ± 0.8(4)	+2.8	
		21	nDA	55.7 ± 3.1(4)	54.3 ± 2.5(4)	-2.5	
CPPAB	2	(-)22	nDA	29.7 ± 3.2(6)	26.6 ± 2.7(5)	-10.4	NSD[b]
		23	nDA	44.5 ± 9.7(6)	40.4 ± 0.2(3)	-9.2	
		24	DA	2.1 ± 0.0(6)	2.2 ± 0.1(3)	+6.7	
CPLAB	2	(+)25	DA	2.1 ± 0.1(6)	2.2 ± 0.2(5)	+6.8	NSD[b]
		26	nDA	30.1 ± 0.4(4)	30.3 ± 2.3(5)	+0.6	
		27	nDA	21.3 ± 2.2(5)	19.8 ± 1.5(6)	-6.8	
VEHICLE		(+)28	DA	4.9 ± 0.8(5)	4.9 ± 0.9(4)	+1.7	NSD[b]
		29	nDA	27.4 ± 1.4(5)	26.0 ± 1.7(4)	-4.9	
		(-)30	DA	1.9 ± 0.1(4)	1.7 ± 0.2(3)	-8.6	
		31	nDA	32.9 ± 2.5(5)	33.6 ± 4.0(3)	+2.2	

[a]Significantly different from vehicle (Dunnetts: CPPAB 2 μmol $p<0.01$; CPPAB 1 μmol $p<0.05$; CPAB 1 μmol $p<0.01$). [b]Paired T-test comparing pre- to post-drug. NSD- No Significant Difference; DA-dopaminergic; nDA-non-dopaminergic. Firing frequencies are reported as means ± standard deviation. The number of 10 min bins (in which data were collected) appear in parenthesis. Post-drug data collection began 20 min after the termination of drug infusion and extended to the end of the recording period. Reprinted with permission Elsevier Science Publishers B. V. (Biomedical Division). Bier et. al. European Journal of Pharmacology Vol. 142 (1987) 321-330

experiments 1-12 a difference between the response of dopaminergic and non-dopaminergic cells was observed. The increase in spontaneous firing frequency of the dopaminergic cells (48.4% +/- 18.9 (n=5)) is significantly greater than the increase in the non-dopaminergic spontaneous firing rate (22.5% +/- 11.7 (n=7); paired t-test, $p<0.05$). It should be noted that the baseline firing frequency of dopaminergic cells is lower than that of the non-dopaminergic cells, and the absolute increase in firing frequency is lower for dopaminergic than for the non-dopaminergic cells (2.6 +/- 0.39 Hz vs 7.2 +/- 1.4 Hz; t-test, $p<0.05$).

A.3. Infusion of Vehicle:

Excitatory and Inhibitory Responses

Infusion of 2 umol CPLAB i.c.v. , a compound structurally related to CPAB and CPPAB but two orders of magnitude less potent as an endopeptidase inhibitor ($K_i=2.6 \times 10^{-4}$ M), had no significant effect on the firing frequency of cells exhibiting (+) responses; range of responses was -6.8% to 6.8%. By comparison, experiments in which vehicle was infused i.c.v. (both (+) and (-) responses) yielded the expected non-significant effect; the range of response was -8.6% to 2.2% (Table 1). Note that the 2 umol injection of CPLAB in a volume of 20 uL serves as a volume control for the experiment in which 20 uL of CPPAB (2 umol) was infused i.c.v.

A.4. Time Course of the Drug Effect

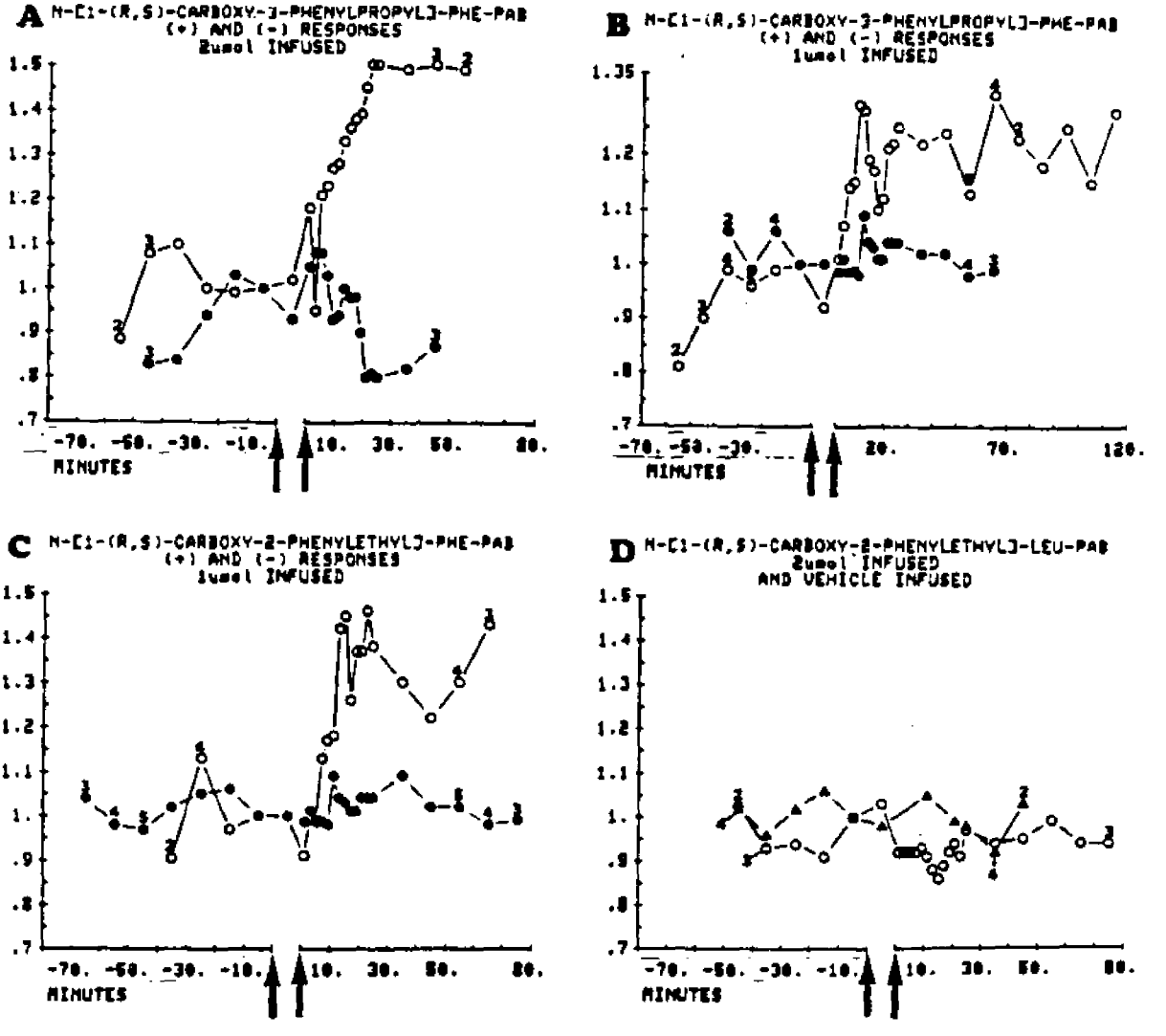
The time course of the drug effect is shown in figure 2. The onset of the drug effect, for cells exhibiting (+) responses, occurred within 5 min of termination of the infusion for 1 umol infusions (10 uL) and near the end of the infusion for the 2 umol (20 uL) infusions. In every experiment the firing frequency reached an apparent maximum by 20 min after the end of the infusion period. The infusion period was 6 to 21.5 minutes for the 1 umol (10 uL) infusions and 12.5 to 21 minutes for the 2 umol (20 uL) infusions. In no case was the recording period long enough to detect any reversal of the drug effect.

A.4. Biochemical Analysis

Experiments were performed to verify that i.c.v. application of 1 umol CPAB (a dose sufficient to produce a statistically significant change in the firing frequency of cells exhibiting (+) responses) could also inhibit the enzyme in vivo. Enzyme activity in the controls was about three times higher in the substantia nigra than in the striatum. Infusion of 1 umol of CPAB i.c.v. significantly inhibited the enzyme in both striatum and substantia nigra when compared to controls (Table 2). As this reversible inhibitor is diluted five fold in the assay tube, it is likely that inhibition in vivo is much greater.

Figure 2. Time course of the change in firing frequency in response to inhibitors of endopeptidase-24.11. Open circles indicate cells exhibiting (+) responses. Closed circles indicate cells exhibiting (-) responses. Closed triangles in D (control experiments in which vehicle was infused) indicate both (+) and (-) responses to striatal stimulation. Ordinate: normalized mean firing frequency. Each curve was generated by pooling the normalized firing frequencies of several experiments: the number of experiments for each point is shown above that point. Each point represents either two minute averages (reported for the first 26 minutes following drug infusion) or 10 minute averages. In each experiment the firing frequencies were normalized to the mean firing frequency in the 10 minutes preceding the infusion. Arrows indicate the end of the infusion period. Infusion times: 6-21.5 minutes (10 uL), 12.5-21 minutes (20 uL). The firing frequency of the cells before drug infusion was compared to the firing frequency >20 minute after the end of the infusion (table 1). $p<0.05$ (paired t-test) for (+) responses. NSD for controls, (-) responses and N-[1-(R,S)- Carboxy-2-Phenylethyl]-Leu-pAB. Reprinted with permission, Elsevier Science Publishers B. V. (Biomedical Division), Bier et al., European Journal of Pharmacology, Vol. 142 (1987) 321-330.

FIGURE 2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N-[1-(R,S)-CARBOXY-3-PHENYLPROPYL]-PHE-PAB
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(+) AND (-) RESPONSES
1umol INFUSED
1.35
1.2
1.1
1.
.9
.8
.7
-70. -50. -30. 20. 70. 120.
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C
N-[1-(R,S)-CARBOXY-2-PHENYLETHYL]-PHE-PAB
(+) AND (-) RESPONSES
1umol INFUSED
1.5
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AND VEHICLE INFUSED
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1.4
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1.
.9
.8
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-70. -50. -30. -10. 10. 30. 50. 80.
MINUTES

TABLE 2

Inhibition of endopeptidase-24.11 in the substantia nigra and caudate-putamen after i.c.v. administration of 1 μmol N-[1-(R,S)-carboxy-2-phenylethyl]-Phe-pAB (CPAB).

CAUDATE		NIGRA	
VEHICLE	CPAB	VEHICLE	CPAB
4.06 ± 0.50 (5)	2.60 ± 0.40 (5)	11.92 ± 3.79 (8)	4.72 ± 0.92 (4)

Nigra: vehicle significantly different from inhibitor (CPAB) treatment ($p<0.005$, T-test). Caudate: vehicle significantly different from inhibitor (CPAB) treatment ($p<0.001$, T-test). Results are expressed as means ± standard deviation. Activity was determined with glutaryl-Ala-Ala-Phe-pAB as described in methods. Data represent specific activities expressed in nmole product/hr /mg tissue. Vehicle and CPAB were administered at a rate of 10 μl in 10 min. Numbers in parenthesis indicate number of experiments performed.

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B. Monopolar Study: Evoked and Spontaneous Responses

The next set of experiments were performed in order to determine if responses evoked in the substantia nigra using monopolar stimulation (1 ms pulses 50 to 200 uA at 1 or 2 Hz) were affected by the application of inhibitors of 24.11 in a manner consistent with the increases in spontaneous activity previously observed. Only the (+) cells were examined. Experiments were also performed in order to begin to localize the site of action of the inhibitors (effect of local application of inhibitors on spontaneous activity and evoked responses) and to begin to characterize the possible transmitter actions underlying our results using naloxone as a probe for enkephalin action.

Recordings, for these experiments were made through the entire rostral-caudal extent of the substantia nigra. The stimulation and paired recording sites appear in the appendix along with the tables which show the results for individual experiments. Of the cells reported, 54 (45%) were classified as dopaminergic and 65 (55%) as non-dopaminergic. The pre-treatment dopamine cell firing was (6.6 +/- 0.1 Hz; range: 2.5 to 12.6 Hz), irregular and often appeared in bursts of spikes (decreasing in amplitude); spikes were biphasic, 3 to 5 milliseconds in duration with prominent negative phase. Non-dopaminergic cells had faster firing frequencies (23.7 +/- 2.9 Hz; range: 12 to 37 Hz) with a more regular firing pattern than

the dopamine cells; spikes were biphasic with durations of no more than one millisecond. All of the reported cells were spontaneously active.

B.1. Intracerebroventricular Infusion of Inhibitors of 24.11:

B.1.a. Spontaneous Activity.

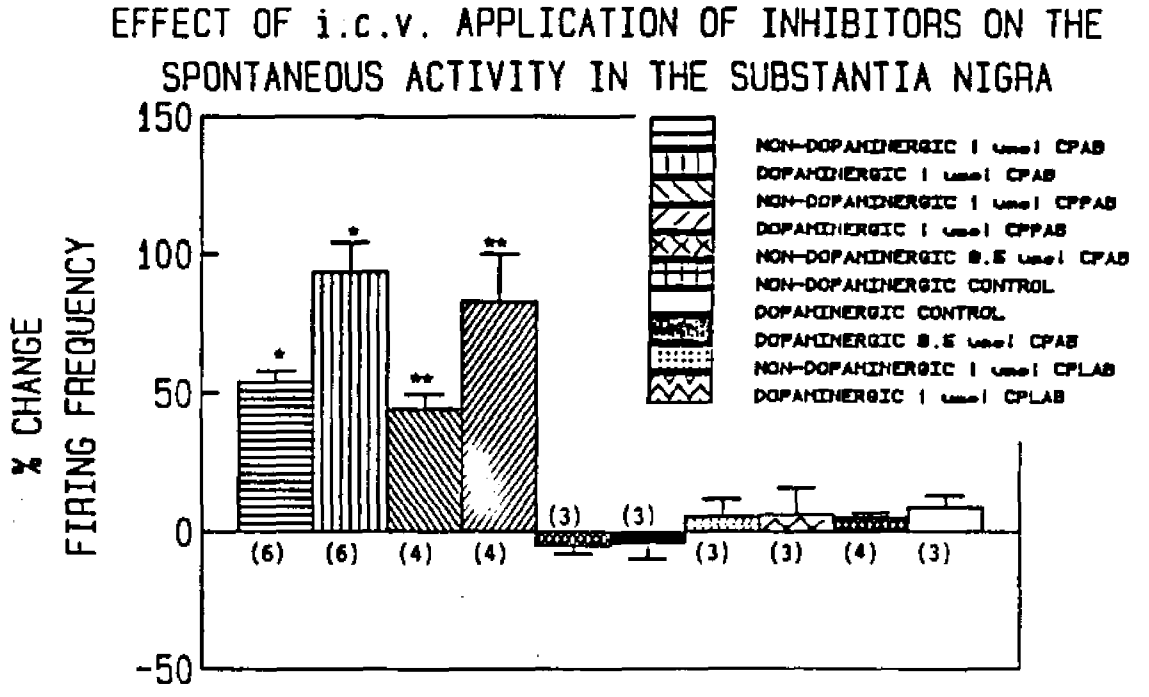
Figure 3 shows the effect of i.c.v. infusion of 1 umol CPAB, 1 umol CPPAB, 1 umol CPLAB, 0.5 umol CPAB or vehicle (0.9% saline or H_2O) on the spontaneous activity of non-dopaminergic and dopaminergic cells in the substantia nigra. Significant increases in spontaneous activity for both dopaminergic and non-dopaminergic cells, pre- to post-drug, were observed for pooled values in those experiments in which 1 umol CPAB i.c.v. or 1 umol CPPAB i.c.v. was applied. No significant effect on the spontaneous activity was observed following application of 0.5 umol CPAB i.c.v.(Tables 7 and 8), 1 umol CPLAB i.c.v. (Tables 9 and 10) or control infusions of 10 ul i.c.v. of 0.9% saline or H_2O (Tables 11 and 12). These results are in agreement with the previous findings.

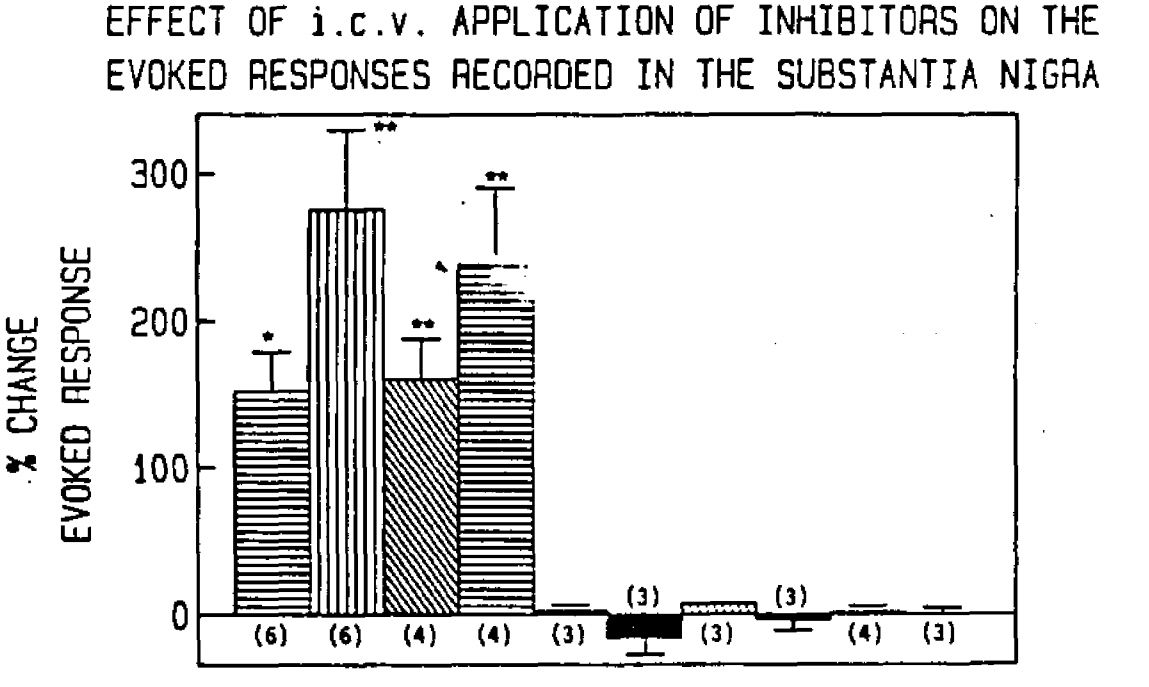
The mean increase in the spontaneous firing frequency of non-dopaminergic cells following application of 1 umol CPAB i.c.v. was 54.0% +/- 4.0 (n=6) (paired t-test, $p<0.01$) with a response range of 20.0% to 91.7% (Table 3 ; Figure 3). Application of 1 umol CPPAB i.c.v. increased the mean

Figure 3 Effect of intracerebroventricular infusion of inhibitors of metalloendopeptidase 24.11 on the spontaneous activity of cells in the substantia nigra. The graph shows the mean +/- SEM of the change in firing frequency from pre- to post-drug. The number of experiments represented by each bar is shown in parenthesis. *p<0.01, paired t-test. ** p<0.05, paired t-test.

Figure 4 Effect of the intracerebroventricular infusion of inhibitors of metalloendopeptidase 24.11 on the magnitude of the evoked response of cells in the substantia nigra. The graph shows the mean +/- SEM of the change in the magnitude of the evoked response from the pre- to post-drug. The number of individual experiments represented by each bar is shown in parenthesis. In each experiment evoked responses to 500 stimuli were summed both before and after administration of drug or vehicle. Stimuli were 100-150 uA pulses, 1 millisecond in duration. *p<0.01, paired t-test ** p<0.05, paired t-test).

FIGURES 3 and 4





spontaneous firing of non-dopaminergic cells 44.1% +/- 5.4 (n=4) (paired t-test, $p<0.05$) with a range of 29.8% to 52.8% (Table 4 : Figure 3). The mean increase in spontaneous firing frequency of dopaminergic cells following the application of 1 umol CPAB i.c.v. was 93.7% +/- 10.6 (n=6) (paired t-test, $p<0.01$) with a response range of 72.2% to 141.7% (Table 5 : Figure 3) and 1 umol CPPAB i.c.v. increasing the firing rate 83.0% +/- 17.1 (n=4) (paired t-test, $p<0.05$) with a range of 62.3% to 104.2% (Table 6 : Figure 3). The increases in spontaneous firing frequency observed following i.c.v. application of equivalent doses of CPAB and CPPAB are not statistically different from one another for either non-dopaminergic or dopaminergic cells (Newman-Keuls multiple range test).

When the values for mean increase in the firing frequency of dopaminergic and non-dopaminergic cells following application of 1 umol CPAB i.c.v. or 1 umol CPPAB i.c.v. were compared with values following application of 0.5 umol CPAB i.c.v., 1 umol CPLAB i.c.v. or vehicle they were found to have significant differences among each pair tested (Newman-Keuls multiple range test; $p<0.01$). The results obtained from i.c.v. infusion of 0.9% saline, H_2O, 1 umol CPLAB or 0.5 umol CPAB are not statistically different from one another (Newman-Keuls multiple range test).

As in our initial study the value of the mean increase in spontaneous firing frequency of the dopaminergic cells (pooled responses following application of 1 umol CPAB i.c.v. and 1 umol CPPAB i.c.v. was 89.4% +/- 7.2 (n=10)) was significantly greater than the mean increase in non-dopaminergic spontaneous rate (50.0% +/- 6.7 (n=10); t-test, $p<0.001$). Again, the baseline firing frequency of dopaminergic cells (6.1 +/- 0.9 Hz (n=10)) is lower than that of the non-dopaminergic cells (22.4 +/- 1.9 Hz (n=10)) and the absolute increase in firing frequency is lower for dopaminergic than for the non-dopaminergic cells (5.4 +/- 0.9 Hz vs 10.5 +/- 2.8 Hz; t-test, $p<0.05$).

B.1.b. Evoked Responses

Responses evoked in the substantia nigra following striatal stimulation were affected by the application inhibitors of 24.11. Significant increases in the responses of dopaminergic and non-dopaminergic cells were seen following application of 1 umol CPAB i.c.v. or 1 umol CPPAB i.c.v. but not 0.5 umol CPPAB i.c.v., 1 umol CPLAB i.c.v. or infusions of vehicle.

Infusion of 1 umol CPAB i.c.v. increased the evoked responses in non-dopaminergic cells by 152.3% +/- 26.6 (n=6) (paired t-test, $p<0.01$) with a response range of 93.0% to 277.9% (Table 3 ; Figure 4). Dopaminergic cells responded with a mean increase of nearly twice that of the non-dopaminergic cells (275.6% +/- 53.9 (n=6); paired

t-test, $p<0.05$) with a range of 108.2% to 433.9% (Table 5 : Figure 4). There was no significant difference between the magnitude of the baseline (pre-drug) evoked response of the dopaminergic and non-dopaminergic cells; dopaminergic (99.2 +/- 25.8 counts (n=6)), non-dopaminergic cells (113.2 +/- 24.6 counts (n=6)). Thus, it may be fair to compare the effect of the inhibitors of 24.11 on the evoked response across these cell groups.

Infusion of 1 umol CPPAB i.c.v. increased the evoked responses in non-dopaminergic cells by a mean of 160.5% +/- 27.4 (n=4) (Table 4 : Figure 4, paired t-test, $p<0.05$) with a range of 100.0% to 219.6%. Dopaminergic cells responded with a mean increase of nearly twice that of the non-dopaminergic cells (238.4% +/- 51.8 (n=4); paired t-test, $p<0.05$) with a range of 165.4% to 386.6% (Table 6 : Figure 4). There was no significant difference in the magnitude of the baseline (pre-drug) evoked response between dopaminergic (87.0 +/- 26.9 counts (n=4)) and non-dopaminergic cells (95.4 +/- 12.2 counts (n=4)).

There was no apparent relation between the initial condition (pre-drug evoked response) and the increase in the evoked response observed following application of 1 umol CPAB i.c.v. or 1 umol CPPAB i.c.v. for dopaminergic or non-dopaminergic cells (Figs. 5 and 6).

B.2. Application of Inhibitors of 24.11 into the Substantia Nigra

Figure 5 Relationship between the magnitude of the evoked response before application of 1 umol CPAB i.c.v and the change in the magnitude of the evoked response induced by the drug. The circles represent individual experiments which appear in tables 3 and 5. Closed circles: non-dopaminergic cells. Open circles: dopaminergic cells. The X-axis, magnitude of the pre-drug evoked response, is reported as the number of counts as defined in the methods.

FIGURE 5

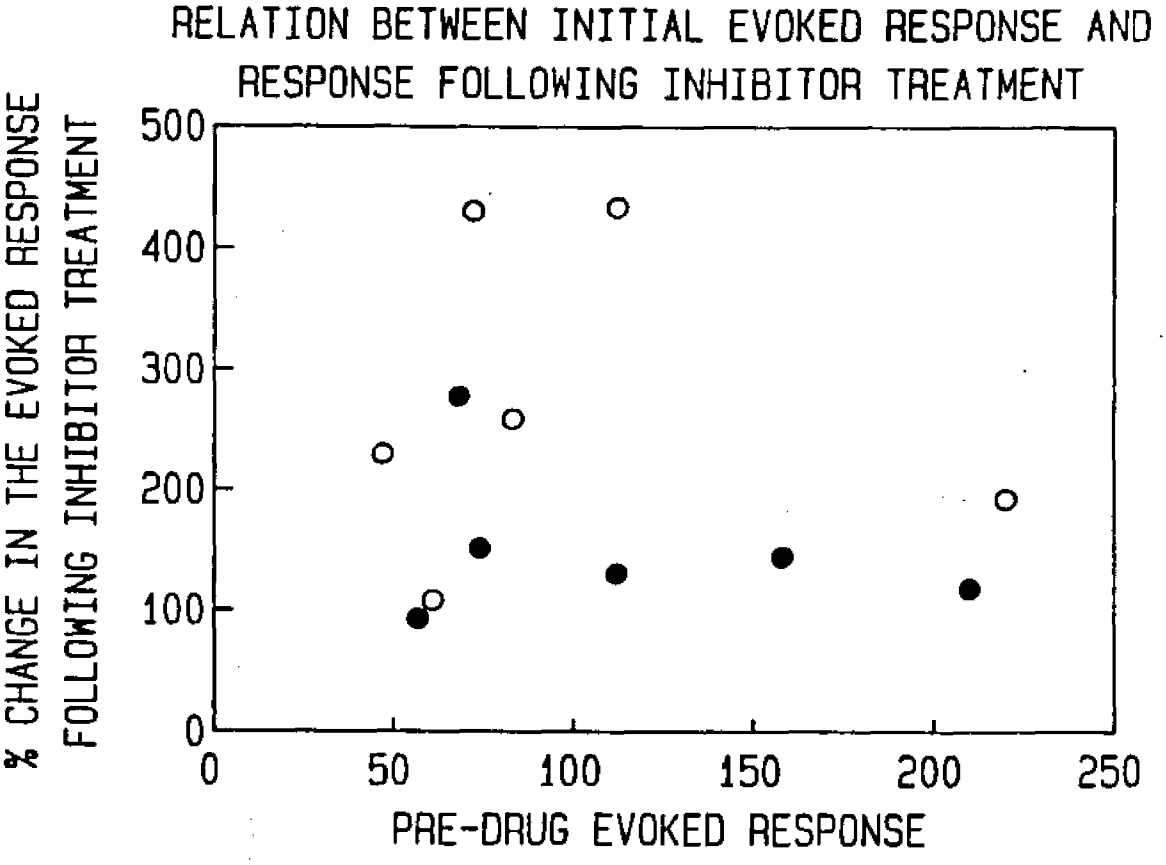
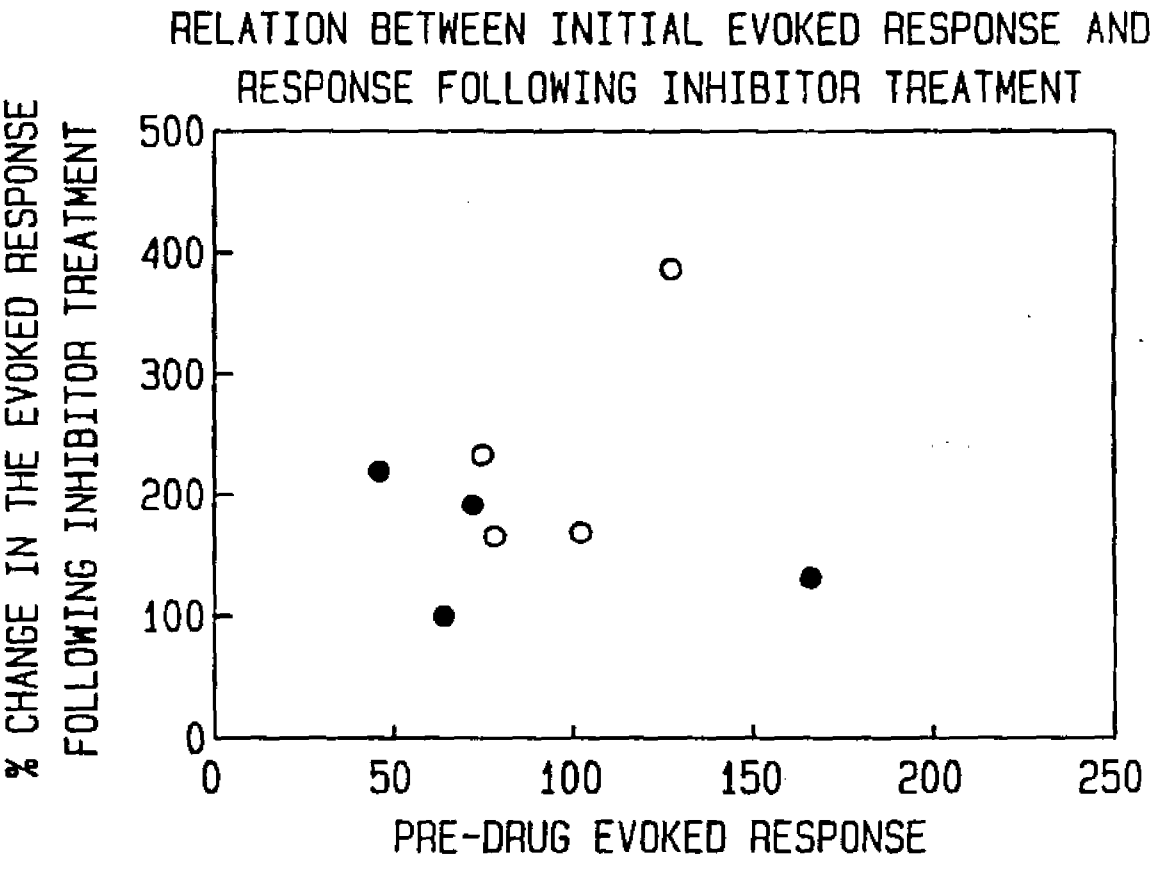

RELATION BETWEEN INITIAL EVOKED RESPONSE AND
RESPONSE FOLLOWING INHIBITOR TREATMENT
% CHANGE IN THE EVOKED RESPONSE
FOLLOWING INHIBITOR TREATMENT
500
400
300
200
100
0
0
50
100
150
200
250
PRE-DRUG EVOKED RESPONSE

Figure 6 Relationship between the magnitude of the evoked response before application of 1 umol CPPAB i.c.v and the change in the magnitude of the evoked response induced by the drug. The circles represent individual experiments which appear in tables 4 and 6. Closed circles: non-dopaminergic cells. Open circles: dopaminergic cells. The X-axis, magnitude of the pre-drug evoked response, is reported as the number of counts as defined in the methods.

FIGURE 6


RELATION BETWEEN INITIAL EVOKED RESPONSE AND
RESPONSE FOLLOWING INHIBITOR TREATMENT
% CHANGE IN THE EVOKED RESPONSE
FOLLOWING INHIBITOR TREATMENT
500
400
300
200
100
0
0
50
100
150
200
250
PRE-DRUG EVOKED RESPONSE

Experiments were performed in order to determine if the effects on the spontaneous firing frequency and evoked responses observed following i.c.v. application could be replicated by applying inhibitors directly into the striatum or the substantia nigra.

B.2.a. Biochemical Analysis

Experiments were performed to determine the dose of CPAB which, when applied locally into the substantia nigra, would inhibit the enzyme to the same degree as application of 1 umol CPAB i.c.v. (60.4% inhibition). Each experiment shows the results of a single punch of tissue taken from a single rat. Table 13 shows the results of these experiments. Initial experiments, numbers 1, 2 and 3 were performed to determine the range of doses which might yield 60% inhibition. There was clearly no inhibition produced following infusion of 0.01 umol CPAB. There was an apparent decrease in enzyme activity upon administration of 0.02 and 0.04 umol CPAB. The large standard error precluded statistical significance. This error may be attributed to the small amounts of tissue assayed, dilution of the inhibitor and/or small sample size. Experiments were repeated at the 0.04 umol dose (experiments 4 and 5) yielding significant inhibition. Average inhibition at that dose was 58.19% +/- 4.27 which was the dose used in subsequent experiments.

TABLE 13

Inhibition of endopeptidase-24.11 in the substantia nigra following local administration of N-[1-(R,S)-carboxy-2-phenylethyl]-Phe-pAB (CPAB).

	DOSE	NIGRA		%
	CPAB	VEHICLE	CPAB	CHANGE
1.	0.010 umol[1]	11.72 +/- 0.27 (3)	11.65 +/- 0.20 (3)	+ 1.1%
2.	0.020 umol[2]	2.73 +/- 0.63 (2)	0.33 +/- 0.90 (2)	- 87.91%
3.	0.040 umol[3]	9.37 +/- 3.15 (4)	3.88 +/- 0.86 (4)	- 58.59%
4.	0.040 umol[4]	1.56 +/- 0.1 (2)	0.54 +/- 0.11 (2)	- 65.38%
5.	0.040 umol[5]	7.33 +/- 0.58 (3)	3.62 +/- 0.16 (2)	- 50.61%

1, 2 and 3 vehicle not significantly different from inhibitor. 4. Vehicle significantly different from inhibitor ($p<0.02$, t-test). 5. Vehicle significantly different from inhibitor treatment ($p<0.02$, t-test). Results expressed as means +/- SEM. Activity was determined with glutaryl-Ala-Ala-Phe-pAB as described in methods. Data represent specific activities expressed in nmole product/hr/mg tissue. Vehicle and CPAB were administered at a rate of 1 uL in 2-3 minutes. Numbers in parenthesis indicate number of experiments performed.

The extent of enzyme inhibition in the striatum following direct application of 0.04 umol CPAB was determined in initial experiments to yield non-significant reductions in enzyme activity of 27.5% (0.40 +/- 0.5 (n=3) vs 0.29 +/- 0.12 (n=2) nmol/hour/mg tissue). These experiments were not continued, in order to obtain statistically significant results, for the following reasons. First, it had previously been determined that enzyme activity, measured colormetrically, was about three times higher in the substantia nigra than the striatum (Table 2). Further, the control readings, for baseline enzyme activity, obtained from the nigral tissue were small with large variance (11.72 to 1.56 nmol/mg protein). Readings for the activity of the enzyme in the striatum were very low with large variance. Thus, the accuracy of the readings at the extreme low end of the scale was in question. Considering these factors, low enzyme activity and the dilution of the inhibitor during assay, it was decided that any further attempted measurement of the effect of direct application of inhibitor into the striatum would be wasteful.

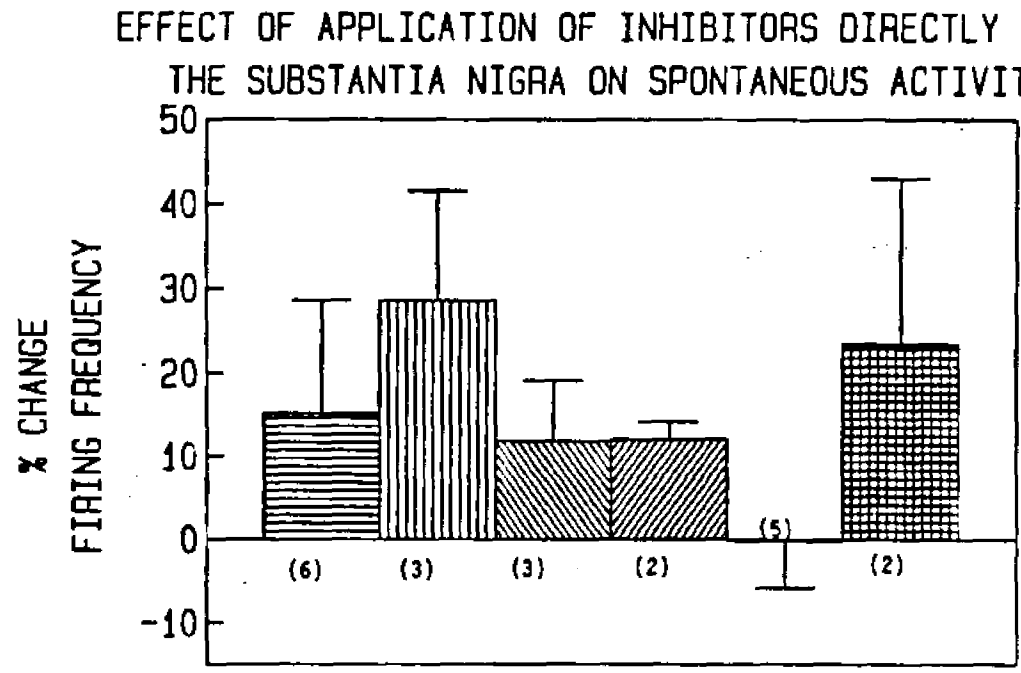
B.2.b. Effect on Spontaneous Activity

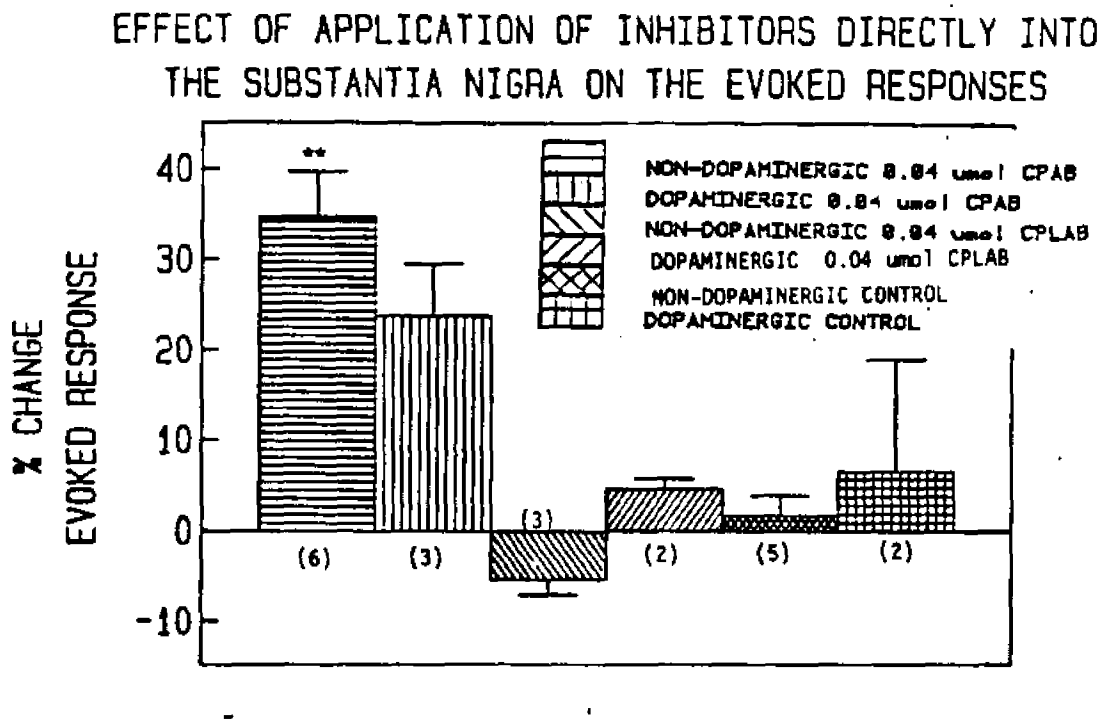
Figure 7 shows the effect of local infusion of 1 uL of a 40 millimolar solution of (0.04 umol) CPAB (Tables 14 and 15), 1 uL of a 40 millimolar solution of (0.04 umol) CPLAB (Tables 16 and 17) and control infusions, 1 uL of either

Figure 7 Effect of direct infusion of inhibitors of metalloendopeptidase 24.11 into the substantia nigra on the spontaneous activity of cells in the substantia nigra. Each bar of the graph represents the mean of the change in firing frequency from pre- to post-drug. The bars are presented +/- SEM. There was no significant effect on the spontaneous activity following any treatment. See figure 8 for the key to cell type and drugs used.

Figure 8 Effect of direct application of inhibitors of metalloendopeptidase 24.11 on the magnitude of the evoked responses in the substantia nigra. The graph shows the mean +/- SEM of the change in the magnitude of the evoked response from the pre- to post-drug. The number of individual experiments represented by each bar is shown in parenthesis. In each experiment evoked responses to 500 stimuli were summed both before and after administration of drug or vehicle. Stimuli were 100-150 uA pulses, 1 millisecond in duration. ** $p<0.05$, paired t-test.

FIGURES 7 and 8





0.9% saline or H_2O (Tables 18 and 19), on the spontaneous activity of non-dopaminergic and dopaminergic cells in the substantia nigra. No statistically significant changes in spontaneous activity for either dopaminergic or non-dopaminergic cells were observed following application of CPAB, CPLAB or controls (paired t-test). Saline, H_2O, 0.04 umol CPLAB and 0.04 umol CPAB values are not statistically different from one another (Newman-Keuls multiple range test).

The mean baseline (pre-drug) firing frequency, in those experiments in which 0.04 umol CPAB was infused, is lower for the dopaminergic cells (5.6 +/- 2.3 Hz (n=3)) than the non-dopaminergic cells (21.0 +/- 2.0 Hz (n=6)). These baseline values, for both dopaminergic and non-dopaminergic cells, are not statistically different from the baseline values obtained, for dopaminergic and non-dopaminergic cells, in the experiments in which the inhibitors were administered i.c.v (Newman-Keuls multiple range test). Thus, the presence of the cannulae in the substantia nigra, in close proximity to the recording electrode did not, in itself, change the initial conditions compared to those experiments in which the infusion cannula was in the lateral ventricle. There is no statistical difference in the absolute change in firing frequency, following infusion of 0.04 umol CPAB into the substantia nigra, between the dopaminergic and non-dopaminergic cells (1.0 +/- 0.4 Hz vs 2.8 +/- 2.8 Hz).

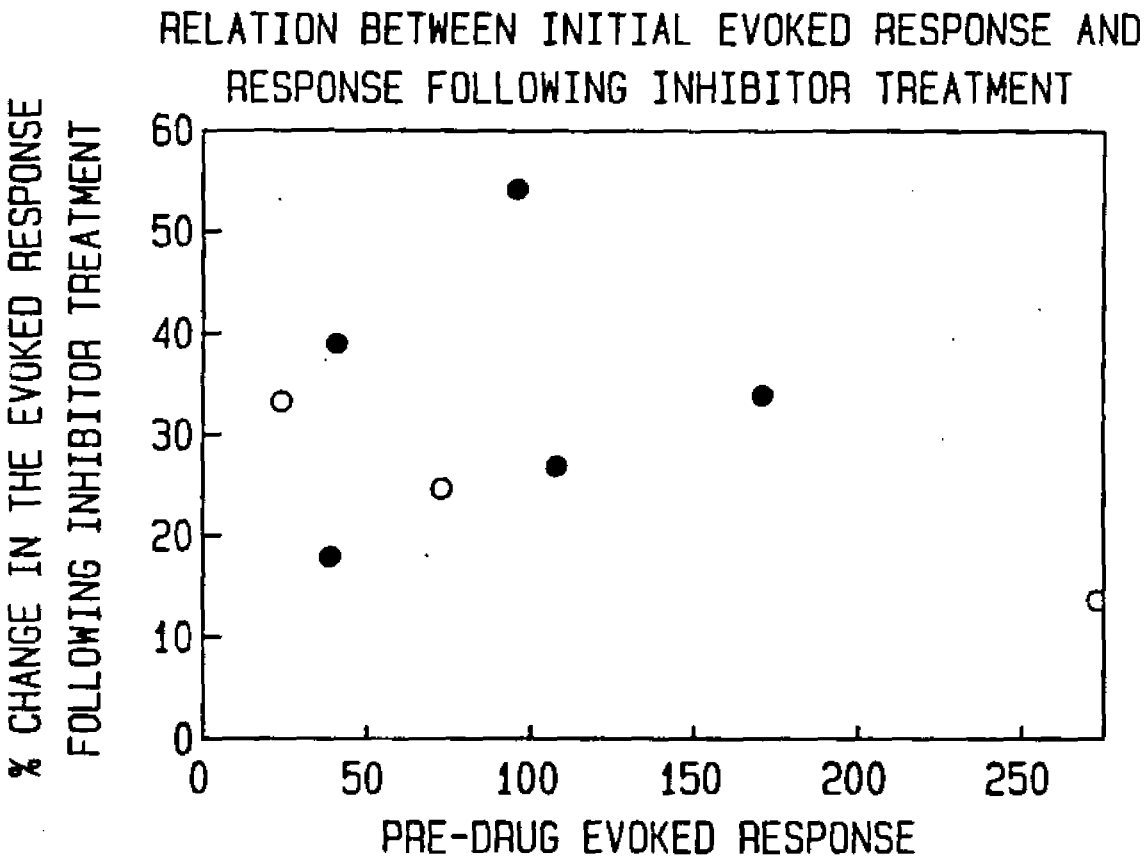
B.2.c. Effect on the Magnitude of Evoked Responses

Infusion of 0.04 umol CPAB into the substantia nigra increased the evoked responses of non-dopaminergic cells with a mean increase of 34.7% +/- 5.0 (n=6) (paired t-test, $p<0.05$) and a response range of 17.9% to 54.2% (Table 14 : Figure 8). The evoked responses of the dopaminergic cells were not significantly changed following the application of 0.04 umol CPAB into the substantia nigra: mean of (23.8% +/- 5.7 (n=3)) with a range of 13.6% to 33.3% (Table 15 : Figure 8). The non-significance of the result is supported by the finding that there is no statistical difference between the effect of infusing CPAB compared to the pooled values of vehicle and CPLAB (Mann-Whitney). There was no significant difference between the magnitude of the baseline (pre-drug) evoked responses; dopaminergic (123.3 +/- 76.2 (n=3)), non-dopaminergic cells (113.7 +/- 30.3 (n=6)). There was no relation between the initial condition (pre-drug evoked response) and the change in the evoked response observed following application of 0.04 umol CPAB directly into the substantia nigra for either the dopaminergic or non-dopaminergic cells (Figure 9).

Infusions of 0.04 umol CPLAB or 1uL vehicle (0.9% saline and H_2O) had no significant effect on the evoked responses of dopaminergic or non-dopaminergic cells (Tables 16, 17, 18 and 19 : Figure 8). Four experiments were performed on dopaminergic cells, two with 1uL vehicle, and

Figure 9 Relationship between the magnitude of the evoked response before direct application of 0.04 umol CPAB directly into the substantia nigra and the change in the magnitude of the evoked response induced by the drug. The circles represent individual experiments which appear in tables 4 and 6. Closed circles: non-dopaminergic cells. Open circles: dopaminergic cells. The X-axis, magnitude of the pre-drug evoked response, is reported as the number of counts as defined in the methods.

FIGURE 9


RELATION BETWEEN INITIAL EVOKED RESPONSE AND
RESPONSE FOLLOWING INHIBITOR TREATMENT
% CHANGE IN THE EVOKED RESPONSE
FOLLOWING INHIBITOR TREATMENT
60
50
40
30
20
10
0
0
50
100
150
200
250
PRE-DRUG EVOKED RESPONSE

two with 0.04 umol CPLAB infused into the substantia nigra. These values were determined to be not statistically different from the control values seen following administration of 0.5 umol CPAB i.c.v., 1 umol CPLAB i.c.v, or vehicle i.c.v. These values were also no different from the values observed in the dopaminergic cells evoked responses following application of 0.04 umol CPAB into the substantia nigra (Newman-Keuls multiple range test).

B.3. Application of Inhibitors of 24.11 into the Striatum

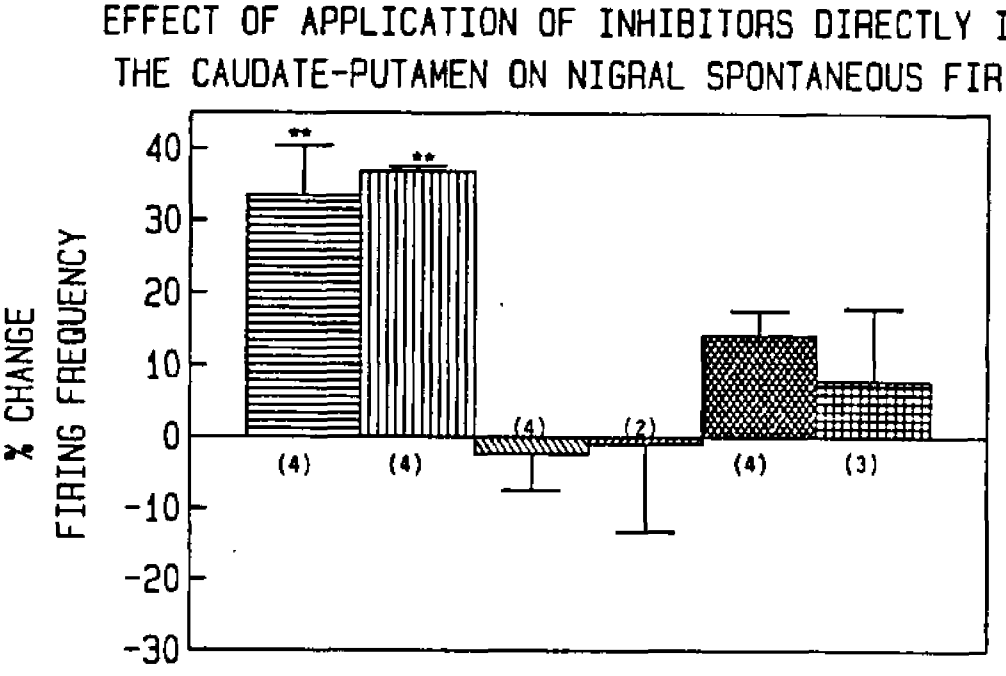
B.3.a. Spontaneous Activity

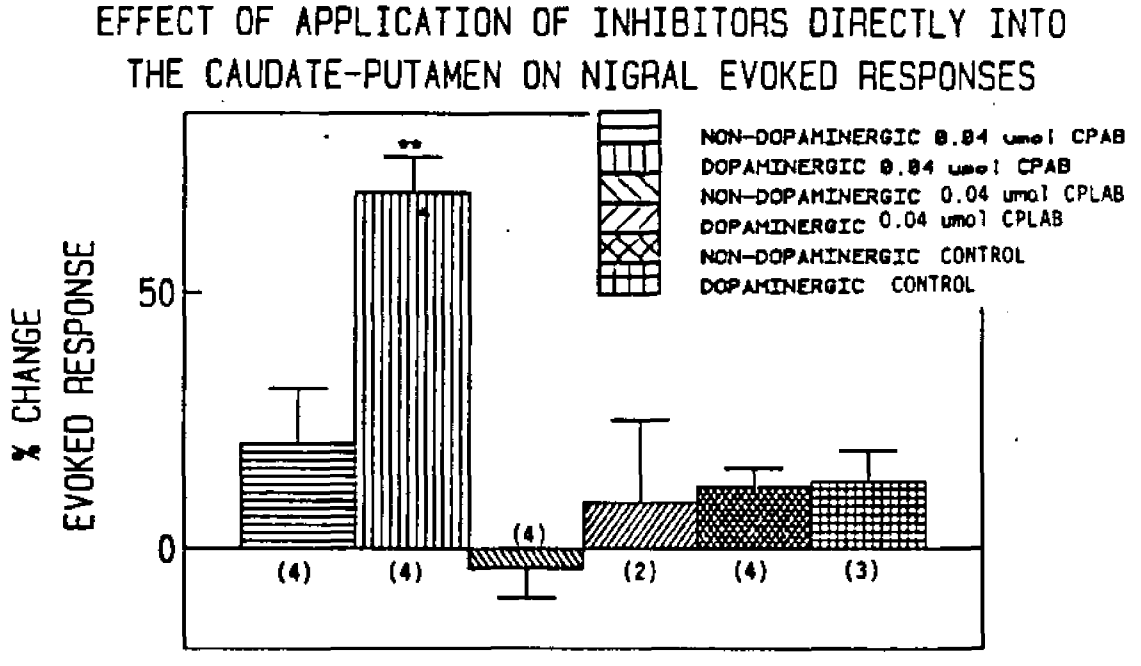
Figure 10 shows the effect of local infusion into the striatum of 1 uL of a 40 millimolar solution (0.04 umol) of CPAB, 1 uL of a 40 millimolar solution (0.04 umol) of CPLAB and control infusions 1 uL of either 0.9% saline or H_2O on the spontaneous activity of non-dopaminergic and dopaminergic cells in the substantia nigra. Significant increases in spontaneous activity were observed in dopaminergic and non-dopaminergic cells following application of 0.04 umol CPAB directly into the striatum. No significant effect on the spontaneous activity in either dopaminergic or non-dopaminergic cells was observed following application of 0.04 umol CPLAB (Tables 22 and 23 : Figure 10) or 1 uL of vehicle (0.9% saline or H_2O) (Tables 24 and 25 : Figure 10).

Figure 10 Effect of direct infusion of inhibitors of metalloendopeptidase 24.11 into the caudate-putamen on the spontaneous activity of cells in the substantia nigra. The graph shows the mean +/- SEM of the change in the firing frequency from pre- to post-drug. The number of experiments represented by each bar is shown in parenthesis. ** $p<0.05$, paired t-test. See figure 11 for the key to cell type and drugs used.

Figure 11 Effect of direct infusion of inhibitors of metalloendopeptidase 24.11 into the striatum on the magnitude of the evoked responses in the substantia nigra. The graph shows the mean +/- SEM of the change in the magnitude of the evoked response from the pre- to post-drug. The number of individual experiments represented by each bar is shown in parenthesis. In each experiment evoked responses to 500 stimuli were summed both before and after administration of drug or vehicle. Stimuli were 100-150 uA pulses, 1 millisecond in duration. ** $p<0.05$, paired t-test.

FIGURES 10 and 11





The mean increase in the spontaneous firing frequency of non-dopaminergic cells following the application of 0.04 umol CPAB was (33.5% +/- 6.9 (n=4); paired t-test, p<0.05) with a response range of 18.5% to 52.0% (Table 20 : Figure 10) and a mean percent increase of (36.9% +/- 0.72 (n=4); paired t-test, p<0.05) with a response range of 23.2% to 55.9% for the dopaminergic cells (Table 21 : Figure 10).

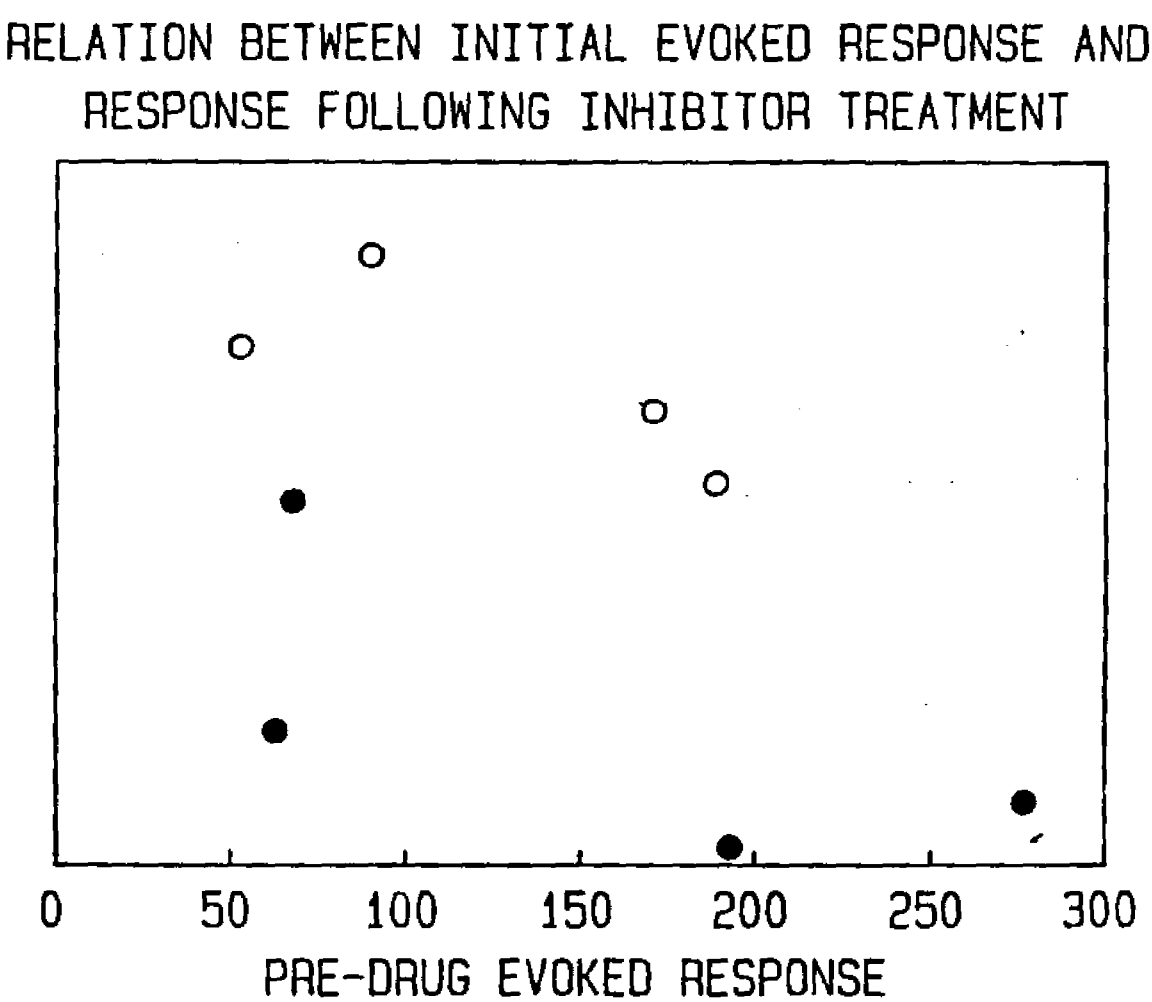
The baseline firing frequency of dopaminergic and non-dopaminergic cells, for those experiments in which 0.04 umol CPAB was administered directly into the striatum, was (7.9 +/- 0.9 Hz (n=4)) and (22.4 +/- 2.2 Hz (n=4)) respectively. These values are not statistically different from the baseline spontaneous firing frequency values, for dopaminergic and non-dopaminergic cells, for those experiments in which 1 umol CPAB i.c.v, 1 umol CPPAB i.c.v. or 0.04 umol CPAB was applied locally into the substantia nigra (Newman-Keuls multiple range test). There is no difference in the absolute change in firing frequency, between the dopaminergic and non-dopaminergic cells, for those experiments in which 0.04 umol CPAB was administered directly into the striatum (3.0 +/- 0.7 Hz vs 7.5 +/- 1.9 Hz).

B.3.b. Evoked Responses

Infusion of 0.04 umol CPAB directly into the striatum significantly increased the mean evoked response of dopaminergic cells (69.6% +/- 7.0 (n=4); paired t-test,

Figure 12 Relationship between the magnitude of the evoked response before direct application of 0.04 umol CPAB directly into the striatum and the change in the magnitude of the evoked response induced by the drugs. The circles represent individual experiments which appear in tables 20 and 21. Closed circles: non-dopaminergic cells. Open circles: dopaminergic cells. The X-axis, magnitude of the pre-drug evoked response, is reported as the number of counts as defined in the methods.

FIGURE 12


RELATION BETWEEN INITIAL EVOKED RESPONSE AND
RESPONSE FOLLOWING INHIBITOR TREATMENT
0
50
100
150
200
250
300
PRE-DRUG EVOKED RESPONSE

$p<0.05$) with a response range of 54.0% to 86.7% (Table 21 : Figure 11). There was no significant increase in the evoked response for non-dopaminergic cells (Table 20 : Figure 11). There was no significant difference in the magnitude of the baseline (pre-drug) evoked responses between the dopaminergic (125.8 +/- 32.5 (n=4)) and non-dopaminergic cells (150.3 +/- 51.9 (n=4)). There was no apparent relation between the initial condition (pre-drug evoked response) and the change in the evoked response observed following application of 0.04 umol CPAB directly into the striatum for either the dopaminergic or non-dopaminergic cells (Figure 12).

Infusions of 0.04 umol CPLAB or vehicle (0.9% saline and H_2O) had no significant effect on the evoked responses (Tables 22, 23, 24 and 25 : Figure 11).

B.4. Effect of Administration of Naloxone 1 mg/kg, I.v.

B.4.a. Spontaneous Activity

The administration of naloxone 1 mg/kg, I.v. had no significant effect on either the spontaneous or evoked responses in the substantia nigra. The mean change in the non-dopaminergic cells spontaneous firing frequency following the application of naloxone I.v. was (0.6% +/- 1.0 (n=4)) with a response range of - 2.6% to 2.0% (Table 26) and a mean change of (0.2% +/- 4.6 (n=4)) with a response range of -11.6% to 9.4% for the dopaminergic cells

(Table 27). These values are not different from the mean changes recorded for those experiments in which 0.9% saline or H_2O were administered i.c.v.

B.4.b. Evoked Responses

Infusion of naloxone 1 mg/kg, i.v. was without significant effect on the evoked responses of dopaminergic cells, with a mean change in the magnitude of the evoked response of (2.2% +/- 6.2 (n=4)) and a response range of -13% to 12.5% (Table 27). Non-dopaminergic cells were also not significantly responsive to naloxone 1 mg/kg, i.v., with a mean change in the magnitude of the evoked response of - 11.4% +/- 3.8 (n=4) with a range of -5.2% to -22.0% (Table 26). These values are not different from the mean of the changes in the magnitude of the evoked responses obtained in those experiments in which 0.9% saline or H_2O were administered i.c.v. There is a difference between the magnitude of the baseline (pre-naloxone) evoked responses of the non-dopaminergic (226.0 +/- 53.1 counts) and dopaminergic (56.3 +/- 16.5 counts) cells ($p<0.05$, t-test). Again the pre-treatment evoked responses do not effect the outcome of the drug effect; there is no correlation between large or small evoked responses and large or small drug effects.

B.5. Application 1 umol CPAB i.c.v. in the Presence of Naloxone 1 mg/kg, i.v.

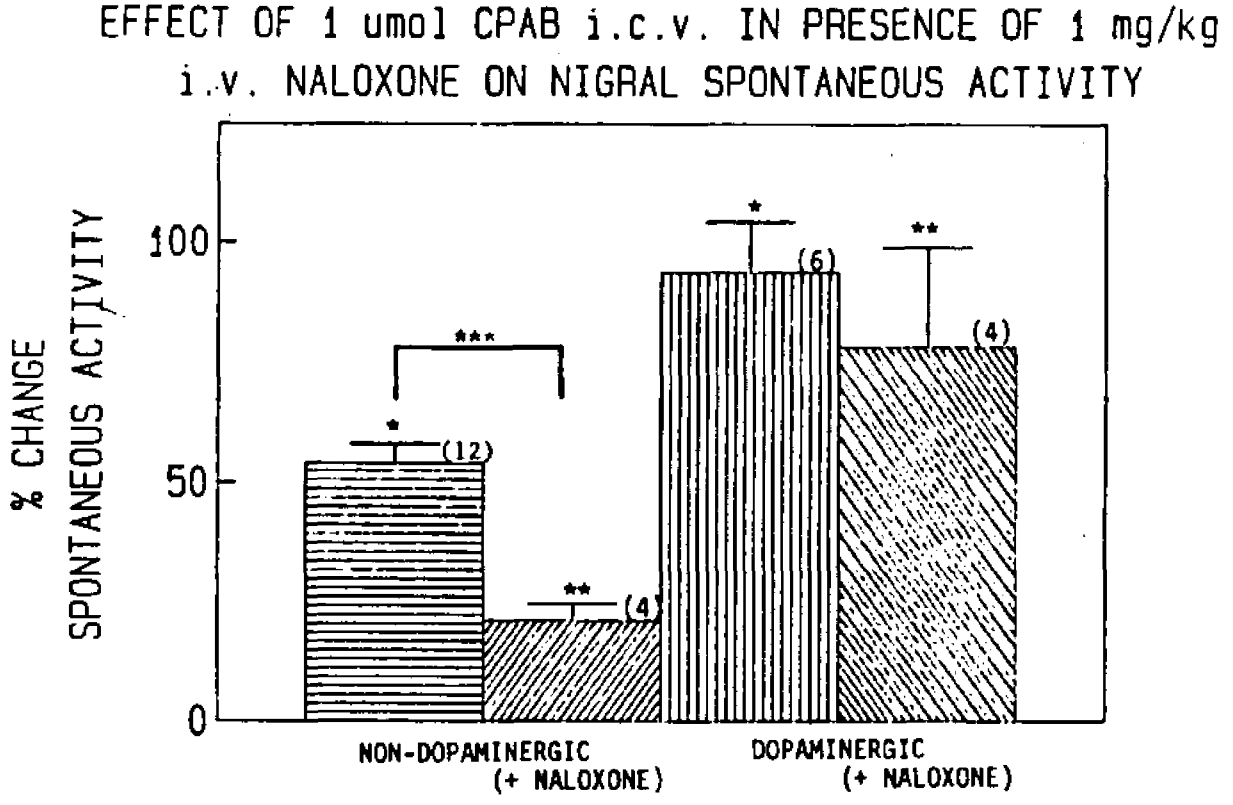
B.5.a. Spontaneous Activity

Naloxone 1 mg/kg, i.v. was administered and a baseline firing frequency was established for 30 minutes before administration of 1 umol CPAB i.c.v. It was established, in the previous set of experiments, that 1 mg/kg, i.v. naloxone had no significant effect on the spontaneous or evoked responses recorded in dopaminergic or non-dopaminergic cells in the substantia nigra. The present experiments were performed in order to determine if the presence of naloxone 1 mg/kg, i.v. could modify the increases in spontaneous and evoked responses recorded in both non-dopaminergic and dopaminergic cells induced by the administration of 1 umol CPAB i.c.v.

Figure 13 shows the effect of infusion of 1 umol CPAB i.c.v., on the spontaneous activity of non-dopaminergic and dopaminergic cells in the substantia nigra in the presence of naloxone 1mg/kg, i.v. Significant increases in spontaneous activity pre- to post-drug were observed, for both dopaminergic and non-dopaminergic cells, following administration of 1 umol CPAB i.c.v. The mean increase in spontaneous activity of non-dopaminergic cells following the application of 1 umol CPAB i.c.v. in the presence of naloxone 1mg/kg, i.v. was (21.3% +/- 3.5 (n=4); paired t-test, $p<0.05$) with a response range of 11.3% to 27.6% (Table 28 : Figure 13). The mean increase in firing frequency of the dopaminergic cells following the

Figure 13 Effect of infusion of 1 umol CPAB i.c.v. in the presence of naloxone 1 mg/kg, i.v. on the spontaneous activity of cells in the substantia nigra. The graph shows the mean +/- SEM of the change in the firing frequency form pre- to post-drug for the dopaminergic and non-dopaminergic cells. The graph contrasts the effects of application of CPAB i.c.v. taken from experiments described earlier (Figure 3) and CPAB i.c.v. in the presence of naloxone. The number of experiments represented by each bar is shown in parenthesis. ** Pre-CPAB compared to post-CPAB (all in the presence of naloxone) $p<0.05$, paired t-test. * $p<0.01$ paired t-test. *** $p<0.01$, Mann-Whitney.

FIGURE 13


EFFECT OF 1 umol CPAB i.c.v. IN PRESENCE OF 1 mg/kg i.v. NALOXONE ON NIGRAL SPONTANEOUS ACTIVITY
% CHANGE SPONTANEOUS ACTIVITY
100
50
0
***
*
(12)
**
(4)
*
(6)
**
(4)
NON-DOPAMINERGIC (+ NALOXONE)
DOPAMINERGIC (+ NALOXONE)

application of 1 umol CPAB was (78.1% +/- 20.8 (n=4); paired t-test, p<0.05) with a response range of 20.6% to 110.2% (Table 29 : Figure 13). The change in firing frequency of the non-dopaminergic cells in the presence of naloxone are significantly smaller than those seen in the absence of naloxone when compared to all experiments in which measures for the change in spontaneous firing following 1 umole CPAB were obtained (pooling 1 umol CPAB alone and experiments in which CPAB was administered prior to application of naloxone into the substantia nigra and striatum). This pooling gives an increase in the spontaneous firing frequency of 60.0% +/- 5.6 (n=12) and 21.3% vs 60.0% for non-dopaminergic spontaneous firing frequency are significantly different (p<0.01; Mann-Whitney). The application of naloxone 1 mg/kg i.v., is able to antagonize the increase in non-dopaminergic cell firing induced by 1 umol CPAB i.c.v.

Alternatively the dopaminergic firing was found to be not significantly different from pooled CPAB i.c.v. (78.1% vs 96.6% +/- 8.6 (n=12, Mann-Whitney). Thus, although small reductions in the firing frequency of dopaminergic cells are seen, the presence of naloxone 1 mg/kg, i.v. does not significantly modify the increases in spontaneous activity induced by 1 umol CPAB i.c.v.

The mean increase in spontaneous firing frequency of the dopaminergic cells following application of 1 umol CPAB i.c.v. in the presence of naloxone 1mg/kg, i.v. (78.1% +/-

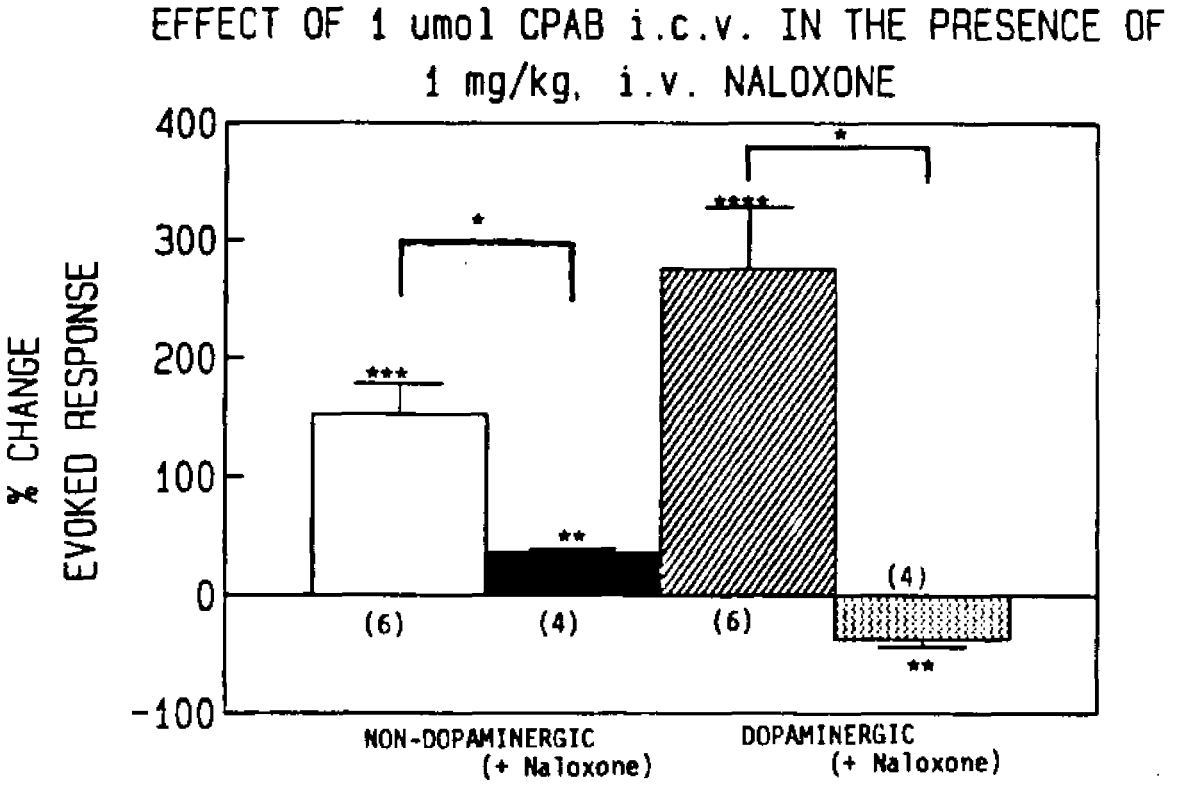
20.8 (n=4)) is different from the increase in non-dopaminergic spontaneous rate (21.3% +/- 3.5 (n=4)); t-test, p<0.05). The apparent increased responsiveness of the dopaminergic cells, as compared to the non-dopaminergic cells, to the i.c.v. application of 1 umol CPAB, seen in the earlier experiments, is not altered in the presence of naloxone 1 mg/kg, i.v. The baseline firing frequency of dopaminergic cells (8.0 +/- 1.8 Hz) is lower than that of the non-dopaminergic cells (26.5 +/- 2.6 Hz). The absolute increase in firing frequency is not significantly different for dopaminergic and non-dopaminergic cells (5.5 +/- 1.6 Hz vs 5.7 +/- 1.2 Hz). This is in contrast to the earlier experiments in which 1 umol CPAB was applied i.c.v., in the absence of naloxone, and the absolute change in the firing frequency induced by the inhibitor was significantly lower for the dopaminergic than non-dopaminergic firing frequency.

B.5.b. Evoked Responses

Infusion of 1 umol CPAB in the presence of naloxone 1mg/kg, i.v. increased the magnitude of the evoked responses in non-dopaminergic cells with a mean increase of 36.2% +/- 2.7 (n=6) (paired t-test, p<0.05) and a response range of 27.1% to 41.9% (Table 28 : Figure 14). Although this is a significant CPAB effect, the magnitude of the effect is lower than that obtained with 1 umol CPAB i.c.v. alone (152.3% vs 36.2%; Newman-Keuls; p<0.01). The evoked

Figure 14 Effect of infusion of 1 umol CPAB i.c.v. in the presence of naloxone 1 mg/kg, i.v. on the magnitude of evoked responses of cells in the substantia nigra. The graph shows the mean +/- SEM in the magnitude of the evoked responses form pre- to post-drug for the dopaminergic and non-dopaminergic cells. The graph contrasts the effects of application of CPAB i.c.v. taken from experiments described earlier (Figure 4) and CPAB i.c.v. in the presence of naloxone. The number of experiments represented by each bar is shown in parenthesis. ** Pre-CPAB compared to post-CPAB (all in the presence of naloxone) $p<0.05$, paired t-test. * $p<0.01$, Newman-Keuls. *** $p<0.05$, paired t-test. **** $p<0.01$, paired t-test.

FIGURE 14


EFFECT OF 1 umol CPAB i.c.v. IN THE PRESENCE OF
1 mg/kg, i.v. NALOXONE
% CHANGE
EVOKED RESPONSE
400
300
200
100
0
-100
*
***
**
****
*
(6)
(4)
(6)
(4)
**
NON-DOPAMINERGIC
(+ Naloxone)
DOPAMINERGIC
(+ Naloxone)

response of dopaminergic cells to 1 umol CPAB i.c.v. was reversed by the presence of naloxone 1 mg/kg i.v.(Table 29 : Figure 14). These cells responded with a mean decrease in the magnitude of the evoked response of -37.1% +/- 6.4 (n=4) (paired t-test, $p<0.05$) and a range of -54.6% to -24.7%. This is in contrast to the increased evoked response produced by 1 umol CPAB i.c.v. alone (275.6% vs -37.1%; Newman-Keuls; $p<0.01$). Unlike other experiments in this group there was a significant difference between the baseline (post-naloxone, pre-CPAB) evoked response; dopaminergic (54.8 +/- 14.6 counts (n=4)), non-dopaminergic cells (235.0 +/- 59.5 counts (n=4); t-test, $p<0.05$). The range of pre-CPAB, post-naloxone responses for dopaminergic and non-dopaminergic cells is similar to the baseline responses in those experiments in which CPAB was given alone. Thus, the results cannot be accounted for by sample bias.

B.6. Application of 0.002 nmol Naloxone into the Substantia Nigra or Striatum

B.6.a. Spontaneous Activity

Application of 1 uL of a 2 micromolar solution (0.002 nmol) of naloxone into the substantia nigra or the striatum did not alter the spontaneous firing rates of either dopaminergic or non-dopaminergic cells. The mean change in the spontaneous firing frequency of non-dopaminergic cells

following the application of 0.002 nmol naloxone into the substantia nigra was 1.1% +/- 3.4 (n=3) with a response range of -5.1% to 6.8% (Table 30) and a mean percent change of (15.3% +/- 8.9 (n=3)) with a response range of 5.0% to 33.0% for the dopaminergic cells (Table 31). Application of naloxone into the striatum produced a mean change in the firing frequency of non-dopaminergic cells of (1.4% +/- 3.0 (n=4)) with a response range of -3.1% to 10.2% (Table 32) and a mean change of (5.0% +/- 5.6 (n=3)) for the dopaminergic cells with a range of responses from -3.9% to 15.3% (Table 33). These data were not different from those obtained following infusion of vehicle into the substantia nigra or striatum as previously described (Newman-Keuls multiple range test).

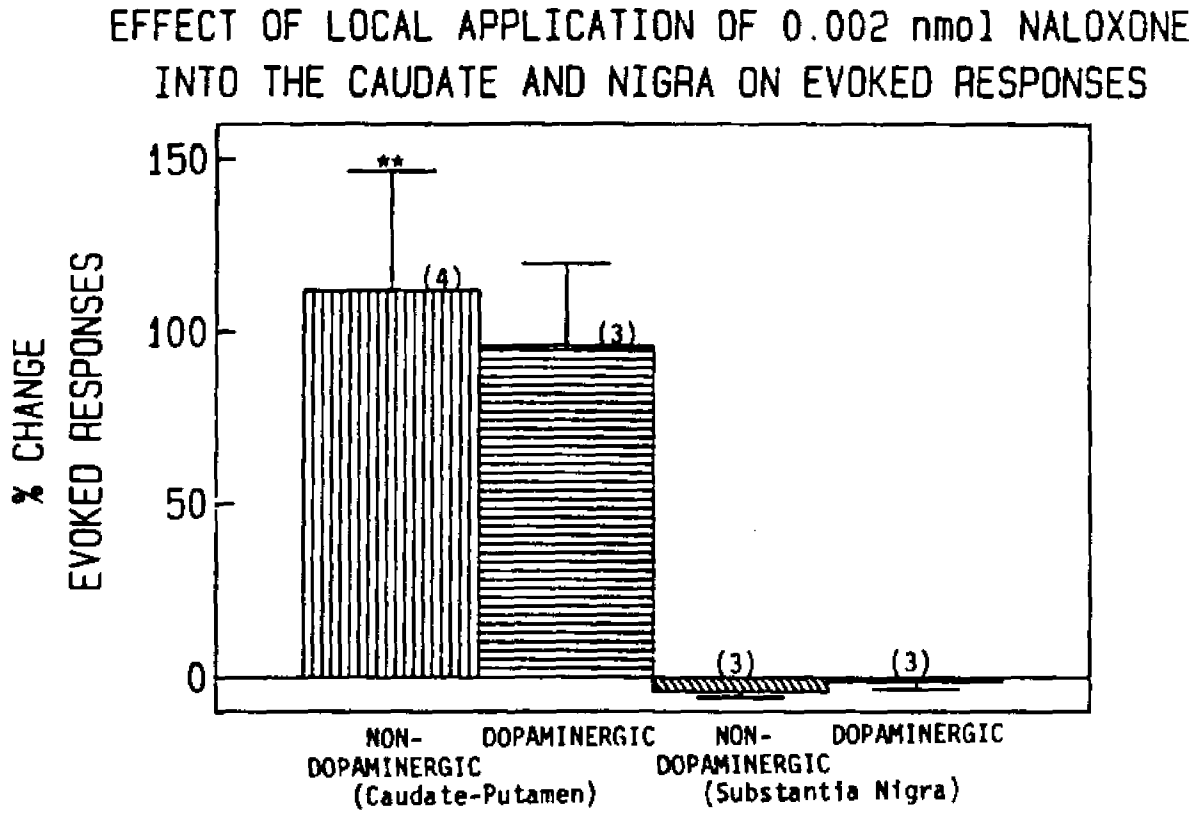
B.6.b. Evoked Responses

Infusion of 0.002 umol naloxone into the striatum significantly increased the magnitude of the evoked responses in non-dopaminergic cells in the substantia nigra (Fig 15 : Table 32). There was no significant effect of the direct application of naloxone into the striatum on the evoked responses of dopaminergic cells nor was application of naloxone into the substantia nigra effective in altering the evoked responses of dopaminergic or non-dopaminergic cells.

The non-dopaminergic cells responded to local application of 0.002 nmol naloxone into the striatum with a

Figure 15 Effect of infusion of 0.002 nmol naloxone into the striatum or substantia nigra on the magnitude of the evoked responses of cells in the substantia nigra. The graph shows the mean +/- SEM of the change in the firing frequency from pre- to post-drug for dopaminergic and non-dopaminergic cells. The number of experiments represented by each bar is shown in parenthesis. In each experiment evoked responses to 500 stimuli were summed both before and after administration of drug or vehicle. Stimuli were 100-150 uA pulses, 1 millisecond in duration. ** $p<0.05$, paired t-test

FIGURE 15


EFFECT OF LOCAL APPLICATION OF 0.002 nmol NALOXONE
INTO THE CAUDATE AND NIGRA ON EVOKED RESPONSES
% CHANGE
EVOKED RESPONSES
150
100
50
0
**
(4)
(3)
(3)
(3)
NON-
DOPAMINERGIC
(Caudate-Putamen)
DOPAMINERGIC
NON-
DOPAMINERGIC
(Substantia Nigra)
DOPAMINERGIC

mean increase in the evoked responses of 111.9% +/- 34.6 (n=4) (paired t-test, $p<0.05$) and a response range of 28.2% to 180.2%. There is a significant difference between the mean change in the magnitude of the evoked responses, induced by application of naloxone into the striatum, observed in the non-dopaminergic cells (which yielded the statistically significant increase of 111.9%; paired t-test) and the response of non-dopaminergic cells to vehicle infusion into the striatum (Newman-Keuls multiple range test; $p<0.01$). The evoked responses of the dopaminergic cells are noteworthy as there was a large increase in the magnitude of the responses following application of naloxone into the caudate which was not significant by a paired t-test (95.7% +/- 23.8 (n=3)) with a range of 55.3% to 137.5% (Fig 15 : Table 33). The non-significance of the result is supported by the finding that there is no significant difference between the mean change of the evoked response of these dopaminergic cells, following application of 0.002 nmol naloxone, and the mean change in the magnitude of the evoked response of dopaminergic cells following infusion of vehicle into the striatum (Newman-Keuls). Also, there is a significant difference between the mean change in the magnitude of the evoked response, following application of naloxone into the striatum, observed in the non-dopaminergic cells (yielding a statistically significant increase of 111.9%; paired t-test) and the response of non-dopaminergic cells to

infusion of vehicle into the striatum (Newman-Keuls; $p<0.01$).

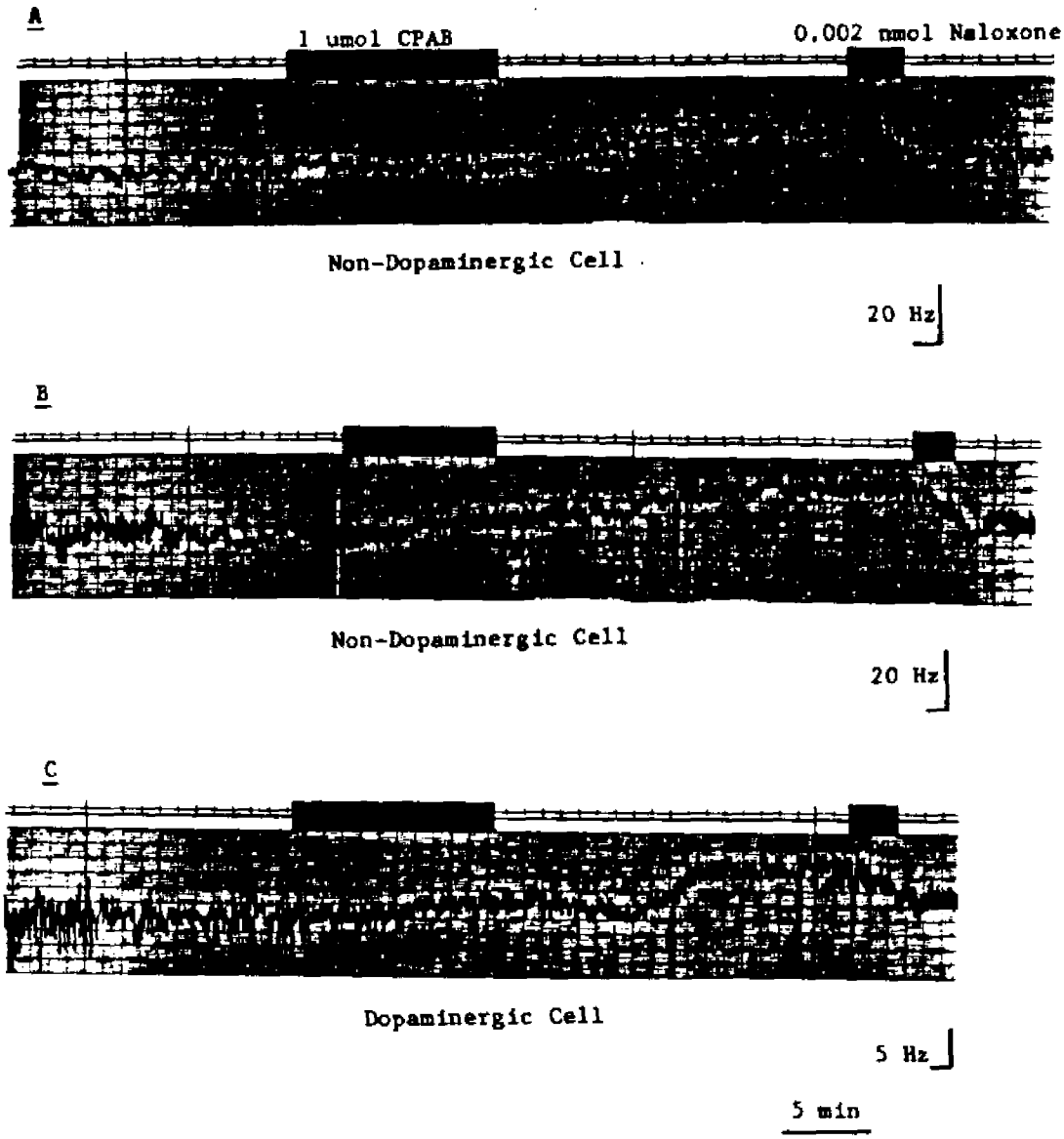
There was no significant difference in the magnitude of the baseline (pre-naloxone) evoked responses between the dopaminergic (125.8 +/- 32.5 counts (n=3)) and non-dopaminergic cells (150.3 +/- 51.9 counts (n=4)). There was no relation between the initial condition (pre-naloxone evoked response) and the change in the evoked responses of dopaminergic or non-dopaminergic cell for those experiments in which 0.002 nmol naloxone was administered directly into the striatum.

B.7. Application of 0.002 nmol Naloxone into the Striatum or Substantia Nigra and 1 umol CPAB i.c.v.

The procedure for these experiments varied from the previously described experiments and the experimental procedure, outlined in the methods, in the following way. Normally, there were two periods of electrical stimulation of the striatum in which evoked data was collected and compared. There was a period of stimulation preceding administration of the inhibitors of 24.11 with a second period of stimulation following the development of the drug effect; typically 20 minutes after the end of drug administration. For the experiments described below there was no period of stimulation directly following the application of 1 umol CPAB i.c.v. Instead the second period of stimulation followed the local administration of

Figure 16 Time course of the change in spontaneous firing frequency of dopaminergic and non-dopaminergic cells in response to application of 0.002 nmol naloxone into the substantia nigra in the presence of 1 umol CPAB i.c.v. A. Response of non-dopaminergic cell appearing in table 34 experiment number 3. B. Response of non-dopaminergic cell appearing in table 34 experiment number 1. C. Response of dopaminergic cell appearing in table 35 experiment number 1.

FIGURE 16


A
1 umol CPAB
0.002 nmol Naloxone
Non-Dopaminergic Cell
20 Hz
B
Non-Dopaminergic Cell
20 Hz
C
Dopaminergic Cell
5 Hz
5 min

naloxone. Naloxone was applied directly into the substantia nigra or the striatum after the CPAB i.c.v. drug effect had stabilized: this usually occurred within 15 minutes after the end of the infusion of the inhibitor and the naloxone was applied 20 to 25 minutes after the end of the inhibitor infusion. The duration of application of 1 uL of a 2 micromolar solution of naloxone (0.002 nmol) was 2 to 3 minutes and the onset of the drug effect occurred within 1 minute after the onset of the perfusion reaching a plateau effect within 3 minutes (Figure 16).

B.8. Application of 0.002 nmol Naloxone into the Substantia Nigra in the Presence of 1 umol CPAB i.c.v.

B.8.a Spontaneous Activity

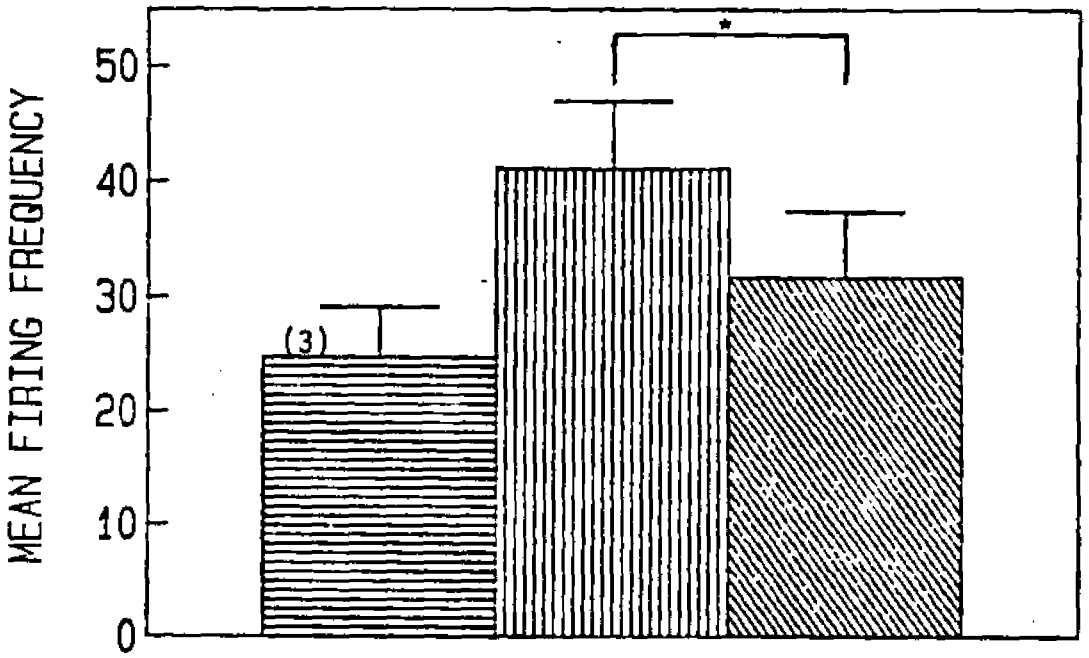
Figures 17 and 18 show the effect on the spontaneous activity of both non-dopaminergic and dopaminergic cells in the substantia nigra following application of 0.002 nmol naloxone directly into the substantia nigra in the presence of 1 umol CPAB i.c.v. The mean change in the spontaneous firing frequency of non-dopaminergic cells following the application of 1 umol CPAB i.c.v., the initial increase in firing frequency, was 68.8% +/- 1.5 (n=3) (paired t-test, $p<0.01$). Application of 0.002 nmol naloxone into the substantia nigra caused a significant drop in the CPAB induced increase in non-dopaminergic firing. The mean

Figure 17 Effect of application of 0.002 nmol naloxone directly into the substantia nigra in the presence of 1 umol CPAB i.c.v. on the spontaneous firing frequency of non-dopaminergic cells in the substantia nigra The graph shows the mean +/- SEM of the change in the firing frequency. The number of experiments represented by each bar is shown in parenthesis. * There was a significant reduction in the firing frequency post-CPAB to post-naloxone $p<0.05$, paired t-test

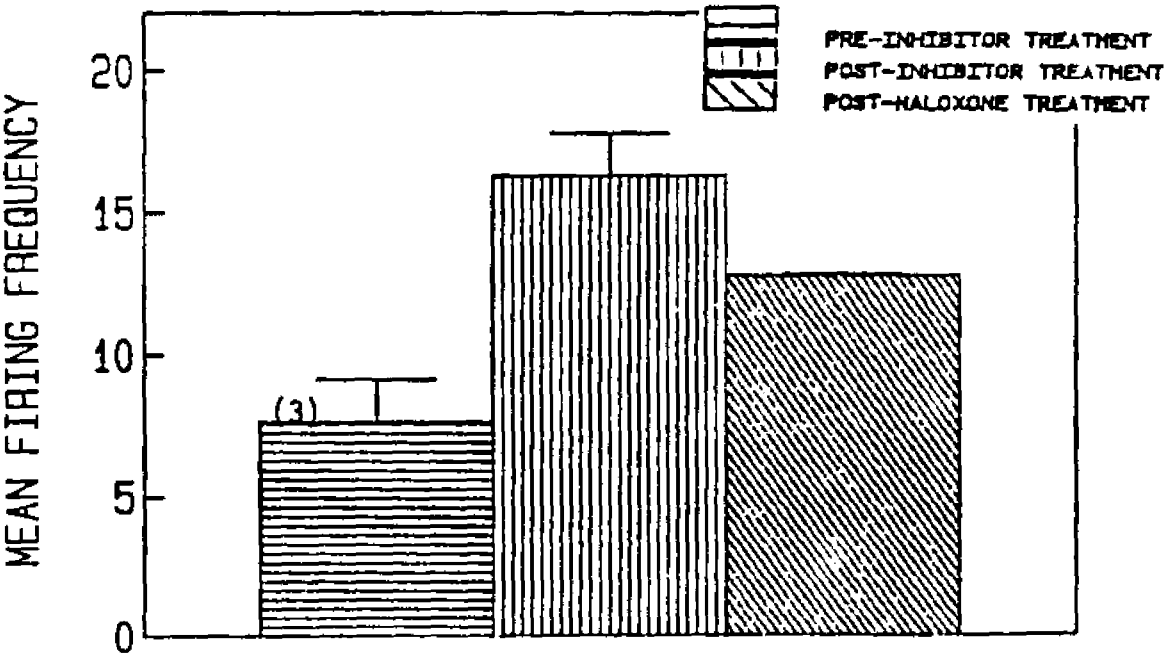
Figure 18 Effect of application of 0.002 nmol naloxone directly into the substantia nigra in the presence of 1 umol CPAB i.c.v. on the spontaneous firing frequency of dopaminergic cells in the substantia nigra The graph shows the mean +/- SEM of the change in the firing frequency. The number of experiments represented by each bar is shown in parenthesis.

FIGURES 17 and 18

EFFECT ON NON-DOPAMINERGIC FIRING OF 0.002 nmol NALOXONE INTO THE NIGRA IN PRESENCE OF CPAB i.c.v.



EFFECT ON DOPAMINERGIC CELL FIRING OF 0.002 nmol NALOXONE INTO THE NIGRA IN PRESENCE OF CPAB i.c.v.



decrease observed was -23.7% +/- 2.9 (n=3) (paired t-test, $p<0.01$) with a range of - 27.5% to -18.0% (Table 34 : Figure 17). But the increase in firing from pre-treatment to post-CPAB-naloxone of 28.5% was significant (paired t-test; $p<0.02$). Further there was a significant difference between the percent increase in firing frequency following local naloxone and CPAB i.c.v. and CPAB alone (pooled values for all CPAB i.c.v. experiments; 60.0% (n=12) vs 28.5%; $p<0.05$; Mann-Whitney). The mean increase in the dopaminergic firing frequency following the application of 1 umol CPAB i.c.v., prior to the application of naloxone was 120.5% +/- 24.8 (n=3) (paired t-test, $p<0.01$). Naloxone also caused a drop in the dopaminergic firing frequency of similar magnitude to the non-dopaminergic cells, with each of the 3 experiments showing decreased firing frequency, but an insufficient number of experiments were performed to yield a statistically significant result by the paired t-test. The mean decrease observed in the dopaminergic cells was -20.3% +/- 3.7 (n=3)) with a range of - 27.5% to -15.5% (Table 35 : Figure 18). The increase in firing from pre-treatment to post-CPAB-naloxone of 76.7% was significant (paired t-test; $p<0.01$). Further there was no significant difference between the percent increase in firing frequency following local naloxone and CPAB i.c.v. and CPAB alone (pooled values for all CPAB i.c.v. experiments; 96.6% (n=12) vs 76.7%; Mann-Whitney).

The baseline firing frequency (pre-CPAB) for the dopaminergic cells (7.7 +/- 1.48 Hz (n=3)) is significantly lower than that of the non-dopaminergic cells (24.7 +/- 4.4 Hz (n=3); t-test, p<0.02). The initial firing rate values, for non-dopaminergic and dopaminergic cells, are not statistically different from the baseline spontaneous firing frequency values obtained prior to application of application of 1 umol CPAB i.c.v, 1 umol CPPAB i.c.v., vehicle administered i.c.v, 0.04 umol CPAB or vehicle administered locally into the substantia nigra or caudate (Newman-Keuls multiple range test). There is no statistical difference, in the mean percent decrease in spontaneous firing following application of 0.002 nmol naloxone into the substantia nigra in the presence of 1 umol CPAB, between dopaminergic and non-dopaminergic cells. There is a statistical difference in the absolute change in firing frequency between non-dopaminergic and dopaminergic cells in this same interval (9.5 +/- 0.8 Hz vs 3.4 +/- 0.9 Hz; t-test, p<0.01).

B.8.b. Evoked Responses

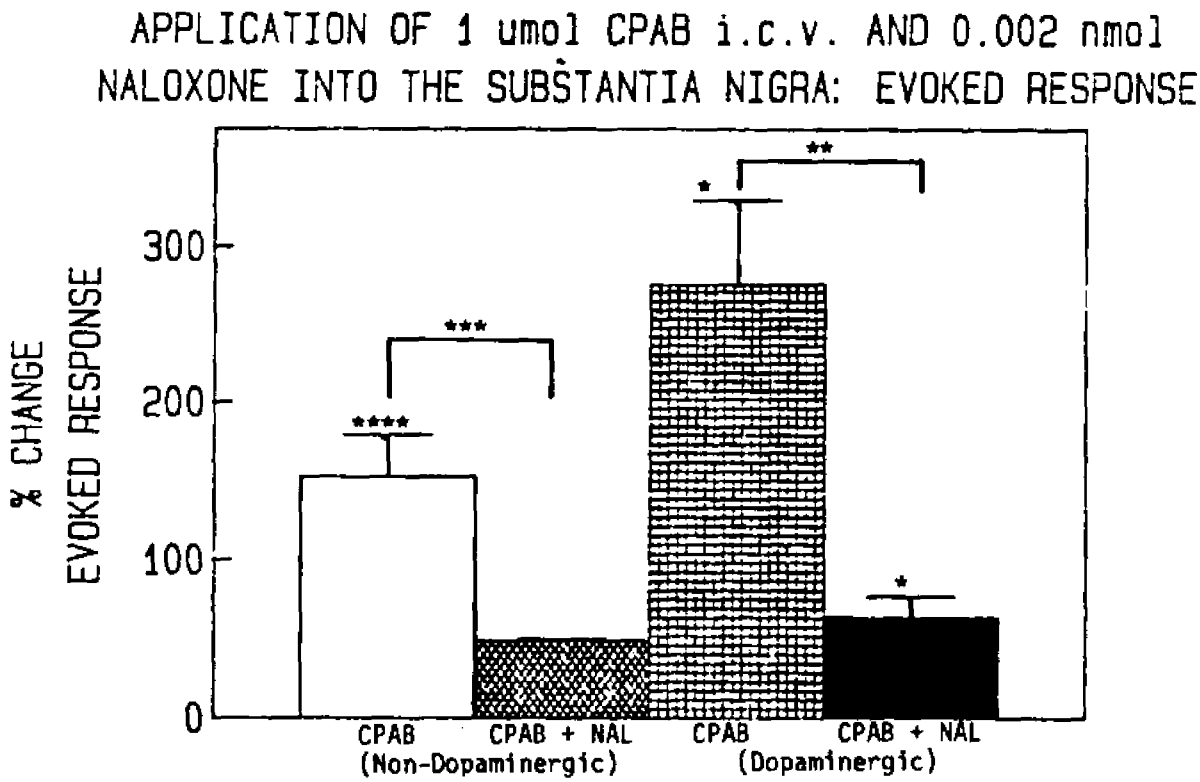
Infusion of 0.002 nmol naloxone into the substantia nigra appeared to antagonize the CPAB induced increases in the magnitude of the evoked responses, of both non-dopaminergic and dopaminergic cells, when compared to the increases in the evoked responses seen in earlier

experiments in which 1 umol CPAB i.c.v was applied in the absence of naloxone.

The dopaminergic cells responded to local application of 0.002 nmol naloxone into the substantia nigra and 1 umol CPAB i.c.v. with mean increase in the evoked response of 64.0% +/- 13.2 (n=3) (paired t-test, $p<0.05$) and a response range of 40.9.8% to 86.6% (Table 41 : Figure 22). This is a significant decrease, in the magnitude of the evoked response, from the mean increase seen in the dopaminergic cells following the application of 1 umol CPAB i.c.v. alone (275.6% vs 64.0%; Newman-Keuls; $p < 0.05$). The non-dopaminergic cells responded to the application of 0.002 nmol naloxone and 1 umol CPAB i.c.v. with a non-significant mean change in the magnitude of the evoked response of 49.0% +/- 1.3 (n=3) with a response range of 46.5% to 51.1% (Table 40 : Figure 22). And, the response of the non-dopaminergic cells following the application of 0.002 nmol into the substantia nigra and 1 umol CPAB i.c.v. is significantly different from the mean increase in the magnitude of the non-dopaminergic evoked response following the application of 1 umol CPAB i.c.v. alone (152.3% vs 49.0%; Newman-Keuls; $p<0.02$). It therefore appears that 0.002 nmol naloxone, when applied directly into the substantia nigra, is able to significantly antagonize the action of 1 umol CPAB i.c.v. on the evoked responses of dopaminergic and non-dopaminergic cells.

Figure 22 Effect of the direct application of 0.002 nmol naloxone into the substantia nigra and 1 umol CPAB i.c.v. on the magnitude of the evoked responses in the substantia nigra. The graph shows the mean +/- SEM of the change in the magnitude of the evoked response from the pre- to post treatment for dopaminergic and non-dopaminergic cells. Experiments shown also contrast the effects of application of 1 umol CPAB i.c.v alone (taken from earlier experiments, figure 4) and experiments in which 0.002 nmol naloxone was applied into the substantia nigra with CPAB i.c.v. The number of individual experiments represented by each bar is shown in parenthesis. In each experiment evoked responses to 500 stimuli were summed both before and after administration of drug or vehicle. Stimuli were 100-150 uA pulses, 1 millisecond in duration. * $p<0.05$, paired t-test. ** $p<0.05$, Newman-Keuls. *** $p<0.02$, Newman-Keuls. **** $p<0.01$, paired t-test.

FIGURE 22


APPLICATION OF 1 umol CPAB i.c.v. AND 0.002 nmol NALOXONE INTO THE SUBSTANTIA NIGRA: EVOKED RESPONSE
% CHANGE EVOKED RESPONSE
300
200
100
0
****
***
*
**
*
CPAB
CPAB + NAL
CPAB
CPAB + NAL
(Non-Dopaminergic)
(Dopaminergic)

There was no significant difference between the magnitude of the baseline (pre-CPAB) evoked responses of dopaminergic (140 +/- 41.3 counts (n=3)) and non-dopaminergic cells (162 +/- 50.1 counts (n=3)).

B.9. Application of 0.002 nmol Naloxone into the Striatum in the Presence of 1 umol CPAB

B.9.a. Spontaneous Activity

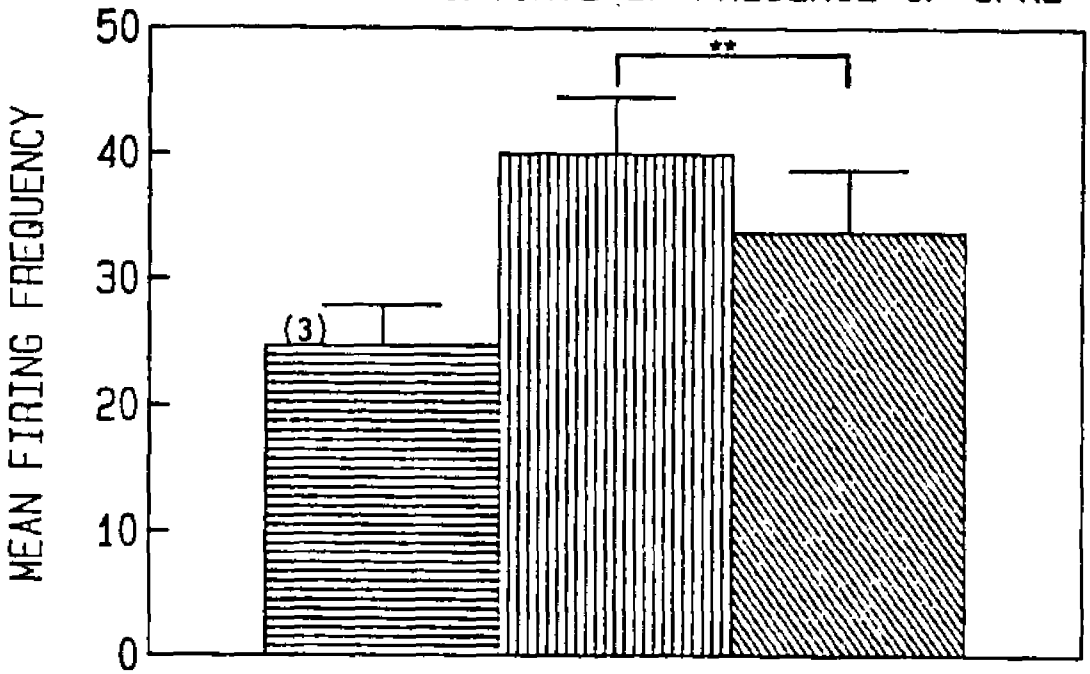
Figures 19 and 20 show the effect on the spontaneous activity of both non-dopaminergic and dopaminergic cells in the substantia nigra following application of 0.002 nmol naloxone directly into the striatum in the presence of 1 umol CPAB i.c.v. The mean increase in non-dopaminergic cell firing following the application of 1 umol CPAB i.c.v., the initial increase in firing frequency, was 63.1% +/- 3.2 (n=3) (paired t-test, $p<0.01$). The application of 0.002 nmol naloxone into the striatum caused a significant drop in the firing increase induced CPAB in the non-dopaminergic cells. The mean decrease in the firing frequency observed was -16.5% +/- 3.5 (n=3) (paired t-test, $p<0.05$) with a range of - 20.5% to - 9.9% (Table 36 ; Figure 19). The increase in firing from pre-treatment to post-CPAB-naloxone of 36.1% was significant (paired t-test: $p<0.05$). Further there was a significant difference between the percent increase in firing frequency following local naloxone and CPAB i.c.v. and CPAB alone (pooled

Figure 19 Effect of application of 0.002 nmol naloxone directly into the striatum in the presence of 1 umol CPAB i.c.v. on the spontaneous firing frequency of non-dopaminergic cells in the substantia nigra The graph shows the mean +/- SEM of the change in the firing frequency. The number of experiments represented by each bar is shown in parenthesis. * There was a significant reduction in the firing frequency post-CPAB to post-naloxone $p<0.05$, paired t-test.

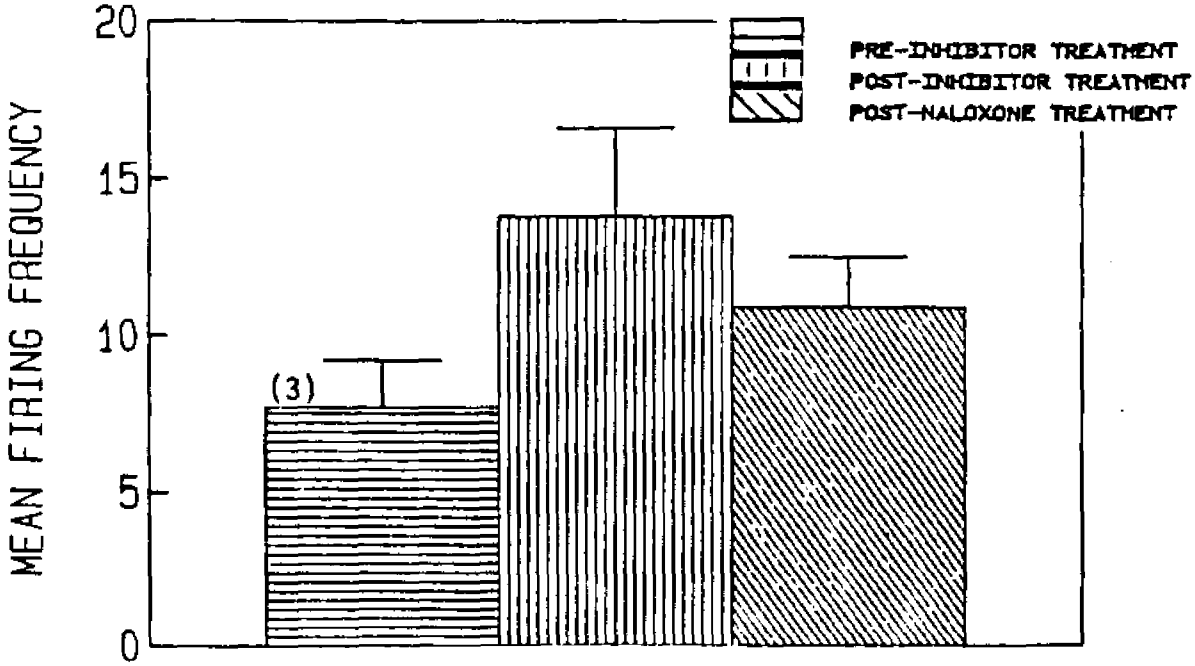
Figure 20 Effect of application of 0.002 nmol naloxone directly into the striatum in the presence of 1 umol CPAB i.c.v. on the spontaneous firing frequency of dopaminergic cells in the substantia nigra The graph shows the mean +/- SEM of the change in the firing frequency. The number of experiments represented by each bar is shown in parenthesis.

FIGURES 19 and 20

EFFECT ON NON-DOPAMINERGIC FIRING OF 0.002 nmol NALOXONE INTO THE CAUDATE IN PRESENCE OF CPAB i.c.v.



EFFECT ON DOPAMINERGIC FIRING OF 0.002 nmol NALOXONE INTO THE CAUDATE IN PRESENCE OF CPAB i.c.v.



values for all CPAB i.c.v. experiments; (60.0% (n=12) vs 36.1%; p<0.05; Mann-Whitney). The mean increase of the dopaminergic cells following the application of 1 umol CPAB i.c.v., prior to the application of naloxone, was 78.4% +/- 2.8 (n=3) (paired t-test, p<0.01). Naloxone also caused a drop in the dopaminergic firing frequency of similar magnitude to the non-dopaminergic cells, with each of the 3 experiments decreasing the firing frequency, but an insufficient number of experiments were performed to yield a statistically significant result by the t-test. The mean decrease observed in the dopaminergic firing frequency was -18.3% +/- 6.9 (n=3) with a range of - 25.3% to - 4.5% (Table 37 : Figure 20). The increase in firing from pre-treatment to post-CPAB-naloxone of 45.3% was significant (paired t-test; p<0.001). However, there was a significant difference between the percent increase in firing frequency following local naloxone and CPAB i.c.v. and CPAB alone (93.7% vs 45.3%; Newman-Keuls). Thus, although not revealed in the paired t-test, an effect of local naloxone on the CPAB induced increase in spontaneous firing was demonstrated.

There is no statistical difference, in the mean decrease in spontaneous firing following application of 0.002 nmol naloxone into the striatum in the presence of 1 umol CPAB, between dopaminergic and non-dopaminergic cells nor is the absolute change in firing frequency, in the same

interval, different (2.9 +/- 1.3 Hz vs 6.4 +/- 1.0 Hz; t-test, $p<0.01$).

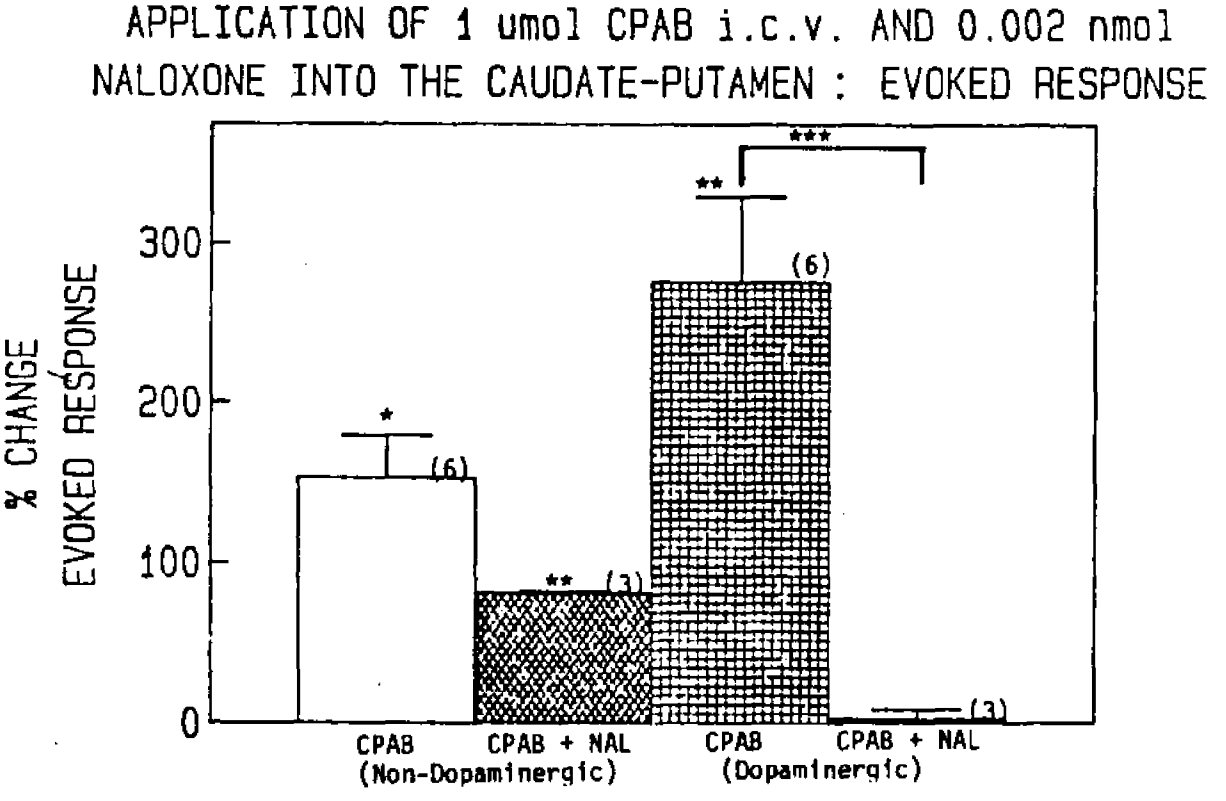
B.9.b. Evoked Responses

Infusion of 0.002 nmol naloxone into the striatum appeared to antagonize the effect of 1 umol CPAB i.c.v. on evoked responses in dopaminergic and non-dopaminergic cells. The mean change in the evoked response when 1 umol CPAB was given i.c.v. and naloxone infused into the striatum was only 3.2% +/- 5.7 (n=3)) with a response range of -7.4% to 12.2% (Table 39 : Figure 21). This decrease in the evoked response in the dopaminergic cells is significantly lower than the mean increase in the evoked response of dopaminergic cells following CPAB i.c.v. alone (275.6% vs 3.2; t-test; $p<0.02$).

There was no significant change in the CPAB i.c.v. induced increases in the evoked response following the infusion of naloxone into the striatum on non-dopaminergic cells. The non-dopaminergic cells responded to local application of 0.002 nmol naloxone into the striatum and 1umol CPAB i.c.v. with a mean increase in the magnitude of the evoked response of 81.4% +/- 1.2 (n=3) (paired t-test, $p<0.05$) and a response range of 29.8% to 140.4% (Table 38). However this is significantly different from the mean increase seen in the non-dopaminergic cells following the application of 1 umol CPAB i.c.v. alone (152.3% vs 81.4%; $p<0.05$; Newman-Keuls).

Figure 21 Effect of the direct application of 0.002 nmol naloxone into the striatum and 1 umol CPAB i.c.v. on the magnitude of the evoked responses in the substantia nigra. The graph shows the mean +/- SEM of the change in the magnitude of the evoked response from the pre- to post treatment for dopaminergic and non-dopaminergic cells. Experiments shown also contrast the effects of application of 1 umol CPAB i.c.v alone (taken from earlier experiments, figure 4) and experiments in which 0.002 nmol naloxone was applied into the striatum with CPAB i.c.v. The number of individual experiments represented by each bar is shown in parenthesis. In each experiment evoked responses to 500 stimuli were summed both before and after administration of drug or vehicle. Stimuli were 100-150 uA pulses, 1 millisecond in duration. *** $p<0.02$, Newman-Keuls. ** $p<0.05$, paired t-test. * $p<0.01$, paired t-test.

FIGURE 21


APPLICATION OF 1 umol CPAB i.c.v. AND 0.002 nmol NALOXONE INTO THE CAUDATE-PUTAMEN : EVOKED RESPONSE
% CHANGE EVOKED RESPONSE
300
200
100
0
*
**
***
(6)
(3)
(6)
(3)
CPAB
CPAB + NAL
CPAB
CPAB + NAL
(Non-Dopaminergic)
(Dopaminergic)

There was no significant difference between the baseline (pre-CPAB) evoked responses of dopaminergic (228.7 +/- 37.7 counts (n=3)) and non-dopaminergic cells (169.7 +/- 64 counts (n=3)). There was no apparent relation between the initial condition (pre-drug evoked response) and the change in the magnitude of the evoked response of the dopaminergic or non-dopaminergic cells for those experiments in which 1 umol CPAB was applied i.c.v. and 0.002 nmol naloxone was infused directly into the striatum.

B.10. Peristimulus Interval Histograms

Examples of peristimulus histograms, representative of several of the groups of the experiments discussed above, appear in figures 28 through 34. The figures are shown not because any particular detail is important, rather these histograms illustrate several important general points concerning the group or experiments as a whole. First, the stimulus intensity used in these experiments 50 to 150 uA at 1 to 2 Hz was sufficient to evoke simple monomodal responses with delay and duration commensurate with previously reported evoked responses (Collingridge and Davies, 1981; Dray et.al., 1976; Frigyesi and Pupura, 1967. Examples 28R, 30R, 31R, 32R, 33R and 34R all show peristimulus histograms of experiments in which the application of inhibitor was effective in altering the magnitude of the evoked response. Figrue 29R shows an example of an experiment in which vehicle was infused

Figure 23 Peristimulus interval histogram The figure shows the pre- to post-drug response of a non-dopaminergic cell (experiment number 1; table 3) in which 1 umol CPAB was applied i.c.v. 100 uA pulses 1 ms in duration were delivered at 2 Hz in 500 sweeps. Arrows mark the stimulus artifact. Amplitide is reported in events per bin with each bin 0.4 ms in duration.

Figure 23

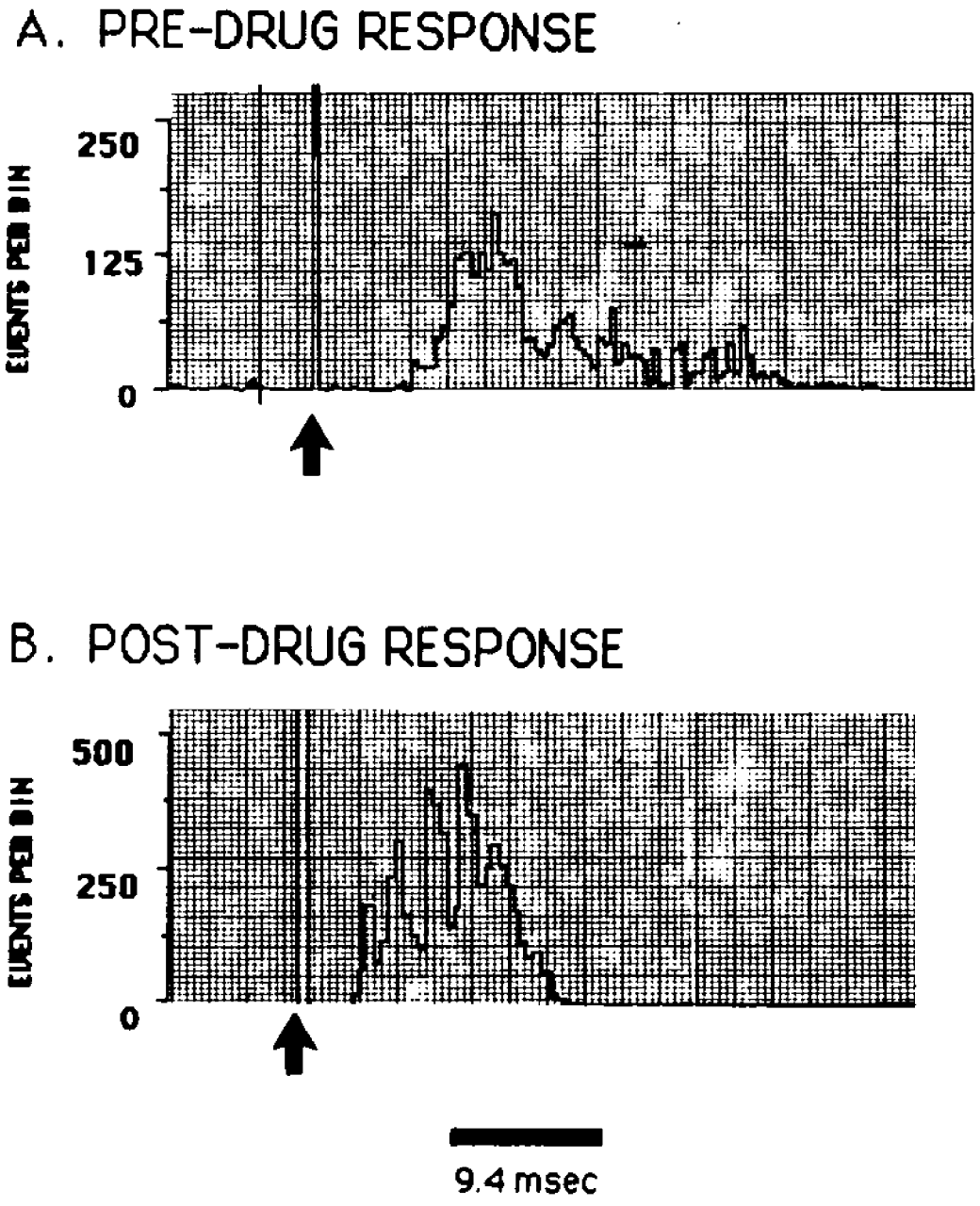

A. PRE-DRUG RESPONSE
EVENTS PER BIN
250
125
0
B. POST-DRUG RESPONSE
EVENTS PER BIN
500
250
0
9.4 msec

Figure 24 Peristimulus interval histogram The figure shows the pre- to post-vehicle response of a non-dopaminergic cell (experiment number 3; table 11) in which vehicle was applied i.c.v. 100 uA pulses 1 ms in duration were delivered at 2 Hz in 500 sweeps. Arrows mark the stimulus artifact. Amplitide is reported in events per bin with each bin 0.4 ms in duration.

Figure 24

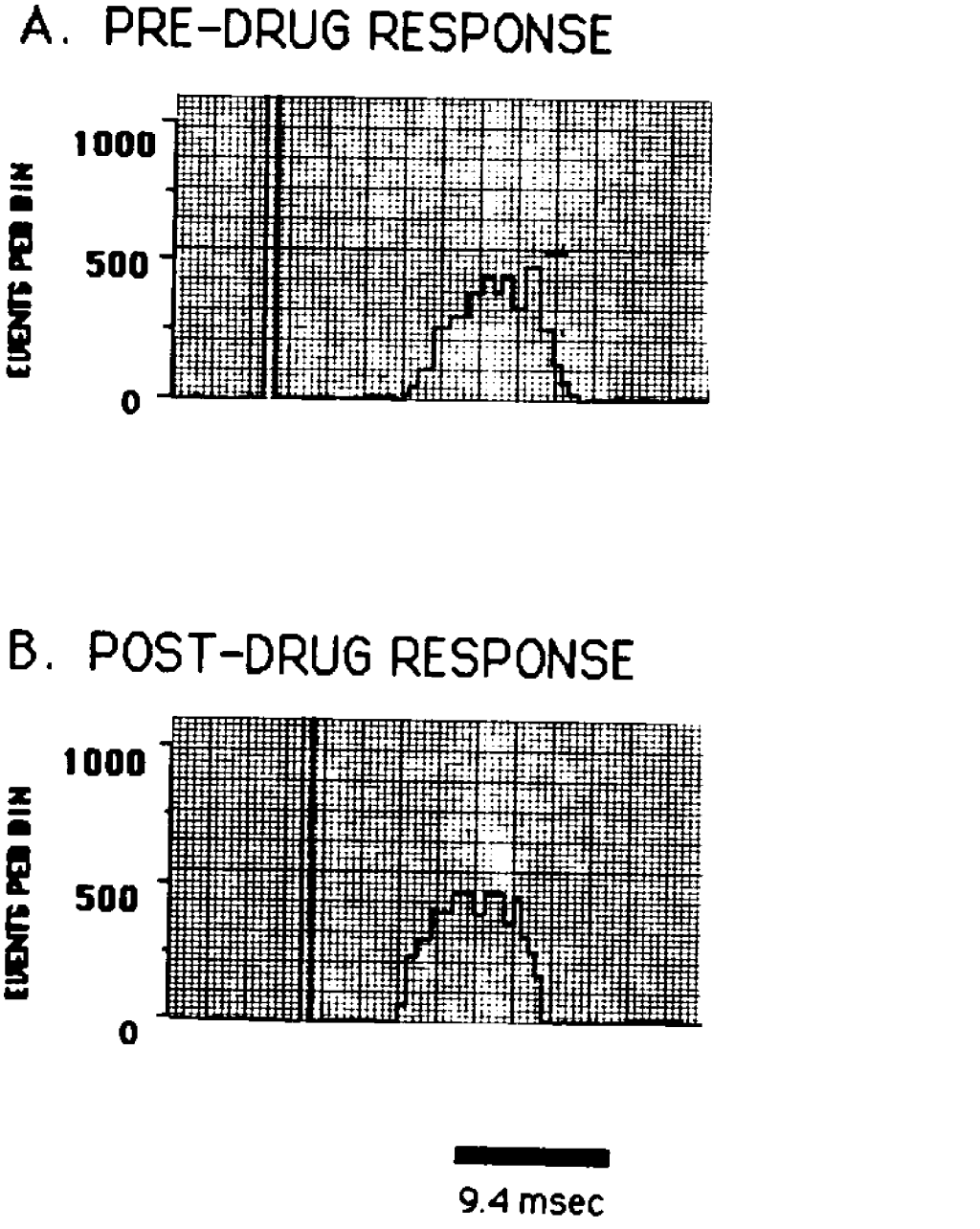

A. PRE-DRUG RESPONSE
EVENTS PER BIN
1000
500
0
B. POST-DRUG RESPONSE
EVENTS PER BIN
1000
500
0
9.4 msec

Figure 25 Peristimulus interval histogram The figure shows the pre- to post-drug response of a non- dopaminergic cell (experiment number 4; table 14) in which 0.04 umol CPAB was applied directly into the substantia nigra. 100 uA pulses 1 ms in duration were delivered at 2 Hz in 500 sweeps. Arrows mark the stimulus artifact. Amplitide is reported in events per bin with each bin 0.4 ms in duration.

Figure 25

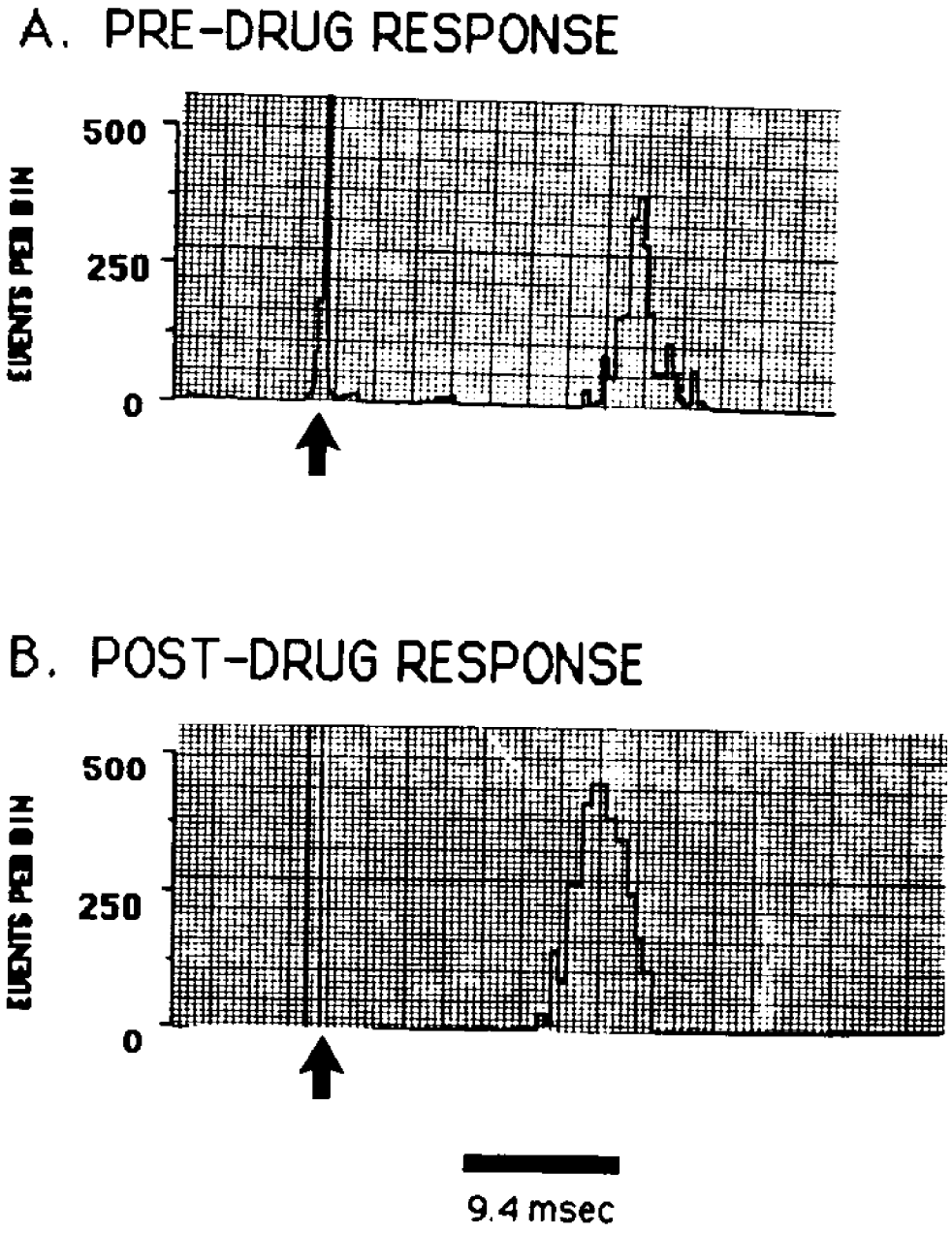

A. PRE-DRUG RESPONSE
500
250
0
B. POST-DRUG RESPONSE
500
250
0
9.4 msec

Figure 26 Peristimulus interval histogram The figure shows the pre- to post-drug response of a dopaminergic cell (experiment number 1; table 21) in which 0.04 umol CPAB was applied directly into the striatum. 100 uA pulses 1 ms in duration were delivered at 2 Hz in 500 sweeps. Arrows mark the stimulus artifact. Amplitide is reported in events per bin with each bin 0.4 ms in duration.

Figure 26

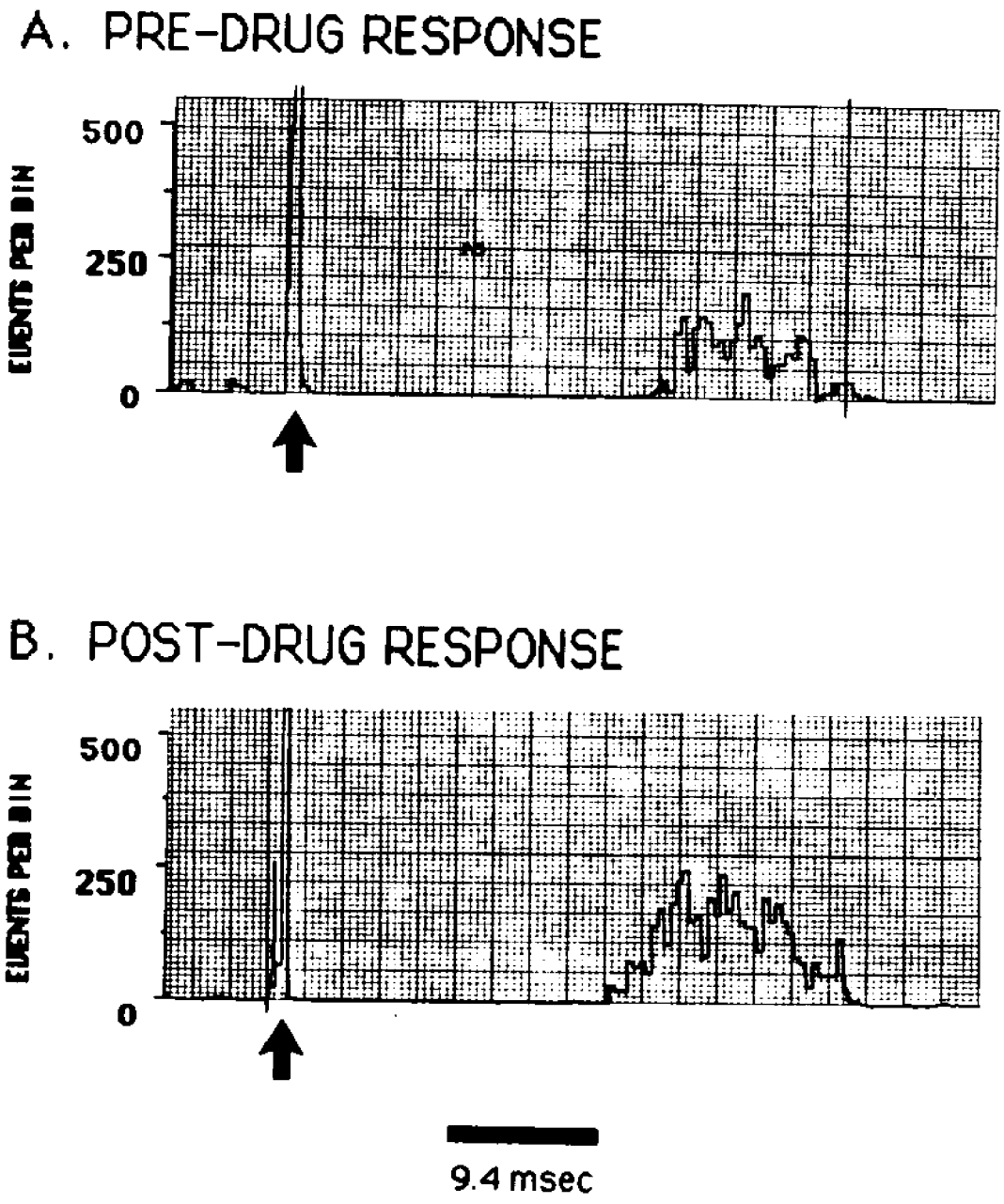

A. PRE-DRUG RESPONSE
500
250
0
EVENTS PER BIN
B. POST-DRUG RESPONSE
500
250
0
EVENTS PER BIN
9.4 msec

Figure 27 Peristimulus interval histogram The figure shows the pre- to post-drug response of a dopaminergic cell (experiment number 2; table 41) in which 0.002 nmol naloxone was applied directly into the substantia nigra in the presence of 1 umol CPAB i.c.v. 100 uA pulses 1 ms in duration were delivered at 2 Hz in 500 sweeps. Arrows mark the stimulus artifact. Amplitide is reported in events per bin with each bin 0.4 ms in duration.

Figure 27

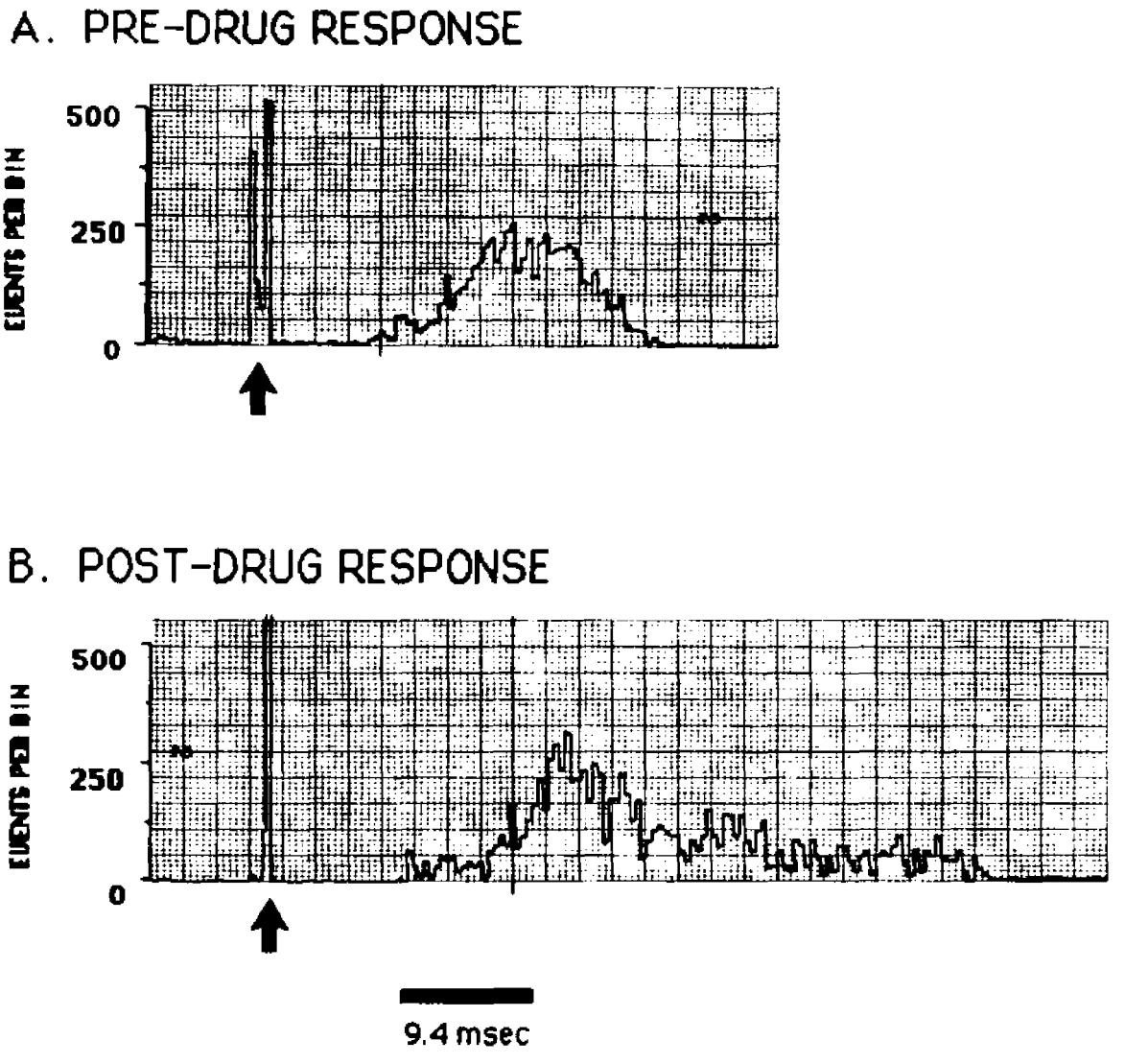

A. PRE-DRUG RESPONSE
500
250
0
EVENTS PER BIN
B. POST-DRUG RESPONSE
500
250
0
EVENTS PER BIN
9.4 msec

Figure 28 Peristimulus interval histogram. The figure shows the pre- to post-drug response of a non-dopaminergic cell (experiment number 3; table 38) in which 0.002 nmol naloxone was applied directly into the striatum in the presence of 1 umol CPAB i.c.v. 100 uA pulses 1 ms in duration were delivered at 2 Hz in 500 sweeps. Arrows mark the stimulus artifact. Amplitide is reported in events per bin with each bin 0.4 ms in duration.

Figure 28

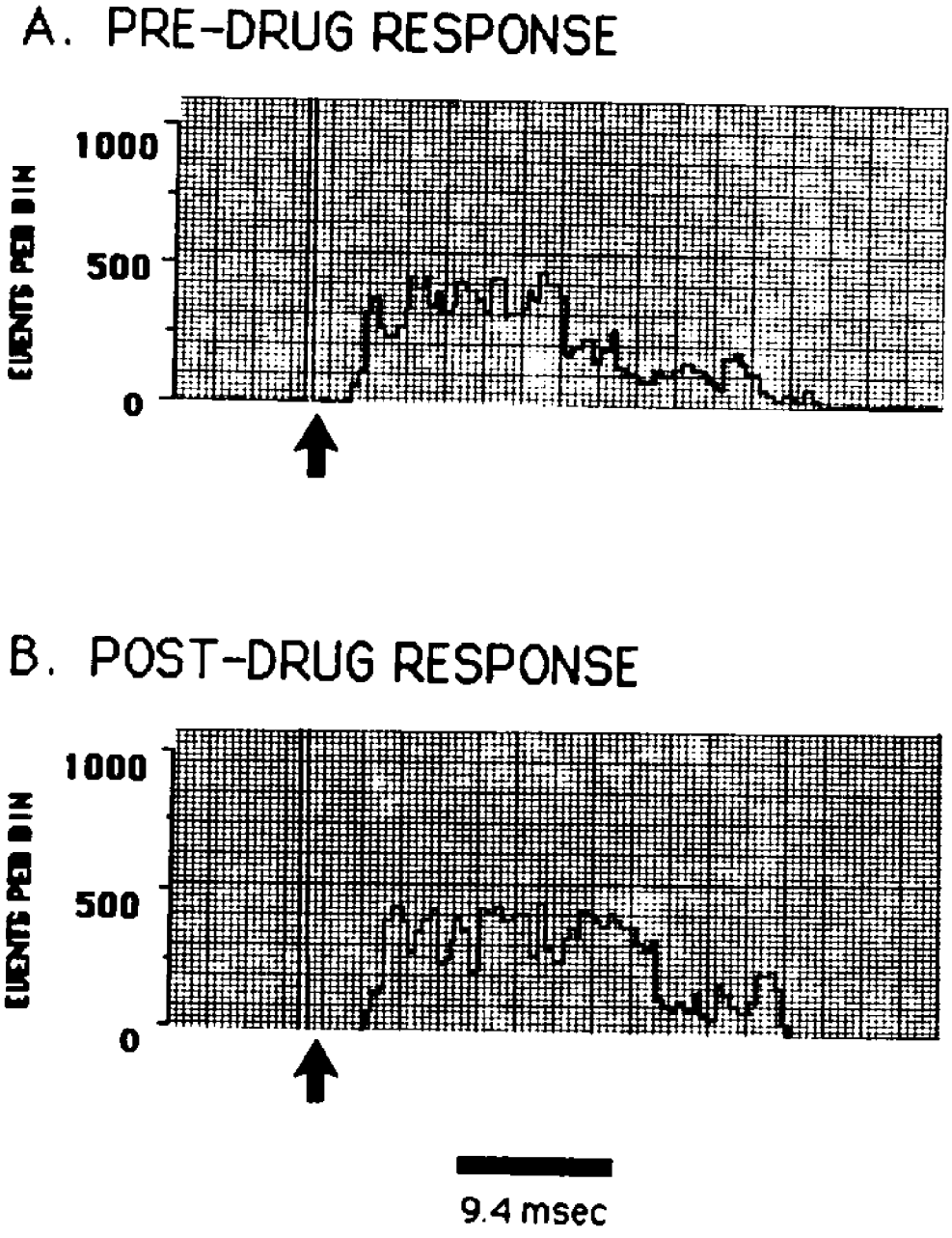
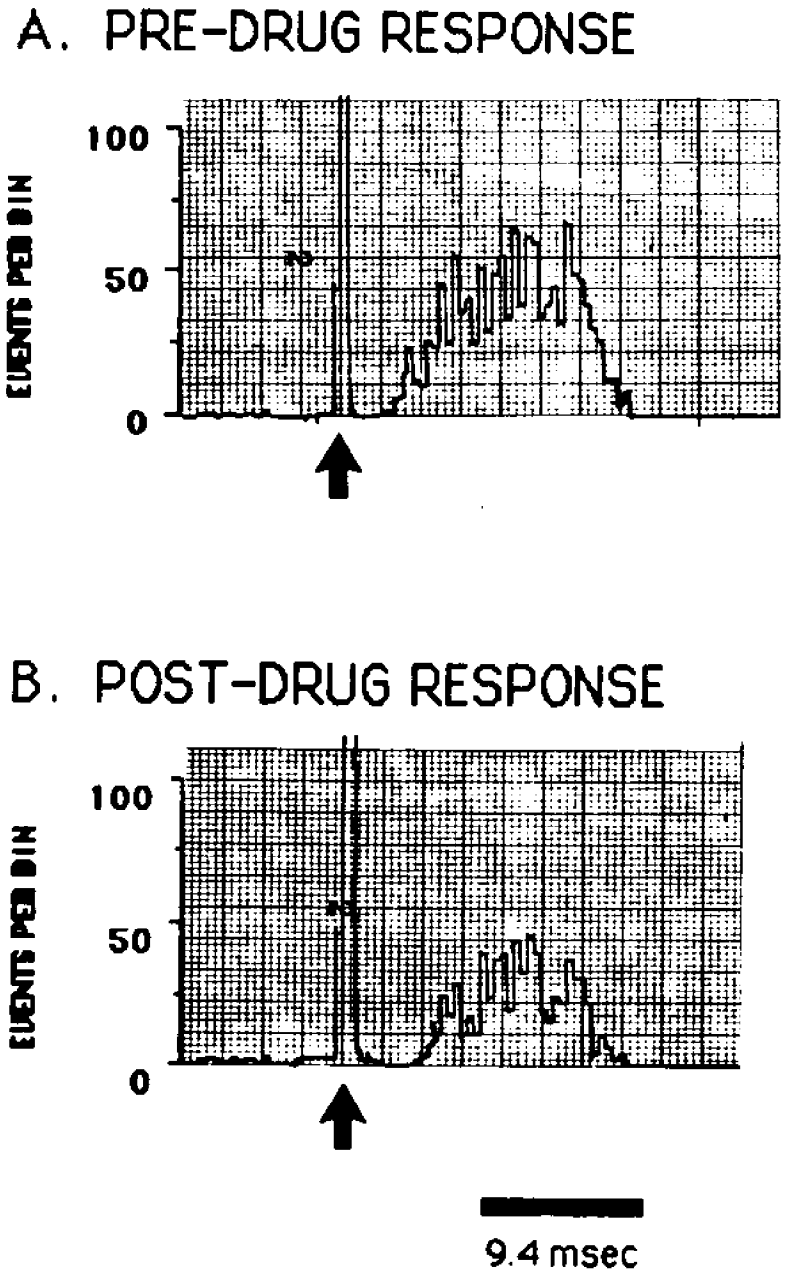

A. PRE-DRUG RESPONSE
1000
500
0
EVENTS PER BIN
B. POST-DRUG RESPONSE
1000
500
0
EVENTS PER BIN
9.4 msec

Figure 29 Peristimulus interval histogram The figure shows the pre- to post-drug response of a non-dopaminergic cell (experiment number 1; table 29) in which 1 umol CPAB was applied i.c.v. in the presence of naloxone 1 mg/kg, i.v. 100 uA pulses 1 ms in duration were delivered at 2 Hz in 500 sweeps. Arrows mark the stimulus artifact. Amplitide is reported in events per bin with each bin 0.4 ms in duration.

Figure 29


A. PRE-DRUG RESPONSE
100
50
0
EVENTS PER BIN
B. POST-DRUG RESPONSE
100
50
0
EVENTS PER BIN
9.4 msec

i.c.v. They are shown to illustrate the point that the inhibitors effected the evoked responses in a simple manner. Complex changes in the shape of the response histograms, such as monomodal to bimodal or reverberations were not introduced by the drugs. The drugs effected the magnitude of the response by increasing either the amplitude or duration of the response.

B.11. Latency and Duration of Evoked Responses

Table 42 shows the relation between the latency and duration of the evoked responses pre- to post-drug for several of the experiments in which application of CPAB caused a significant change in the evoked responses. There was no drug treatment which caused a significant or consistent change in the latency or duration of the responses. Only the magnitude of the evoked response, change in the absolute number of spikes, was significantly changed by the drugs.

Table 42

Relation between the latency and duration of the evoked responses pre- to post-drug.

Treatment	Delay		Duration	
	Pre-Drug	Post-Drug	Pre-Drug	Post-Drug
A)				
DA (6)	7.0 +/- 1.8	8.4 +/- 2.2	46.8 +/- 26.0	51.0 +/- 32.4
nDA (5)	5.4 +/- 1.7	5.5 +/- 2.0	23.8 +/- 10.2	24.8 +/- 22.2
B)				
DA (3)	6.6 +/- 2.3	4.4 +/- 0.9	38.3 +/- 8.1	38.7 +/- 11.5
nDA (6)	10.1 +/- 4.9	13.1 +/- 8.0	23.0 +/- 17.6	22.2 +/- 17.0
C)				
DA (4)	10.7 +/- 7.7	10.1 +/- 6.5	20.6 +/- 9.2	21.4 +/- 9.8
nDA (4)	6.0 +/- 2.5	6.0 +/- 2.3	25.7 +/- 18.3	25.9 +/- 16.9
D)				
DA (3)	6.7 +/- 1.3	7.8 +/- 2.0	19.6 +/- 9.4	23.5 +/- 16.1
nDA NA				
E)				
DA NA				
nDA (3)	4.0 +/- 1.4	4.1 +/- 1.2	28.1 +/- 9.9	23.0 +/- 3.4
F)				
DA (4)	4.0 +/- 1.5	4.4 +/- 1.7	23.4 +/- 11.3	22.4 +/- 12.6
nDA NA				

A) 1 umol CPAB i.c.v. B) 0.04 umol CPAB into the substantia nigra. C) 0.04 umol CPAB into the striatum. D) 0.002 nmol naloxone into the substantia nigra and CPAB i.c.v. E) 0.002 nmol naloxone into the striatum and CPAB i.c.v. F) Naloxone 1 mg/kg, i.v. and CPAB i.c.v. All values are in milliseconds. Number of experiments shown in parenthesis. NA data not available.

DISCUSSION

The results reported here provide evidence for a functional role of the metalloendopeptidase-24.11, commonly called 'enkephalinase' in the regulation of the electrical activity of cells in the substantia nigra of the rat. There is a specific set of cells in the substantia nigra which may be identified by their excitatory responses to striatal stimulation which are responsive to the inhibition of 24.11 (Table 1).

The effect of the inhibitors of endopeptidase-24.11 on the firing frequency of these select cells in the substantia nigra is due to the specific inhibition of endopeptidase-24.11 and not to a non-specific effect for the following reasons. First, 2 umole of CPLAB (an inhibitor structurally similar to CPAB yet two orders of magnitude less potent) had no effect on the firing frequency of cells exhibiting excitatory responses which were expected, based on their classification, to be responsive to the same dose of CPAB (Tables 1, 9 and 10 : Figures 2 and 3). Second, i.c.v. infusion of CPAB (at a dose which is sufficient to significantly increase the firing frequency of responsive cells in vivo) was shown, using an in vitro assay, to inhibit the activity of endopeptidase-24.11 (Tables 2 and 13). Third, the inhibitors used in this study (CPAB and CPPAB) are the most

specific inhibitors yet synthesized against this enzyme. Unlike the other commonly used inhibitors of endopeptidase-24.11, thiorphan and phosphoramidon, the inhibitors used in this study selectively inhibit only endopeptidase-24.11 with no effect on peptidyldipeptidase activity (Fulcher et al., 1982; Matsas et al., 1984). Thiorphan, which has been the most widely used inhibitor of endopeptidase-24.11, is non-selective as its inhibitory potency against angiotensin-converting enzyme (ACE; peptidyldipeptide hydrolase, EC 3.4.15.1) is within two log units of endopeptidase-24.11 (Roques et al., 1983). Angiotensin-converting enzyme (ACE) is present in high concentration in the substantia nigra and is able to hydrolyse substance P and enkephalin. Inhibition of ACE would therefore be expected to affect both peptide metabolism and electrical activity in the nigra. The inhibitors used in this study are also more suitable for in vivo use as they are more biologically stable; phosphoramidon is susceptible to enzymatic degradation while thiorphan is susceptible to oxidation at its sulfhydryl group (Fulcher et al., 1982; Matsas et al., 1984). Thus, inhibitors used in this study avoid effects secondary to degradative products of the inhibitors and spurious enzyme inhibition. Finally, the delayed onset of action of the inhibitors is consistent with the hypothesis that the compounds are exerting their effects through

enzyme inhibition rather than acting as agonists or antagonists (Figure 2).

Initial studies were performed using a relatively large bipolar stimulus. This fixed voltage, high frequency, stimulus was used for the following reasons. First, the release of peptides, in vivo, had been reported to occur under different conditions than those of the classical transmitters (Lundberg et al., 1982a). Peptides, according to the model established following studies of the action of VIP in the periphery, may require a higher frequency of stimulation for release and are released under much more restricted circumstances compared to the non-peptide transmitters (Lundberg et al., 1982b). Secondly, using a single electrode, opposed to the five electrode monopolar electrodes used in later experiments, restricted the area of striatal stimulation. One simple way of increasing the area of the striatum activated, to insure activation of striatal efferents to the cell recorded in the substantia nigra, is increasing the amount of current delivered. Although the stimulus amplitude was high the response patterns observed in the nigra were not different from those reported in the literature and our later results (using much less current) did not contradict the early results. Further, currents delivered, measured post-hoc, 500-1200 uA although large, were not out of range of some studies (Collingridge and Davies 1981).

In the first group of experiments (Table 1) the subset of cells responding to the i.c.v. application of inhibitors of 24.11 was identified. In the experiments which followed, these cells were characterized electrophysiologically (short latency evoked responses) and pharmacologically (response following inhibitor and naloxone treatment). Studies on the possible site of action of the observed effects were also performed. The initial experiments showed significant increases in the spontaneous firing frequency of dopaminergic and non-dopaminergic cells following application of 1 umol CPAB, 1 umol CPPAB or 2 umol CPPAB. The initial hypothesis for the effect was that inhibition of 24.11 resulted in increased tachykinin activity in the substantia nigra. In the experiments which followed, a smaller amplitude lower frequency monopolar stimulus was used in order to characterize the short latency evoked responses. The results of these studies confirmed the earlier results on spontaneous activity.

A The Use of Naloxone

Naloxone was used in these studies in order to assess the effects of possible opioid peptides (enkephalin and/or dynorphin) in the striatum and substantia nigra following attenuated degradation of these peptides due to inhibition of 24.11. As naloxone is able to block multiple opiate receptors, especially at high doses; doses greater than

10^{-4} M induce non-specific effects (Sawynok et al., 1979). The dose administered i.v. and locally was determined to be most selective for enkephalin antagonism. The estimate of the correct dose was based on the following observations reported in the literature. Morphine (10^{-4} M) superfused into the rat striatum increased the release of ^{3}H-dopamine. This effect was antagonized by 10^{-4} M naloxone. Naloxone (1 mg/kg i.v.) was able to antagonize the effect of D-ala-met-enkephalin (20 ug, i.c.v.) on the tail withdrawl in the rat (Roques et al., 1980). Naloxone 1 mg/kg, i.p. was able to antagonize the effect of intrathecal administration of 1 ug of D-ala-met-enkephalin on hot plate and tail flick latency in the rat (Yaksh and Harty 1982). Misra et al. (1976) determined, using radioimmunoassay, that the whole brain concentration of ^{14}C-naloxone following administration of 1 mg/kg s.c. to be 0.5ng/mg. Therefore, 2 uL of a 10^{-4} M solution (0.002 nmol; 0.65 ng) was delivered locally. This volume was determined to cover an area of 1.0 to 2.0 mm^{3} with 1.3 mm^{3} sphere having an average concentration of 0.5 ng/mg assuming 1 mg tissue/mm^{3}.

Naloxone has been demonstrated to have pharmacological effects which may be unrelated to opiate receptor blockade (Sawynok et al., 1979). It has been suggested that naloxone may, at certain doses have agonist effects. For example, naloxone at doses between 10^{-4} and 10^{-8} M is able to block potassium evoked Met-enkephalin release in the rat

striatal slice in a manner similar to the blockade produced by 10^{-4} M morphine (Sawynok et al., 1980). Superfusion of 10^{-4} M naloxone into the striatum of the cat is able to produce changes in dopaminergic release which resemble opiate agonist effects (Chesselet et al., 1983a). However, these apparent agonist like effects may be due to antagonism of receptors other than mu which unmasks activity which is not ordinarily seen. Walker et al. (1987) studied the effects of morphine and the delta agonist U50-448 on the firing frequency of dopaminergic cells in the nigra of the rat. He found that 0.02 mg/kg i.v. naloxone was able to antagonize the morphine effects while 0.28 mg/kg naloxone i.v. was necessary to antagonize the effects of U50-448.

B Peptide facilitation and mechanisms of generating excitatory evoked responses in the substantia nigra following striatal stimulation

The magnitude of the initial response of nigral cells following striatal stimulation (baseline response magnitude) did not affect the response in the nigra following treatment with inhibitor; large initial responses were not associated with large responses to the drug (Figures 5, 6, 9 and 12). This independence raises questions concerning possible mechanisms involved in the generation of excitatory responses in the substantia nigra following striatal stimulation and the role of the peptides

in those responses. If it is assumed that inhibition of 24.11 acts to prolong the activity of one or several of its substrate peptides and that the peptides affected modulate the action of non-peptide transmitters then such modulation may either enhance the effectiveness of the non-peptide transmitter as established in the periphery for vasoactive intestinal polypeptide (VIP) and acetylcholine (Lundberg et al., 1982a,b) or possibly decrease its effectiveness. The results following inhibitor treatment are consistent with the idea that inhibition of 24.11 causes prolonged peptide action which ultimately acts to facilitate the action of non-peptide transmitters as the size of the initial evoked response (presumably largely a measure of the fast non-peptide transmitter action) does not influence the outcome (post-drug change in the evoked response) following inhibition of 24.11. The outcome of the experiment cannot be predicted if one assumes the evoked response is a consequence of peptide action alone but is consistent with facilitation. The drug effect is not stimulus specific, is not additive, but appears to be modulatory in nature. Facilitation of the short latency responses following drug administration (inhibition of 24.11) increases only the overall magnitude of the evoked response. The increased responsiveness to striatal stimulation is not restricted in the time domain (duration of the response); the peristimulus interval shows an increase in the absolute number of spikes with no consistent change in the delay or

duration of the response (Figures 23 to 29 ; Table 42). All responses were classified as short latency; consistent with such classification in the literature for extracellular responses (Collingridge and Davies 1981; Dray et al., 1976).

C Spontaneous Activity; Dopaminergic Cells

The 93.7% increase in the spontaneous firing frequency of dopaminergic cells observed following application of 1 umol CPAB i.c.v. cannot be fully accounted for by inhibition of 24.11 in the substantia nigra or application of 0.04 umol CPAB to the striatum. There is some indication that application of CPAB into the striatum may contribute to this effect as there is a significant increase of 36.9% in dopaminergic cell spontaneous firing frequency following local application of 0.04 umol CPAB there (Figure 10).

The increase in the spontaneous firing frequency of dopaminergic cells following CPAB i.c.v. is partially antagonized by application of 0.002 nmol naloxone into the striatum; 0.002 nmol naloxone in the presence of CPAB i.c.v. compared to CPAB i.c.v. alone (45.3% vs 93.7%, $p<0.05$; Newman-Keuls). This indicates opioid peptide facilitation in the striatum may mediate part of this effect. This conclusion is consistent with the observations of Iwatsubo and Clouet (1977) who showed that infusion of morphine directly into the striatum of the rat

caused a naloxone reversible increase in the spontaneous firing of dopaminergic cells 50% to 300% above baseline. The peak firing frequency of the dopaminergic cells they recorded following treatment with morphine was 15 to 18 Hz. This is close to the fastest firing induced by inhibition of 24.11 in the striatum (14.2 +/-1.3 Hz) with peak dopaminergic cell firing of 19.0 +/1.2 Hz following CPAB i.c.v. and 22.0 +/- 1.3 following CPPAB i.c.v. The effect of i.v. morphine was abolished following kainic acid lesioning of striatum. Thus, potentiation of opioid peptide action in the striatum may be important in generating the effects seen following application of inhibitors of 24.11 i.c.v. or direct application into the striatum.

Application of naloxone 1 mg/kg i.v. was unable to antagonize the action of CPAB i.c.v. on the spontaneous firing of dopaminergic cells. This indicates that there may be an opioid peptide process in the substantia nigra or possibly an area projecting to the striatum that, when blocked, either antagonizes the effect of naloxone in the striatum and/or has direct excitatory effects on the dopaminergic cells in the nigra. But, the results of these experiments do not clarify this issue nor is there a known process which can explain this result.

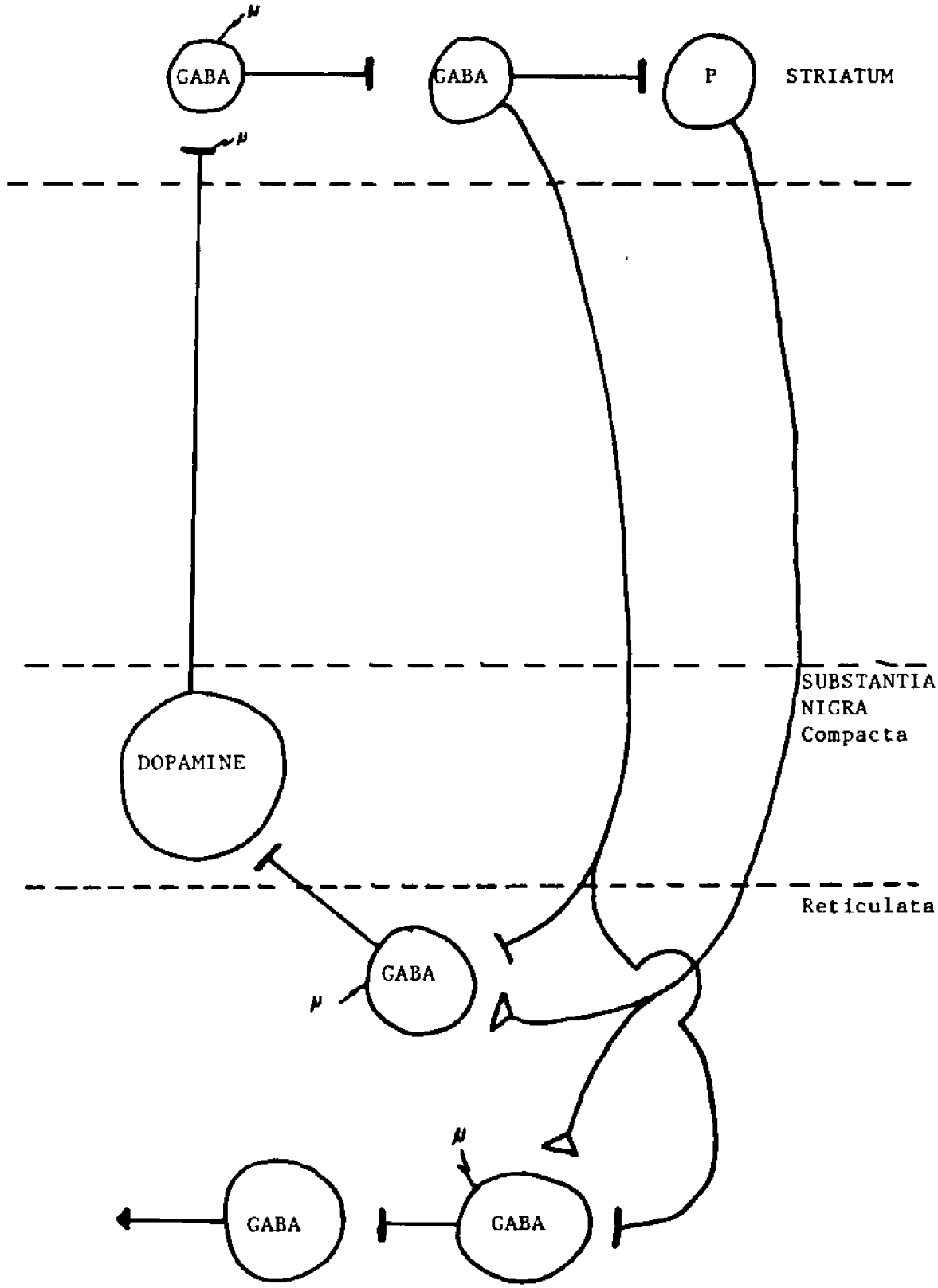
Support for the participation of endopeptidase-24.11 in regulation of striatonigral processes comes from studies using inhibitors of the enzyme which demonstrate that

application of these inhibitors are able to effect changes in dopamine metabolites recovered in the striatum (Algeri et al., 1981; Wood 1982). Wood (1982), using thiorphan i.c.v. showed a naloxone reversible increase in dopamine metabolites DOPAC and HVA. Algeri et al. (1981), using phosphoryl-L-leucyl- L-phenylalanine potassium salt i.c.v., also demonstrated increased dopamine metabolite recovery. These studies suggest that application of inhibitors of endopeptidase-24.11 i.c.v. may act to increase the activity of dopaminergic cells in the SNc. Murthy et al., (1984) using 12 umol/kg i.p. CPAB show increased levels of striatal enkephalin 3 hours post-treatment. The effect of inhibition of 24.11 in the striatum may be mediated by enhanced enkephalin action rather than dynorphin as the kappa agonist U50-488 infused into the striatum decreases dopaminergic cell firing (Walker et al., 1987).

A relation between striatal enkephalinergic functioning and the cell firing recorded in the substantia nigra was first suggested by studies showing that opiates inhibit the spontaneous activity of select striatal (Iwatsubo and Clouet 1977; Finnerty and Chan 1979; Kondo and Iwatsubo 1980) and nigral cells (Collingridge and Davies 1982; Hommer and Pert 1983). The specific cells affected are thought to be GABAergic interneurons in the nigra and striatum which may regulate the nigrostriatal dopaminergic outflow through a disinhibitory process (Figure 30).

Figure 30 Circuit diagram showing the relation of the GABAergic interneuron in the striatum and substantia nigra to the cells involved in the strionigral "loop". These interneurons are hypothesized to be sensitive to inhibition by opiates and opioid peptides. Mu receptors are shown on the soma of the interneurons and terminals of the dopaminergic projection.

Figure 30


GABA
GABA
P
STRIATUM
SUBSTANTIA
NIGRA
Compacta
DOPAMINE
Reticulata
GABA
GABA
GABA

According to a model of striatal functioning proposed by Groves (1983) potentiation of enkephalinergic activity in the striatum would be expected to increase the activity of the spiny type I GABAergic projection neuron (through disinhibition) with a decrease in the activity of the spiny type II tachykinin cells. This increased spiny type I activity may in turn be expected to preferentially inhibit the GABAergic interneuron residing in the reticulata which is thought to regulate the activity of certain dopaminergic cells (Grace and Bunney 1985a,b) and the primary efferent cells of the substantia nigra (Deniau and Chevalier 1985; Hikosaka and Wurtz 1983) (Figure 30). Also of interest is the finding of Grace and Bunney (1985a) that the level of striatal stimulus affects the response recorded in the substantia nigra with lower levels of stimulation (20 to 50 uA) giving consistent SNc excitation; presumably by activation of the GABA sensitive interneuron. This low stimulus result may be analagous to the results obtained following inhibition of 24.11 alone on spontaneous activity. Sustained peptide activity (following inhibition of 24.11) and small amplitude stimuli (effecting low levels of peptide release in the nigra) may have the same effect on nigral cell firing. Larger stimulus apparently activated GABA afferents of the dopaminergic cells leading to the appearance of more inhibiton in the SNc (Figure 30).

Moroni et al. (1978) showed decreased GABA turnover in the striatum of the rat with increased nigral GABA

turnover following subcutaneous application of morphine. This observation supports the model proposed by Groves. Such increased GABA outflow may coincide with decreased substance P activity but this has not been demonstrated experimentally. Increased GABA activity in the SNr could act to disinhibit dopaminergic cells increasing their firing (Grace and Bunney 1985a,b).

Haloperidol has also been demonstrated to increase the activity of dopaminergic cells in the SNc. This activation has been shown to increase dopaminergic cell firing 30% to 400% above baseline following striatal or i.v. application of haloperidol (Iwatsubo and Clouet 1977). Antagonism of the inhibitory action of dopamine by haloperidol on the spiny type I GABAergic projection neurons may contribute to the activation of these striatal projection cells; which is translated to activation of dopaminergic cells in the SNc through disinhibitory process in the nigra. Haloperidol has been reported to raise dopaminergic cell activity through local antagonism in the nigra (Bunney et al., 1973) and to have no effect without prior amphetamine treatment (Piercey et al., 1987). Piercey suggests that this treatment is necessary in situations where dopamine release is low, for example due to the action of anesthetics, and in this case amphetamine is able to rase the level of dopamine release to reveal antagonism by haloperidol. This effect is assumed to be post synaptic. Support for pre-synaptic action comes from studies which show that

dopaminergic terminal excitability is reduced by apomorphine while haloperidol increases dopamine terminal excitability (Tepper et al., 1984). Thus, the release of dopamine from the terminals is a function of the number of impulses reaching the terminal and the pharmacological environment of the terminals. Sustained enkephalin and/or dynorphin activity in the striatum following inhibition of 24.11 may act directly on GABAergic interneurons, inhibiting their activity, which could activate the projection neurons via disinhibition, and/or antagonizing the action of dopamine either directly on striatal dopaminergic nerve endings (Biggio et al., 1978; Moroini et al., 1979) or post synaptically (Kondo and Iwatsubo 1980).

The observation that application of 0.002 nmol naloxone into the substantia nigra does not significantly decrease the effect of CPAB i.c.v. indicates that local opioid peptide action does not contribute significantly to the increase in the spontaneous activity of dopaminergic cells (Figure 18).

Neurotensin and CCK may also contribute to the increase in dopaminergic cell firing observed. Infusion of neurotensin i.c.v. and direct application into the SNc of the rat increases the recovery of striatal dopamine, HVA and DOPAC (Nemeroff et al., 1983). Iontophoretic application of neurotensin into the SNc increases the activity of dopaminergic cells (Pinnock 1985). The sulfated form of CCK appears to have direct excitatory

action on dopaminergic cells while both sulfated and non-sulfated forms facilitate the inhibitory actions of dopamine in the substantia nigra of the rat (Hommer and Skirboll 1983). But, the reduced specificity of these peptides by endopeptidase-24.11 compared to enkephalin and substance P (approximately 10 times lower K_{cat}/K_m relative to Leu-enkephalin (Matsas et al., 1983)) indicates the contribution of these peptides may be small; but such effects cannot be ruled out.

D Spontaneous Activity: Non-Dopaminergic Cells

The initial experiments in which 1 umol CPAB was administered i.c.v. yielded a mean increase in the spontaneous activity of non-dopaminergic cells of 54.0% (Figure 3 : Table 3). This increase can be fully accounted for by inhibition in the striatum (no significant difference between 33.5% increase observed following striatal inhibition and 54.0% increase following CPAB i.c.v. by Newman-Keuls; Figure 10). If the data from the experiments in which 1 umol CPAB i.c.v. was administered prior to application of naloxone into the nigra and striatum (Tables 34 and 36) is combined with data from experiments in which the only treatment is 1 umol CPAB i.c.v. (Table 3) then the increase in spontaneous firing of non-dopaminergic cells following CPAB i.c.v. is 60.0% +/- 5.6 (n=12). The additional experiments were added for the sake of the following statistical analysis. Significant

antagonism of the CPAB induced increase in spontaneous firing of the non-dopaminergic cells is observed following application of naloxone 1 mg/kg, i.v. (21.3% vs 60.0%, $p<0.01$, Mann-Whitney; Figure 13), 0.002 umol into the nigra (28.4% vs 60.0%, $p<0.05$, Mann-Whitney) or 0.002 umol into the striatum (36.1% vs 60.0%, $p<0.05$, Mann-Whitney).

Inhibition of 24.11 in the substantia nigra (Figure 7) following local application of 0.04 umol CPAB did not cause a significant increase (15.2%) in the spontaneous firing frequency there. This result was surprising in light of the studies demonstrating a large tachykinin projection to the SNr, excitatory effects of tachykinins on cells in the reticulata and the high specificity of endopeptidase-24.11 for tachykinins; all suggesting that inhibition of 24.11 in the nigra would cause large increases in the firing of non-dopaminergic cells. The absence of the expected increase indicates that disinhibitory processes may be of primary importance in maintaining the spontaneous firing of non-dopaminergic as well as dopaminergic cells.

A tachykinin induced increase in excitability of GABAergic interneurons in the SNr could make these cells less sensitive to inhibition by striatonigral GABA. Thus, the dual action of tachykinin and GABA on the interneuron would tend to make the disinhibitory process less efficient and may account, in part, for the absence of the expected increase in the spontaneous firing of cells in the SNr following inhibition of 24.11 in the nigra. Sustained

dynorphin action could also contribute to this result by inhibiting SNr cell firing (Walker et al 1987).

The increase in spontaneous activity of the non-dopaminergic cells following 1 umol CPAB i.c.v. is clearly sensitive to antagonism by naloxone. Antagonism of the CPAB induced increase in the spontaneous firing of non-dopaminergic cells following infusion of 0.002 nmol naloxone into the striatum (Figure 19) may be the result of increased activity of the GABAergic interneuron leading to decreased disinhibition of the spiny type I GABAergic cell. This would in turn lead to decreased disinhibition in the SNr which would oppose increases in the firing of neurons in the reticulata (Figure 30). This is analagous to the process affecting the dopaminergic spontaneous firing frequency. Thus, the main influence on dopaminergic and non-dopaminergic spontaneous cell firing appears to be the striatal GABAergic afferent of the interneuron in the reticulata. In this light the observation th treatment of the striatum with 1 umol CPAB is able to effect increases in the activity of dopaminergic and non-dopaminergic cells is not unexpected. The absence of an effect on the spontaneous rate of non-dopaminergic cells following inhibition of 24.11 in the nigra is the result of decreased striatal GABAergic afferent activity (below CPAB stimulated level), increased tachykinin action on the GABAergic interneurons responsible for the disinhibition and sustained dynorphin action on the cells recorded in the

SNr. The cells recorded in the SNr may be facilitated by sustained tachykinin action but the appearance of such facilitation may depend on phasic synaptic input into these cells; excitatory input (Nowak and McDonald 1981; Nakanishi et al., 1987). That nigral application of naloxone following CPAB i.c.v. is able to antagonize the effect of CPAB i.c.v. indicates that opioid peptide activity in the nigra is necessary or permissive in the disinhibitory process. Apparently increased striatal GABAergic activity following treatment of the striatum with 1 umol CPAB is sufficient to significantly increase the spontaneous firing frequency of the non-dopaminergic cells in the reticulata and this effect is dependent on opioid action in the reticulata. As GABA and enkephalin may be colocalized in the spiny type I afferents of the strionigral pathway (Zahm et al., 1985; Aronin et al., 1984; Morelli et al., 1983; Oertel et al., 1983) it is likely that enkephalin is acting to facilitate the effect of GABA on the interneuron in the reticulata that is regulating the spontaneous activity of the cell recorded in the SNr.

E Short Latency Excitatory Responses in Non-Dopaminergic Cells: Possible Mechanisms

The possible mechanisms of generating short latency excitatory responses is much less well characterized for non-dopaminergic cells in the SNr than for the dopaminergic cells in the SNc. As there is little support for a

monosynaptic striatonigral pathway which could account for the short latency excitatory responses in the SNr and SNc It is most likely that such responses are produced locally (polysynaptically) via disinhibitory processes (Gale and Casu 1981; Deniau and Chevalier 1985; Chevalier et al., 1985). The majority of cells recorded in the SNr are inhibited at short latency; ipsp's recorded 1.8-2.2 msec (Grace and Bunney 1985a) which may be a monosynaptic response (Deniau and Chevalier 1985). Cells which may be excited by disinhibition include neurons with excitory afferents from the pedunculopontine tegmental nucleus (Jackson and Crossman 1983; Scarnati et al., 1984) or subthalamic nucleus (Deniau et al., 1978; Nakanishi et al., 1987), cells which are under auto inhibitory processes (Karabelas and Purpura 1980) and cells which are in small networks which may be turned on by disinhibitory processes (Figure 30). Evidence for such networks comes from electrophysiological studies which show rhythmically recurring discharges in SNr cells following striatal stimulation (Frigyesi and Purpura 1967; Collingridge and Davies 1981). The latency of responses recorded extracellularly cannot be used as the only criteria to judge if the responses were monosynaptic or not. The shortest latency ipsp's recorded in the rat are 1.8-2.2 msec (Grace and Bunney 1985a) (which are arguably monosynaptic responses) while the shortest latency inhibitory and excitatory responses recorded

extracellularly in the rat are around 5 msec (Dray et al., 1976; Collingridge and Davies 1981) which is in agreement with the shortest latency excitatory responses observed in the experiments reported here (Table 42).

F Evoked Responses: Non-Dopaminergic Cells

The magnitude of the evoked response of non-dopaminergic cells is significantly increased (152.3%) following administration of 1 umol CPAB i.c.v. (Figure 4). This increase can not be fully accounted for by inhibition of 24.11 in the substantia nigra (34.7% vs 152.3%, $p<0.01$; Newman-Keuls) or treatment of the striatum with 0.04 umol CPAB (20.5% vs 152.3, $p<0.01$; Newman-Keuls). The failure of CPAB treatment in the nigra or striatum to account for the full i.c.v. effect may reflect a combination of opposing influences on the non-dopaminergic cell firing. Some of those influences are revealed in the experiments (described below) in which naloxone was administered alone and in the presence of CPAB i.c.v. It is also possible that either the volume of striatum treated with CPAB was insufficient (approximately 4% of striatum covered by inhibitor) or both striatum and nigra need to be treated simultaneously to get the maximal effect.

One key result which shed some light on the possible mechanisms influencing the evoked response of the non-dopaminergic cells was the significant increase in the magnitude of the evoked response (34.7%) of these cells

following inhibition of 24.11 in the nigra (Figure 8). This increase may reflect the action of increased tachykinin activity on the cell recorded in the SNr due to the combined effect of inhibition of 24.11 and striatal stimulation. Of the experiments performed in which the activity of 24.11 was inhibited in the substantia nigra this is the only case in which significant changes in the activity of nigral cells was observed. If the disinhibitory process, which is hypothesized to regulate the spontaneous activity of non-dopaminergic and dopaminergic cells, is also active in the generation of short latency excitatory responses of the non-dopaminergic cells in the SNr then inhibition of 24.11 in the substantia nigra may be expected to attenuate this response due to the action of tachykinin on the interneuron responsible for the disinhibition (Figure 30). An increase in the firing or excitability of this central SNr interneuron would tend to make the disinhibitory process less efficient. But, at high stimulus intensity (above spontaneous release) direct tachykinin action may become an important influence on the cell recorded in the SNr. The action on non-dopaminergic cells suggests that there may be a mechanism which is able to lower the threshold of excitability in these cells enough to enhance excitatory synaptic drive without affecting the spontaneous activity in those cells. This finding supports the idea that certain SNr cells may be facilitated by tachykinin and the apparance of such

facilitation may depend on phasic synaptic input onto these cells (Nowak and McDonald, 1981; Nakanishi et al., 1987). Thus, even with decreased excitatory input, due to the action of tachykinins on the GABAergic interneurons responsible for disinhibition, the threshold of the cells recorded could be lowered enough to get significant excitation.

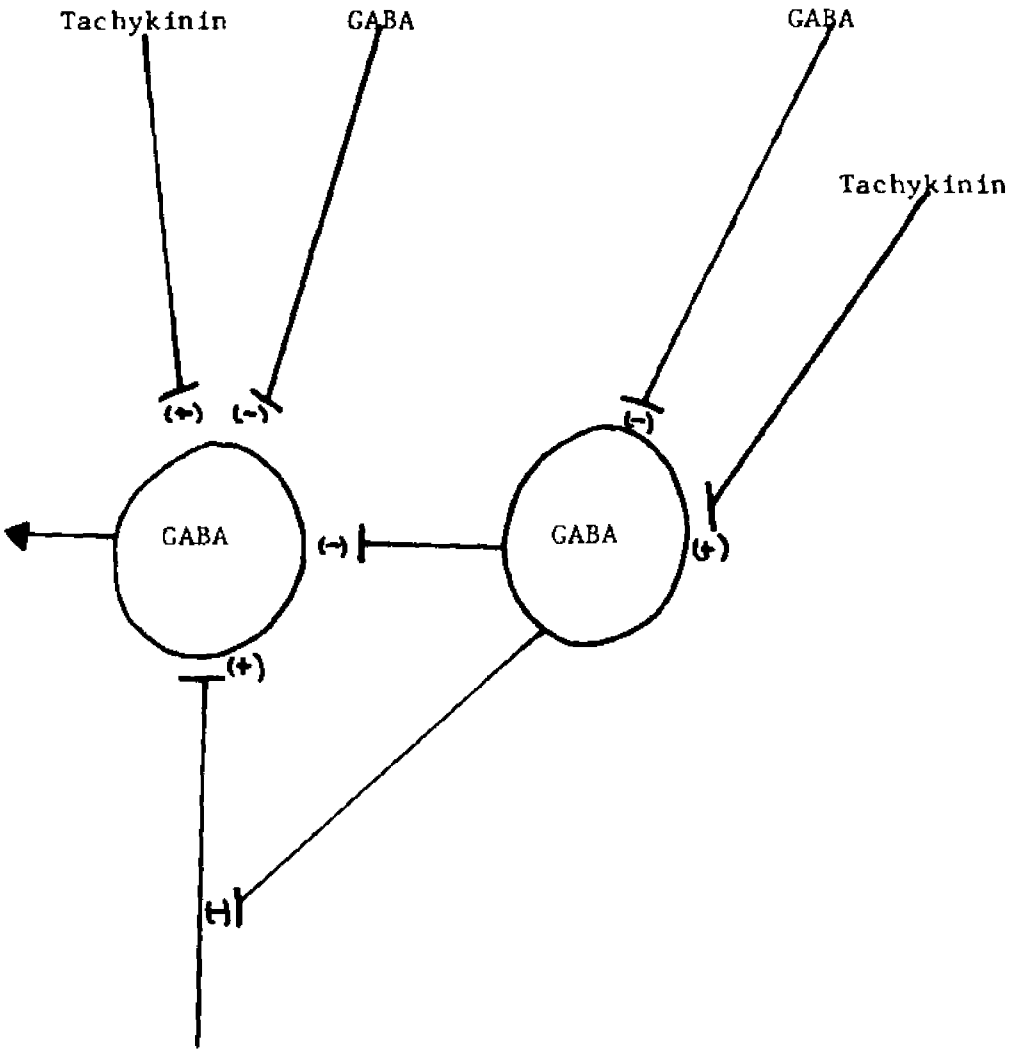
Application of 0.04 umol CPAB into the striatum had no significant effect on the magnitude of the evoked response of non-dopaminergic cells (Figure 11). The finding that infusion of CPAB into the striatum increases the spontaneous but not the evoked activity may appear paradoxical but striatal stimulation coupled with inhibitor treatment causes greater activation of direct GABAergic input onto the cell recorded in the SNr (Figure 30). This is supported by the experiments of Grace and Bunney (1985a) showing that the level of striatal stimulus affects the response recorded in the substantia nigra with lower levels of stimulation (20 to 50 uA) giving consistent SNc excitation; presumably by activation of the GABA sensitive interneuron. The results presented here support the hypothesis that a similar mechanism may be at work in the SNr. This low level of stimulus may be analagous to the results obtained following inhibition of 24.11 alone in that the sustained activity of the peptides which regulate these processes following inhibition of 24.11 may be the same as low levels of stimulus causing low levels of

peptide to be released. Larger stimuli apparently activated GABA afferents of the dopaminergic cells leading to the appearance of more inhibition in the SNc. Such direct GABAergic input could act to antagonize excitatory input onto the same cell. This hypothesis is supported by experiments in which naloxone alone was administered into the striatum causing a significant increase of 111.9% in the magnitude of the evoked response of cells in the SNr (Figure 15). Decreased activity of the GABAergic projection neurons (resulting from the local action of naloxone) along with increased tachykinin activity (due to the stimuli) could cause the increase observed. It appears that the following influences may be active in shaping the evoked responses recorded in the SNr following striatal stimulation; direct tachykinin action, direct GABA action, direct excitatory input which is modulated by disinhibitory processes and GABAergic input which is disinhibitory (Figure 31). The lack of an effect on the magnitude of the evoked response of the non-dopaminergic cells following application of CPAB into the striatum could be explained by the following: antagonism of disinhibition in the SNr by increased tachykinin activity and decreased dynorphin (which inhibits SNr activity) and enkephalin (which may facilitate disinhibition) activity in the SNr.

Application of 0.002 nmol naloxone into the striatum is able to antagonize the CPAB i.c.v. effect (81.4% vs 152.3%, $p<0.05$; Newman-Keuls; Figure 21). In the striatum

Figure 31 Circuit diagram showing possible synaptic influences on the cell recorded in the SNr which are consistent with the results presented.

Figure 31



Pedunculopontine Nucleus
Subthalamic Nucleus

the effect of CPAB (which increases the GABAergic outflow) is opposed by the naloxone. Thus, there is less activation of the direct GABAergic afferent of the cell recorded in the SNr with greater activation of the disinhibitory pathway. But, sustained activity of tachykinin in the SNr opposes the disinhibitory influences on the cell recorded leading to less efficient disinhibitory processes. Further, sustained action of dynorphin could also contribute to reduced excitability of the cell recorded. Application of naloxone into the substantia nigra is also able to antagonize the effect of CPAB i.c.v. (49.0% vs 152.3%, $p<0.01$; Newman-Keuls; Figure 22). This result may be a combination of the local effect of CPAB i.c.v. in the striatum, activating direct GABA influences on the cell recorded as well as antagonism of an opioid peptide which is hypothesized (based on spontaneous results) to be facilitatory or permissive on the disinhibitory process in the SNr. The antagnism of the effect of CPAB i.c.v. following naloxone i.v. (36% vs 152%, $p<0.01$; Newman-Keuls; Figure 14) is thought to be the result of a combination of these processes.

G Evoked Responses; Dopaminergic Cells

The magnitude of the evoked responses of dopaminergic cells is increased 275% following application of 1 umol CPAB i.c.v. (Figure 4). The baseline evoked responses of the dopaminergic and non-dopaminergic cells are not

different and both percent and absolute increases in evoked responses are greater for the dopaminergic cells. Thus, it appears that the dopaminergic cells are approximately 2 times more responsive to evoked stimulation following inhibitor treatment than non-dopaminergic cells. The large increase in the magnitude of the evoked response of the dopaminergic cells can not be accounted for by inhibition of 24.11 in either the substantia nigra (28.8% vs 275.6%, $p<0.01$; Newman-Keuls) or treatment of the striatum with 0.04 umol CPAB (69.6% vs 275.6%, $p<0.01$; Newman-Keuls) (Figures 8 and 11). It is possible that either the volume of striatum treated with CPAB was insufficient (approximately 4% of striatum covered by inhibitor) or both striatum and nigra need to be treated simultaneously to get the maximal effect.

The significant increase in the magnitude of the evoked response of dopaminergic cells of 69.6% following treatment of the striatum with 0.04 umol CPAB is probably a reflection of a decreased inhibitory environment in the striatum due to sustained enkephalin action which may either lower the threshold of stimulation of GABA efferents or increase the number of efferents stimulated. Similar influences are affecting the magnitude of the evoked responses of non-dopaminergic cells and dopaminergic cells. But, there are apparently some important differences in the afferents of these cells which may account for the differences in the responses of these cells following

similar drug treatments. The main difference between these cells is that the dopaminergic cells receive much less direct GABAergic and tachykinin input and are apparently regulated primarily through the disinhibitory process. Therefore, the processes which affect the inhibitory interneuron in the SNr will have a greater effect on the evoked responses of dopaminergic cells than on the responses of non-doapminergic cells. The increase of 69.6% in the magnitude of the evoked response of dopaminergic cells following treatment of the striatum with CPAB is different from the evoked response of the non-dopaminergic cells (where there is no increase in the magnitude of the evoked response following treatment of the striatum with CPAB). This may simply be the result of less direct inhibition by GABAergic afferents. This direct GABAergic influence is postulated to significantly contribute to the absence of an effect on the evoked response of the non-dopaminergic cells. In a likewise manner the significant increase in the magnitude of the evoked response of the non-dopaminergic cells (34.7%) following inhibition of 24.11 in the substantia nigra is postulated to be primairly due to the direct action of tachykinins on these cells. The paucity of a tachykinin projection to the SNc may account for the absence of a significant effect on the evoked response of these cells following inhibition of 24.11 in the nigra.

The reversal of the CPAB i.c.v. effect in the presence of naloxone i.v. (-37.1% vs 275.6%, $p<0.01$; Newman-Keuls; Figure 14) suggests several possible influences on the evoked responses of these cells. First, this result affirms the importance of enkephalin in the striatum in facilitation of this response (also supported by the significant decrease in the magnitude of the evoked response of dopaminergic cells following application of 0.002 nmol naloxone into the striatum in presence of CPAB i.c.v. (3.2% vs 275.6%, $p<0.01$; Newman-Keuls; Figure 21). The difference in the response of dopaminergic cells (with a non-significant increase of 3.2%) and non-dopaminergic cells (significant increase of 81.4%) following the direct application of naloxone into the striatum in the presence of CPAB i.c.v. r also be the result of the influence of the direct GABAergic afferent of the SNr cell. Apparently the main influence on the evoked response of the dopaminergic cells is the process of disinhibition. When that is blocked in the striatum by the naloxone treatment coupled to the sustained action of tachykinin (acting to attenuate the disinhibition) in the SNr (due to the inhibition of 24.11), the result is a great reduction in the magnitude of the evoked response of these cells (Figure 21). But, the non-dopaminergic cells are influenced by the same processes with the additional influence of direct GABAergic inhibition (as evidenced by the non-significant (20.5%) increase in the magnitude of the evoked response of

these cells following infusion of 0.04 umol CPAB into the striatum (Figure 11)). When the effect of application of 0.04 umol CPAB into the striatum is antagonized (following treatment of the striatum with naloxone in the presence of CPAB i.c.v. (Figure 21)) there is less attenuation of the evoked response (due to blockade of the direct GABAergic influence) of the non-dopaminergic cells (81.4% increase) compared to the dopaminergic cells (3.2% increase). Second, the significant reduction in the evoked response of dopaminergic cells following the application of naloxone into the nigra in the presence of CPAB i.c.v. compared to CPAB alone (64% vs 275.6%, $p<0.01$; Newman-Keuls) confirms the necessity of opioid action in the SNr for full expression of this response. Dynorphin, applied to SNr, has been shown to decrease cell firing (Lavin and Garcia-Munoz 1985) and antagonism of this effect by naloxone could contribute to the attenuation of disinhibitory effects in the SNc. The reversal of the evoked response of the dopaminergic cells following application of naloxone i.v. could be the result of blockade of the disinhibitory processes combined with the direct action of GABA on these cells. Although this action would arguably (based on the paucity of direct GABAergic afferents of the SNc) be small this result supports the suggestion that there is some direct GABAergic action on these cells.

H Future Experiments

Marked heterogenities in the distribution of transmitters in the substantia nigra and striatum have recently been described (Graybiel 1983). In the striatum many of the peptide and non-peptide transmitters, as well as endopeptidase-24.11 (Matsas et al., 1986), show compartmentalization which is organized around the striosomal pattern of acetylcholine esterase staining first described by Graybiel and Ragsdale (1978). In light of this neurochemical organization it would be of interest to know how the positions of the stimulating and recording electrodes are related to the outcome of experiments in which inhibitors of endopeptidase-24.11 are used. Approximately 4% of the entire volume of the striatum was profused (in those experiments in which drugs were administered locally) with inhibitors and naloxone. Treatment of this small volume was effective in many experiments in significantly increasing the activity of cells in the substantia nigra: application of naloxone into the striatum was able to partially antagonize the effects of CPAB i.c.v. on the spontaneous and evoked activity of non-dopaminergic and dopaminergic cells (with complete antagonism of the evoked response of dopaminergic cells). But, in other cases the treatment had no effect on the activity of the cells in the nigra: striatal application of CPAB had no effect on the evoked responses of

non-dopaminergic cells. The question that arises concerns the specific location or critical volume which is necessary to induce maximal effects. For example, would increasing the volume of the striatum treated with naloxone cause complete antagonism of the effect of CPAB on the activity of cells in the substantia nigra. Also, how does the area treated relate to the peptide transmitter profile in the striatum.

The inability of naloxone 1 mg/kg, i.v. to antagonize the action of CPAB i.c.v. on the spontaneous activity of dopaminergic cells, while the application of naloxone directly into the striatum was able to antagonize the effect of CPAB i.c.v., suggests that there is an opioid dependent process in the nigra (or elsewhere) that, when blocked, either antagonizes the effect of naloxone in the striatum and/or has direct excitatory effects on the dopaminergic cells in the nigra. This question could be addressed by infusion of naloxone into the substantia nigra following application of naloxone into the striatum (in the presence of CPAB i.c.v.). This treatment may be expected to replicate the effect of naloxone i.v. if there is an opioid dependent process active in the nigra.

Central to the proposed mechanism of action is the existence of an opioid peptide sensitive GABAergic interneuron in the striatum and SNr. These neurons are hypothesized to exert direct inhibitory control over the spiny type I GABAergic efferent of the striatum,

dopaminergic cells in the SNc and the cells recorded in the SNr. The disinhibitory regulation of the dopaminergic cell (by an opioid sensitive cell intrinsic to the SNr) has been described in some detail (Finnerty and Chan 1979; Collingridge and Davies 1982; Hommer and Pert 1983; Grace and Bunnney 1979, 1985a,b). But, cells exhibiting disinhibitory control over the spiny type I striatal cells have not been described. Disinhibitory mechanisms have been suggested in order to explain striatally evoked short latency excitatory responses in the SNr and 'paradoxical' excitatory responses to profusion of the SNr with GABA (Chevalier et al., 1985).

Critical to the conformation of the hypothesis presented in this thesis is the electrophysiological and pharmacological characterization of the interneurons acting on the spiny type I cells in the striatum and the SNr cells exhibiting excitatory responses to the striatal stimulation in the SNr. The interneuron in the SNr would be expected to be inhibited by striatal stimulation and local application of GABA. But, the interneurons controlling the dopaminergic cells of the SNc and the primary efferents of the SNr would exhibit the same characteristics. Simultaneous recording from the interneuron and the cell disinhibited in the SNr, the kind of study performed by Grace and Bunney (1985a,b) on the disinhibition of the dopaminergic cell, would need to be performed. Replication

of the pharmacological studies using inhibitors of endopeptidase-24.11 could then be performed.

Application of CPAB into the substantia nigra had no effect on the spontaneous firing of non-dopaminergic cells. This is attributed to the action of tachykinins on the interneurons. The tachykinins are hypothesized to increase the excitability of these interneurons making them less sensitive to inhibition by striatonigral GABA afferent activity. Direct iontophoretic application of tachykinins onto the interneurons responsible for disinhibition should have no effect on the spontaneous firing of the cell while profusion of the interneuron and the cell recorded with tachykinin is expected to increase the magnitude of the evoked response (situation analogues to evoked studies on non-dopaminergic cells in which CPAB was infused into the nigra). The best test of this hypothesis would be to antagonize the effect of the tachykinin. This depends on the availability of an acceptable tachykinin antagonist.

Sustained action of opioid peptides in the striatum (following inhibition of the activity of endopeptidase-24.11) has been postulated to account for many of the effects observed in these experiments. For example, direct application of naloxone into the striatum is able to partially antagonize the effects of CPAB i.c.v. on the spontaneous firing of dopaminergic cells. One of the hypothesized actions of the opioid peptides is their direct inhibitory action on the striatal GABAergic

interneurons which are in direct contact with the spiny type I GABAergic efferents. As striatal application of CPAB is able to increase the spontaneous firing frequency of non-dopaminergic and dopaminergic cells one simple test which may confirm the action of opioid peptides in the striatum is the striatal administration of naloxone following striatal application of CPAB. Naloxone would be expected to antagonize the CPAB induced increase in firing of the nigral cells. Profusion of the striatum with enkephalin following striatal administration of CPAB may increase the spontaneous firing of nigral cells above the increases induced by CPAB alone. But, this is not necessarily expected as the effect of CPAB may be maximal.

If the hypothesis are to be tested, cells in the striatum which fit the criteria of the proposed interneurons should be recorded from. These cells would be expected to decrease their activity following local intracerebroventricular administration of CPAB. This decreased activity should be antagonized by naloxone if the inhibition is directly mediated by opioid peptides. Recording from the spiny type I GABAergic projection neurons should show increased activity following treatment with CPAB. This activity should be antagonized by naloxone and this antagonism should be reversed by bicuculline if it is mediated by the increases activity of GABAergic interneurons. Sustained opioid peptide activity could also act pre-synaptically on dopaminergic terminals in the

striatum. The contribution of the pre-synaptic effect could be studied using the methods of Tepper et al., (1984). The current necessary to antidromically activate dopamine cells in the SNc from their terminal fields in the striatum is assessed before and after treatment with CPAB. Any decrease in the excitability is interpreted to arise from autoreceptor mediated hyperpolarization of the terminals (Tepper et al., 1984). One could then perform experiments in order to determine which peptide or peptides might replicate this effect.

Striatal application of CPAB was able to increase the spontaneous firing frequency of non-dopaminergic and dopaminergic cells. The magnitude of the evoked response of dopaminergic cells but not non-dopaminergic cells was increased following this treatment. In order to explain the inability of striatal application of CPAB to increase the magnitude of the evoked response of non-dopaminergic cells it is postulated that treatment of the striatum with CPAB promotes the activation of GABA afferents of the non-dopaminergic cells. Increased activity of these afferents is postulated to antagonize increases in evoked activity due to disinhibition. The increase in the magnitude of the evoked response of the dopaminergic cells is attributed the paucity of striatonigral GABA efferents to the SNc. This is supported by anatomical studies showing the bulk of striatonigral GABAergic efferents terminating in the SNr (Brownstein et al., 1977; Somogy and

Smith 1979; Ribak et al., 1980). Iontophoretic application of bicuculline onto the cell recorded in the SNr should reverse the effect of striatal CPAB on the evoked response of this cell. Similar treatment of the dopaminergic cells in the SNc should have less effect compared to the non-dopaminergic cells. Activation of the GABAergic afferent of the SNr is attributed to a decreased inhibitory environment in the striatum due to sustained enkephalinergic action. The enkephalins are postulated to lower the threshold of stimulation of these cells. Lowering the amplitude of stimulation would be expected to reverse the effect of CPAB treatment on the evoked response of the non-dopaminergic cells.

I Summary and Conclusions

A. Inhibition of the activity of membrane-bound endopeptidase-24.11 in the CNS of the rat is able to effect significant increases in the spontaneous firing frequency and the magnitude of striatally evoked responses of cells displaying excitatory responses in the SNr and SNc.

1. The inhibition of the enzyme by 1 umol CPAB i.c.v. was verified through in vitro assay.

2. The increased firing frequency and evoked response was shown to result from the inhibition of the enzyme, as infusion of N-[1-(R,S)-carboxy-2- phenyl-ethyl] Leu-pAB (CPLAB), an inhibitor structurally related to CPAB and

CPPAB yet two orders of magnitude less potent, was without effect on the activity of nigral neurones.

3. The increase in the spontaneous activity reached an apparent maximum approximately 20 minutes after the end of infusion which is consistent with enzyme inhibition rather than agonist or antagonist action.

B. The striatum may be an important site of action following application of CPAB i.c.v. in the generation of the increases in spontaneous activity observed in dopaminergic and non-dopaminergic cells and may also contribute to, but can not fully account for, the increase in the magnitude of the evoked response of dopaminergic cells.

1. Perfusion of approximately 4% of the structure with 1 uL of a 40 millimolar solution of CPAB produces 60% of the response on spontaneous activity of non-dopaminergic and 30% of the response of dopaminergic cells obtained following i.c.v. administration of CPAB.

2. Treatment of approximately 4% of the striatum with CPAB produces 25% of the evoked response of dopaminergic cells obtained following CPAB i.c.v.

C. The increase in the spontaneous activity and magnitude of the evoked response of non-dopaminergic or dopaminergic cells following 1 umol CPAB i.c.v. can not be

accounted for by inhibition of the activity of endopeptidase-24.11 in the substantia nigra alone.

1. Application of 0.04 umol CPAB into the substantia nigra has no significant effect on the spontaneous firing of dopaminergic or non-dopaminergic cells.

2. Application of 0.04 umol CPAB into the substantia nigra was demonstrated to inhibit the activity of the enzyme in the substantia nigra in vitro to an equivalent degree (60%) as 1 umol CPAB i.c.v.

3. The increase in the magnitude of the evoked response of non-dopaminergic cells (34.7%) is significantly less than that observed following CPAB i.c.v.

D. The increase in the spontaneous and evoked activity of dopaminergic and non-dopaminergic cells observed following CPAB i.c.v. is dependent, in part, on enhanced opioid peptide (enkephalin and/or dynorphin) activity in the striatum and substantia nigra. In every circumstance naloxone (1 mg/kg, i.v. or local infusion of 0.002 nmol into the striatum or substantia nigra) significantly reduced CPAB i.c.v. induced increases in activity with two exceptions. Increases in the spontaneous activity of dopaminergic cells following CPAB i.c.v. was not antagonized by application of naloxone i.v. or local application into the substantia nigra.

1. Application of 0.002 nmol of naloxone into the striatum antagonizes the CPAB i.c.v. induced increases in

the spontaneous and evoked activity of dopaminergic and non-dopaminergic cells.

2. Application of 0.002 nmol naloxone to the substantia nigra or 1 mg/kg, i.v. is unable to antagonize the effect of CPAB i.c.v on the spontaneous activity of dopaminergic cells.

3. Application of 1 mg/kg, i.v. naloxone is able to antagonize the effect of CPAB i.c.v. on the magnitude of the evoked response of non-dopaminergic cells.

4. Application of 1 mg/kg, i.v. naloxone is able to reverse the effect of CPAB i.c.v. on the magnitude of the evoked response of dopaminergic cells.

5. Application of naloxone into the substantia nigra or striatum is able to antagonize the effect of CPAB i.c.v. on the evoked response of dopaminergic cells but both treatments are significantly different from the reversal of the effect of CPAB i.c.v. following application of 1 mg/kg, i.v., naloxone.

Table 3

Effect on non-dopaminergic cells of intracerebroventricular infusion of 1 umol CPAB

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	57	110	+ 93.0	18 +/- 3.2	26 +/- 2.8	+ 44.4
2	210	456	+ 117.4	25 +/- 1.7	35 +/- 1.2	+ 40.0
3	156	386	+ 144.3	12 +/- 0.5	23 +/- 0.7	+ 91.7
4	112	258	+ 130.4	22 +/- 0.8	33 +/- 0.2	+ 50.0
5	74	186	+ 151.4	25 +/- 1.2	30 +/- 1.3	+ 20.0
6	68	257	+ 277.9	18 +/- 0.9	32 +/- 1.2	+ 77.8
		MEAN	+ 152.3			+ 54.0

* Post-drug significantly different from pre-drug (Paired T-test: p<0.01)
** Post-drug significantly different from pre-drug (Paired T-test: p<0.01)

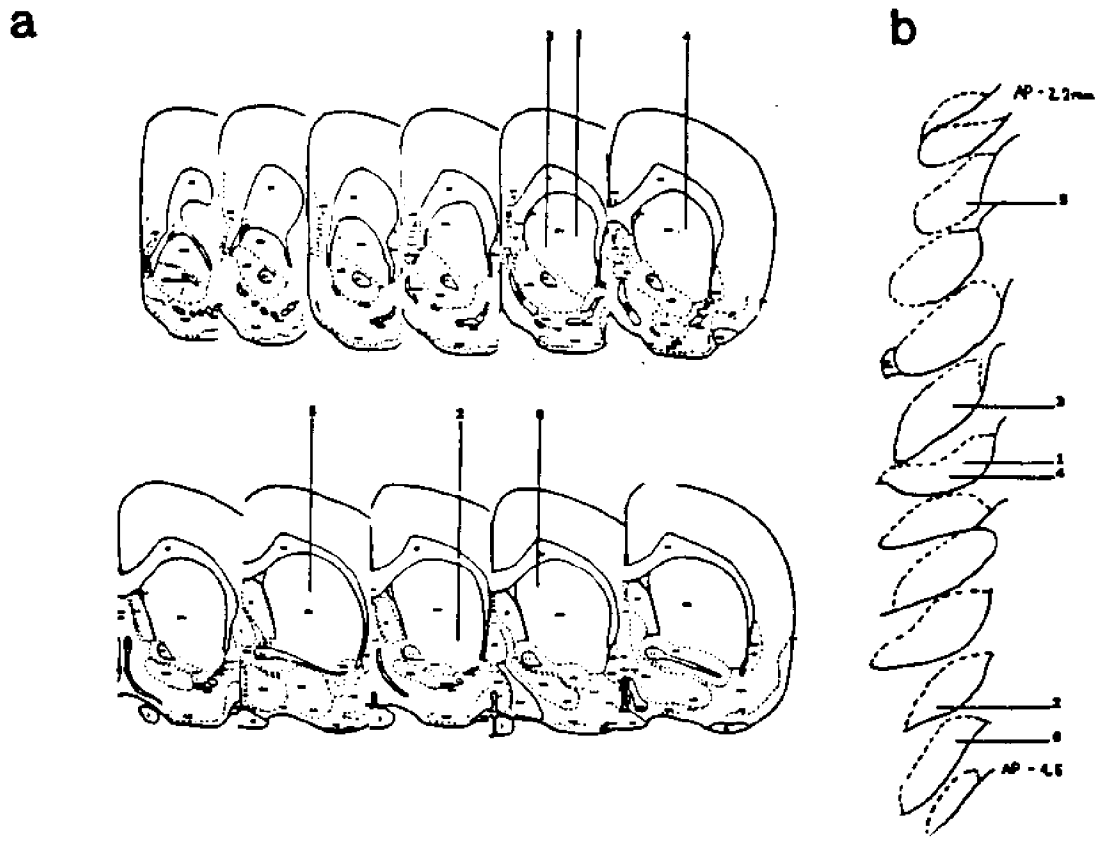


Table 4

Effect on non-dopaminergic cells of intracerebroventricular infusion of 1 umol CPPAB

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	72	210	+ 191.7	18.0 +/- 0.8	27.5 +/- 1.4	+ 52.8
2	46	147	+ 219.6	33.5 +/- 2.7	51.0 +/- 2.2	+ 52.2
3	166	383	+ 130.7	28.5 +/- 1.3	37.0 +/- 1.7	+ 29.8
4	64	128	+ 100.0	24.0 +/- 0.5	34.0 +/- 1.3	+ 41.7
		MEAN	+ 160.5			+ 44.1

* Post-drug significantly different from pre-drug (Paired T-test: p<0.05)
** Post-drug significantly different from pre-drug (Paired T-test: p<0.05)

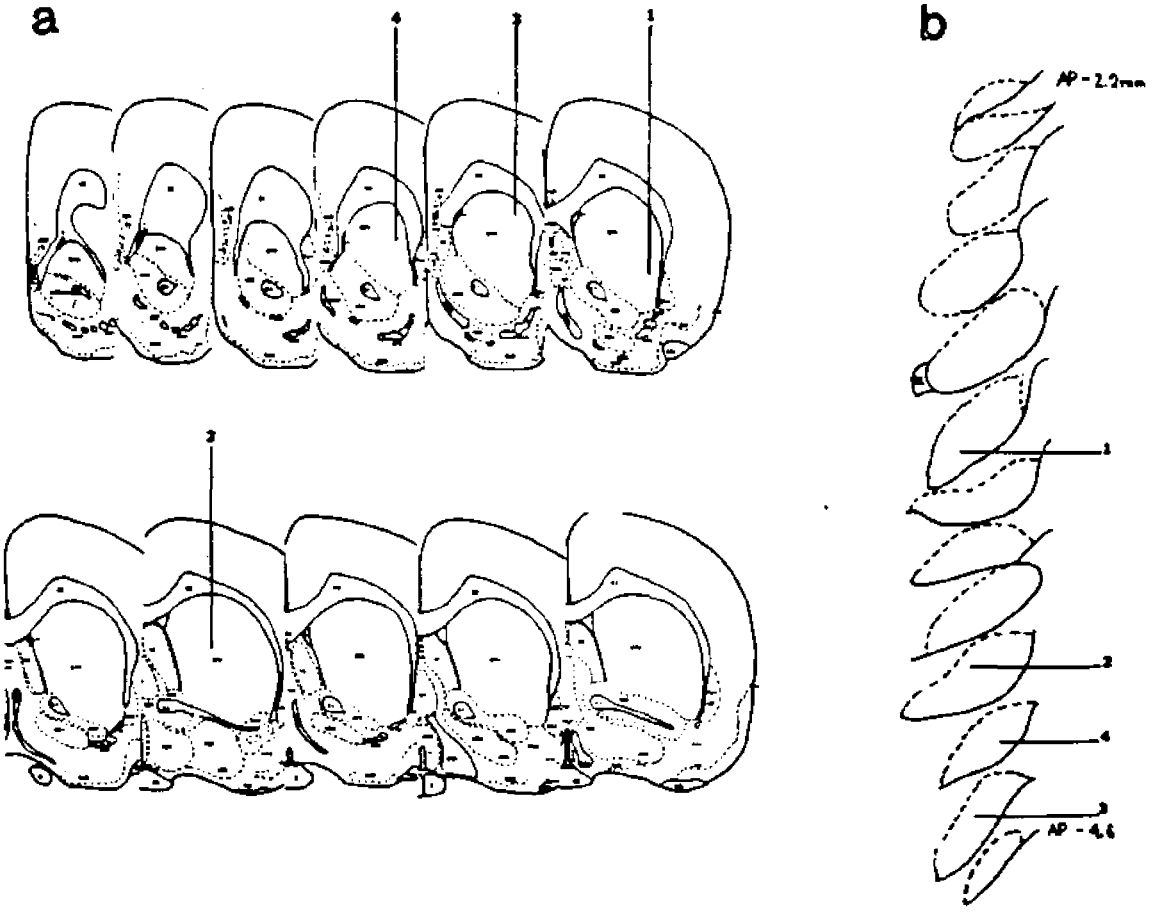


Table 5

Effect on dopaminergic cells of intracerebroventricular infusion of 1 umol CPAB

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	61	127	+ 108.2	8.0 +/- 0.9	14.5 +/- 0.4	+ 81.3
2	112	598	+ 433.9	5.2 +/- 1.1	9.0 +/- 0.9	+ 73.1
3	72	382	+ 430.6	4.8 +/- 0.1	11.6 +/- 0.3	+ 141.7
4	220	643	+ 192.3	9.8 +/- 1.0	19.0 +/- 1.2	+ 93.9
5	83	298	+ 259.0	3.6 +/- 0.5	6.2 +/- 0.6	+ 72.2
6	47	155	+ 229.8	2.8 +/- 0.9	5.6 +/- 0.4	+ 100.0
		MEAN	+ 275.6			+ 93.7

* Post-drug significantly different from pre-drug (Paired T-test: p<0.05)
** Post-drug significantly different from pre-drug (Paired T-test: p<0.01)

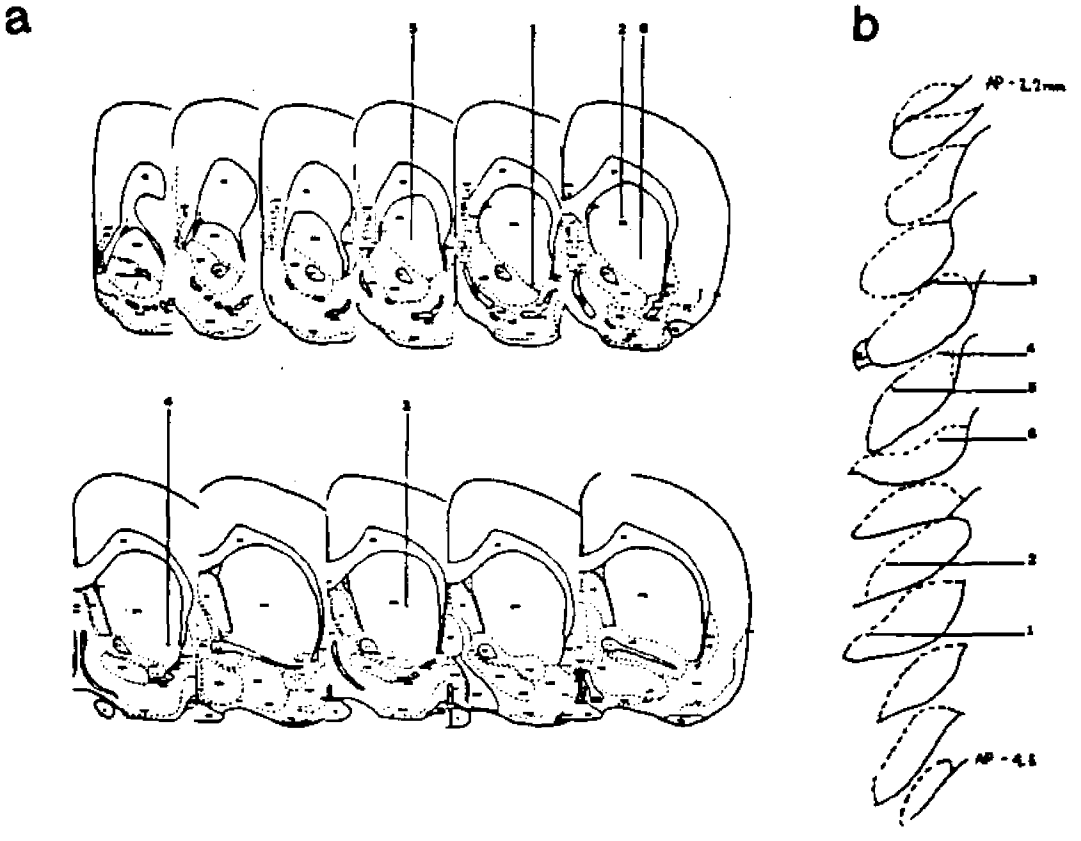


Table 6

Effect on dopaminergic cells of intracerebroventricular infusion of 1 umol CPPAB

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	102.0	383	+ 168.6	4.5 +/- 0.2	8.2 +/- 0.5	+ 82.2
2	74.5	248	+ 232.9	12.0 +/- 0.9	22.0 +/- 1.9	+ 83.3
3	127.0	618	+ 386.6	4.8 +/- 0.6	9.8 +/- 0.4	+ 104.2
4	78	207	+ 165.4	5.3 +/- 0.1	8.6 +/- 0.3	+ 62.3
		MEAN	+ 238.4			+ 83.0

* Post-drug significantly different from pre-drug (Paired T-test: p<0.05)
** Post-drug significantly different from pre-drug (Paired T-test: p<0.05)

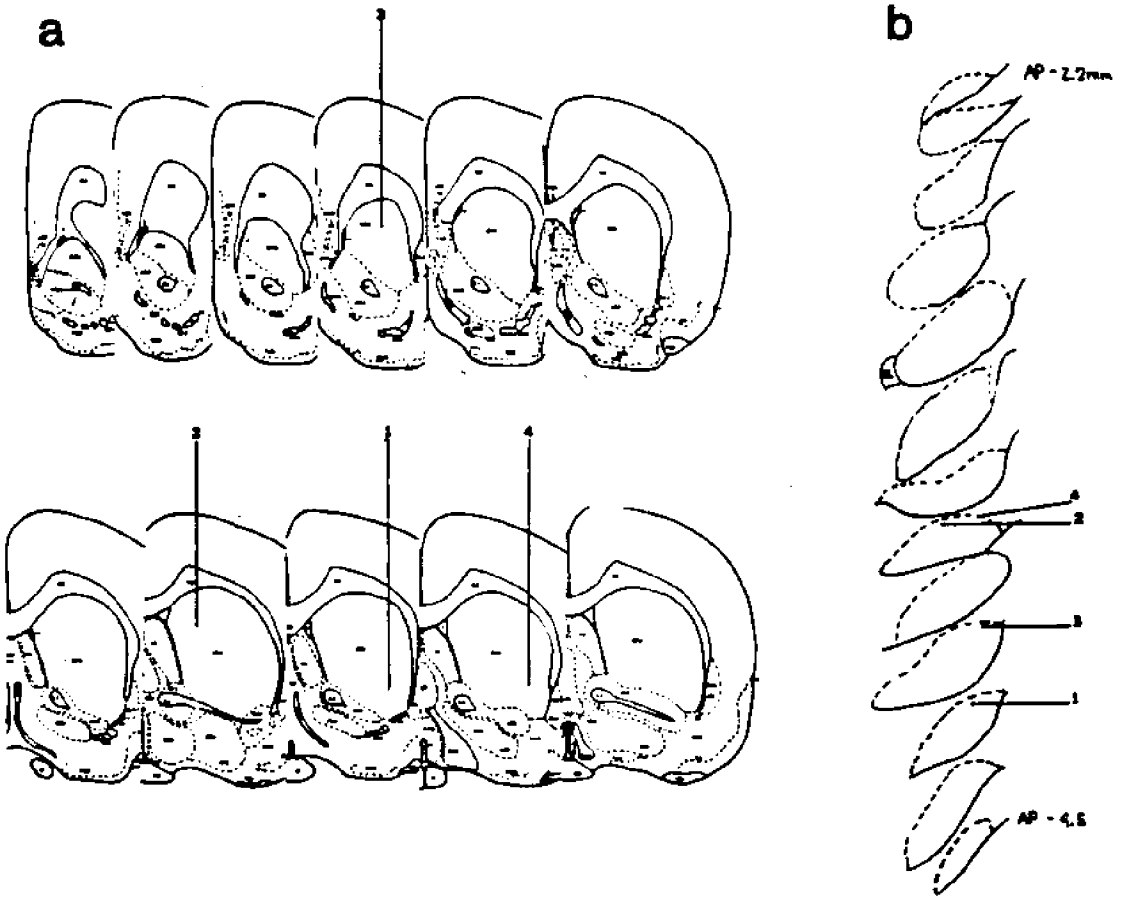


Table 7

Effect on non-dopaminergic cells of intracerebroventricular infusion of 0.5 umol CPAB

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	120	119	- 0.8	29.0 +/- 2.8	29.0 +/- 2.3	0
2	193	187	- 3.1	25.0 +/- 1.3	23.5 +/- 0.7	- 6.0
3	56	62	+ 10.7	22.0 +/- 0.4	19.8 +/- 0.6	- 10.0
		MEAN	+ 2.3			- 5.3

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

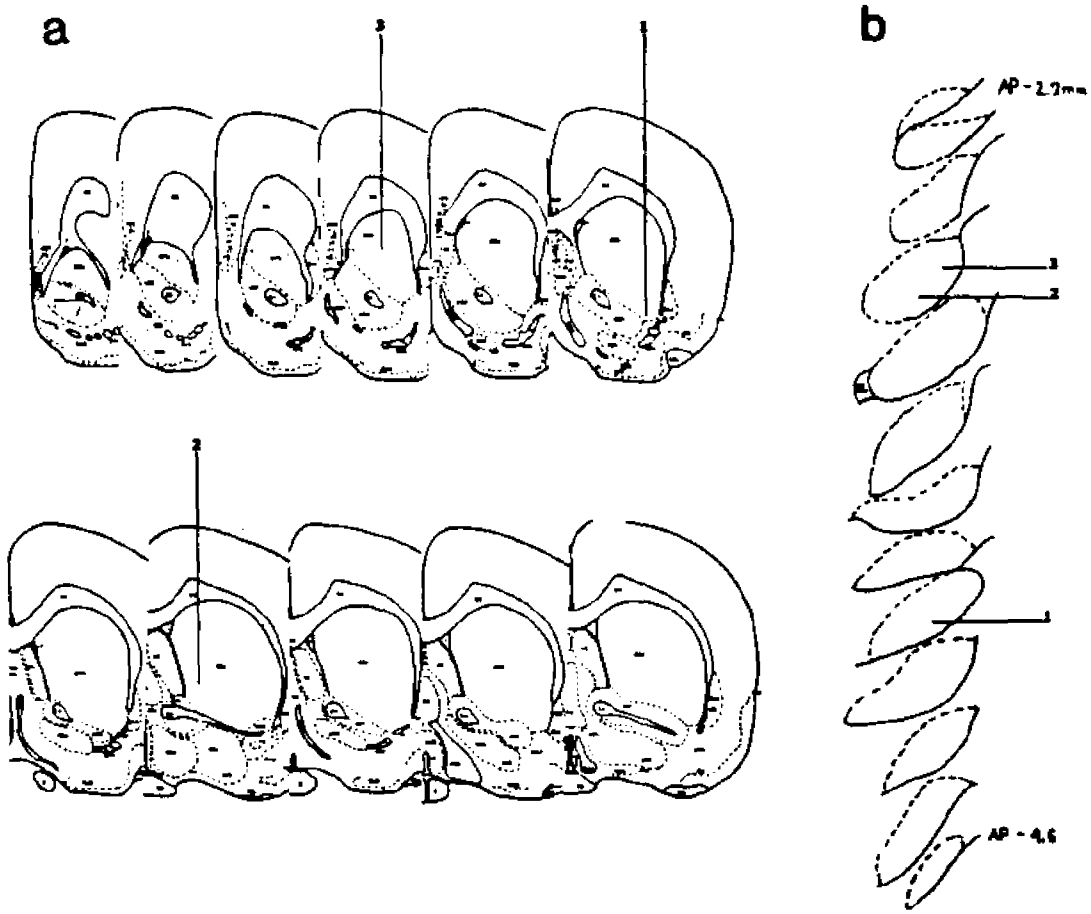


Table 8

Effect on dopaminergic cells of intracerebroventricular infusion of 0.5 umol CPAB

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	33	28	- 15.2	5.6 +/- 0.6	6.0 +/- 0.6	+ 3.2
2	63	65	+ 3.2	4.5 +/- 0.2	4.5 +/- 0.1	0
3	89	56	- 37.0	3.8 +/- 0.2	9.2 +/- 0.2	- 15.8
		MEAN	- 16.3			- 4.2

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

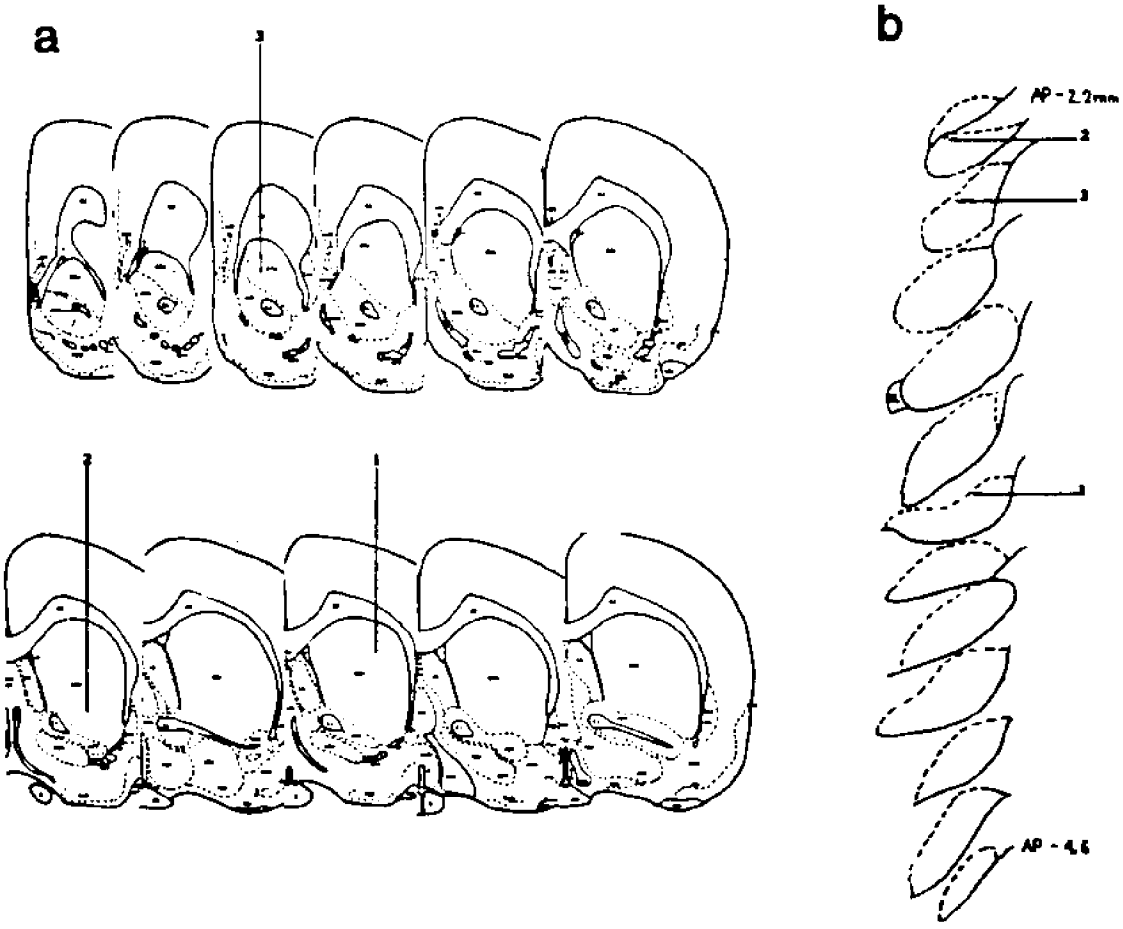


Table 9

Effect on non-dopaminergic cells of intracerebroventricular infusion of 1 umol CPLAB

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	172	184	+ 6.98	23 +/- 0.8	25 +/- 1.6	+ 8.7
2	91	98	+ 7.7	28 +/- 1.3	32 +/- 0.4	+ 14.3
3	261	282	+ 8.1	21 +/- 0.2	19.5 +/- 0.5	- 7.1
		MEAN	+ 7.6			+ 5.3

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

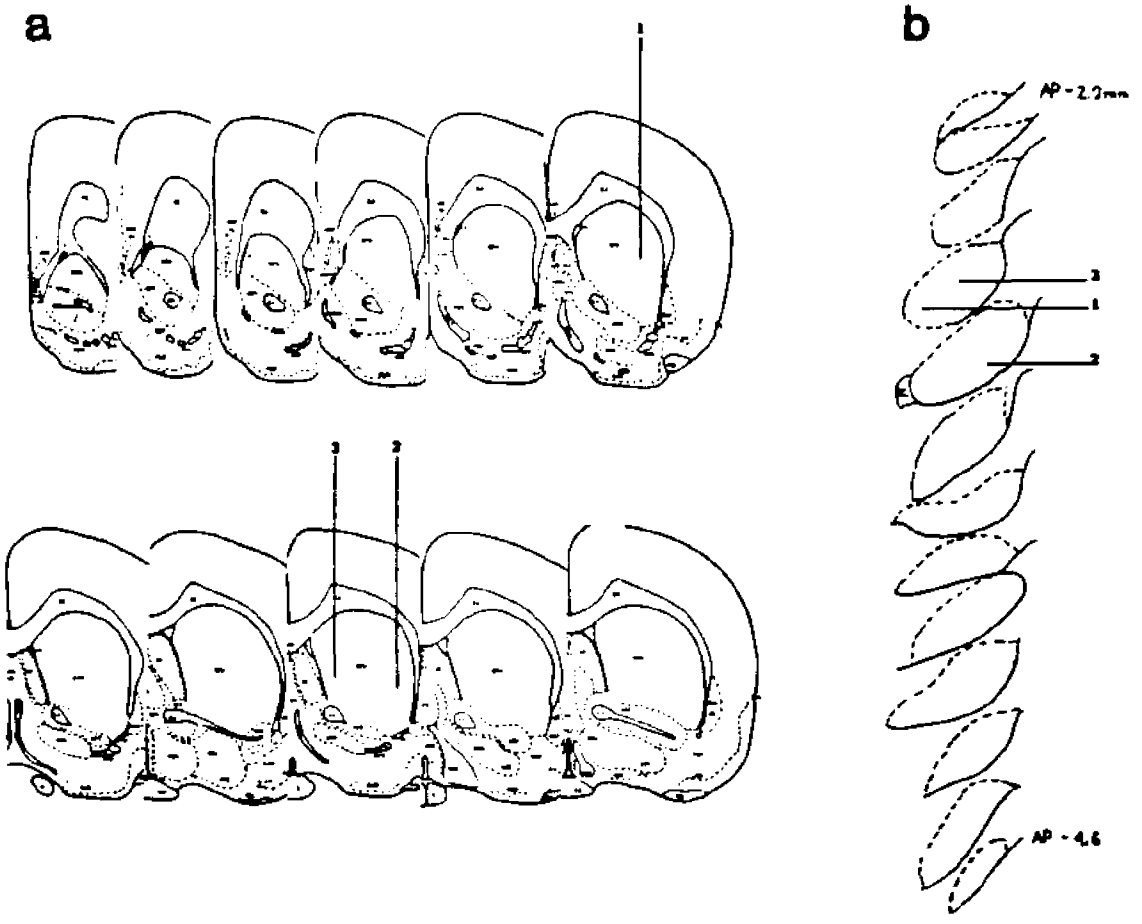


Table 10

Effect on dopaminergic cells of intracerebroventricular infusion of 1 umol CPLAB

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	38	32	- 15.8	12.0 +/- 0.1	10.5 +/- 0.2	- 12.5
2	18	20	+ 11.1	7.5 +/- 0.4	9.0 +/- 0.3	+ 20.0
3	98	93	- 5.1	4.5 +/- 0.3	5.0 +/- 0.5	+ 11.1
		MEAN	- 3.3			+ 6.2

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

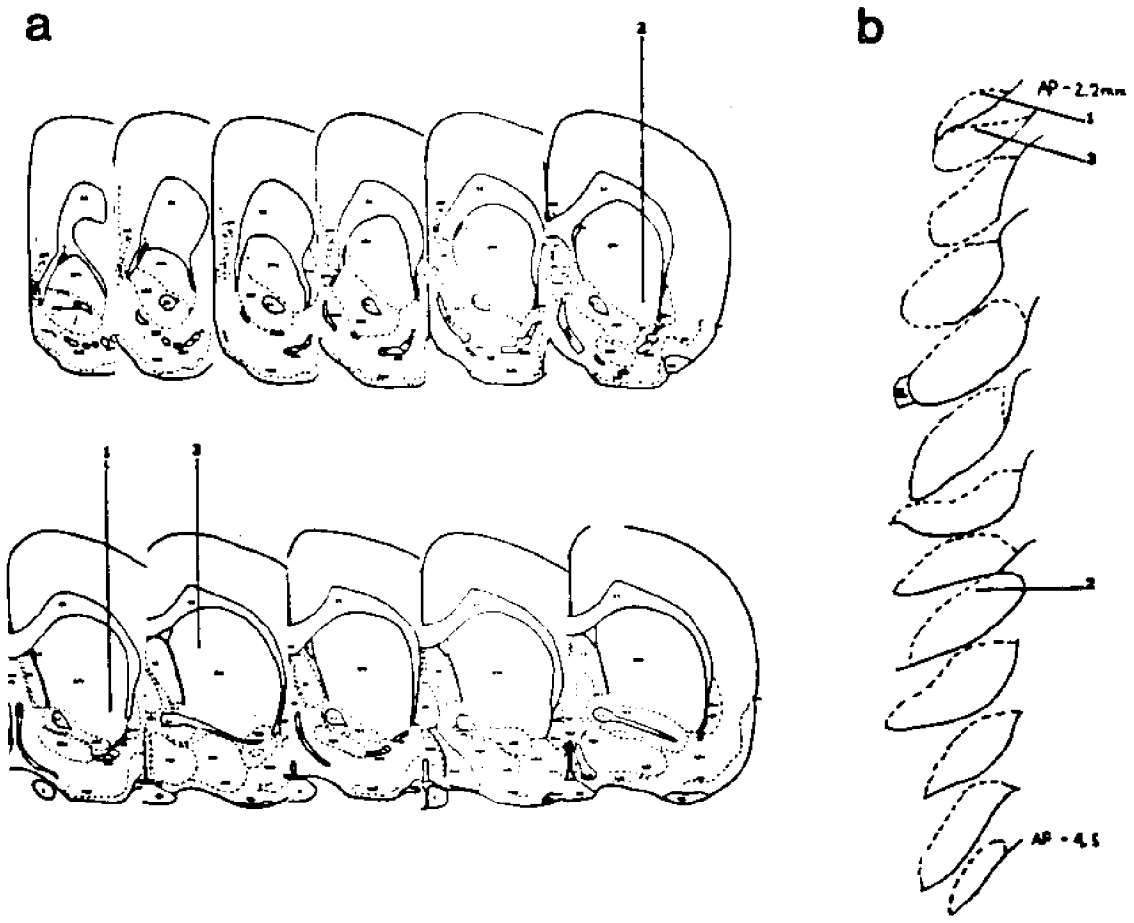


Table 11

Effect on non-dopaminergic cells of intracerebroventricular infusion of 0.9% saline* or H_2O*

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1*	79.5	82.0	+ 3.1	28.0 +/- 0.2	28.0 +/- 0.1	0
2*	185.0	168.0	- 9.2	32.8 +/- 1.3	34.0 +/- 1.0	+ 3.7
3*	54.5	59.3	+ 8.8	19.2 +/- 0.4	19.8 +/- 0.8	+ 3.1
4*	98.0	102.0	+ 4.0	22.0 +/- 1.5	25.0 +/- 0.9	+ 13.6
		MEAN	+ 1.7			+ 5.1

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

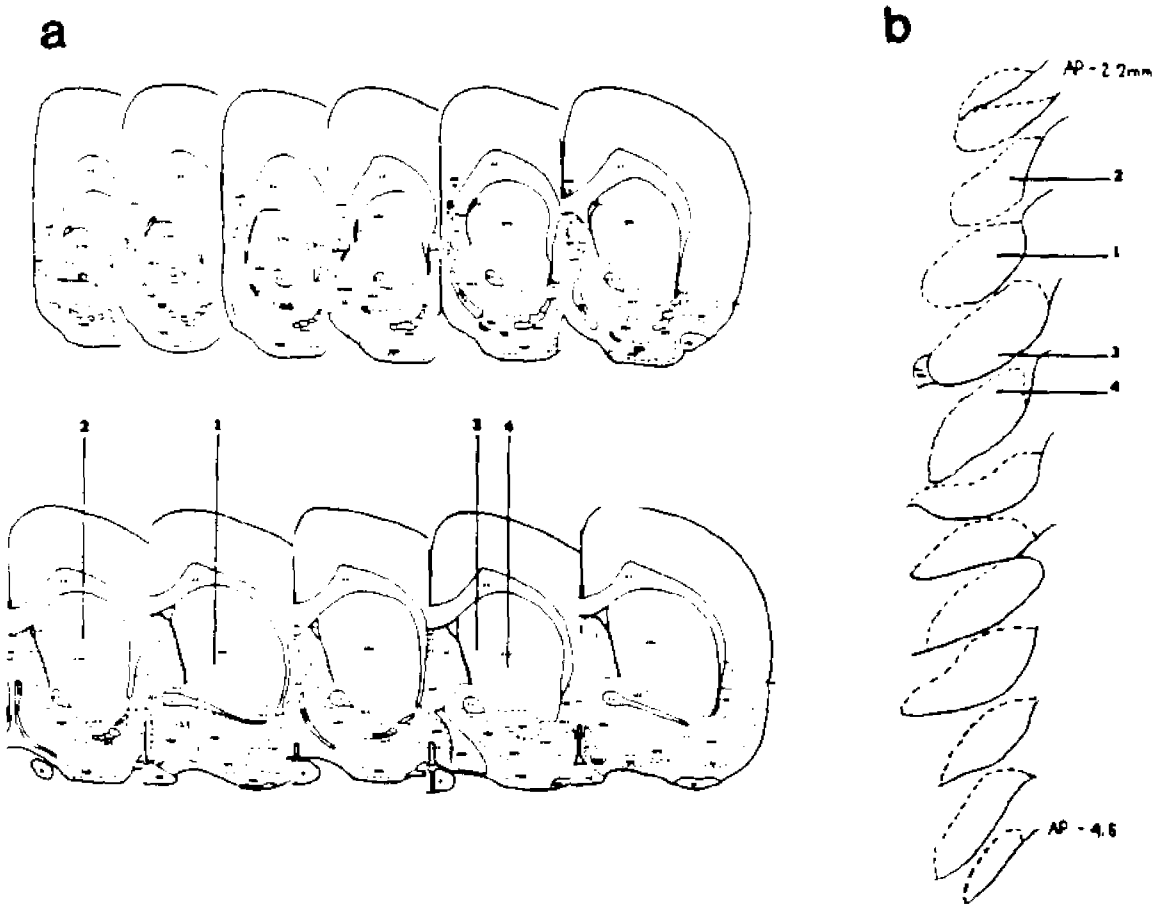


Table 12

Effect on dopaminergic cells of intracerebroventricular infusion of 0.9% saline* or H_2O**

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1*	78	71	- 8.9	4.3 +/- 0.2	4.9 +/- 0.2	+ 14.0
2**	129	136	+ 5.8	6.5 +/- 0.7	7.3 +/- 0.4	+ 12.3
3*	147	153	+ 4.1	7.0 +/- 0.1	7.0 +/- 0.1	0
		MEAN	+ 0.2			+ 8.8

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

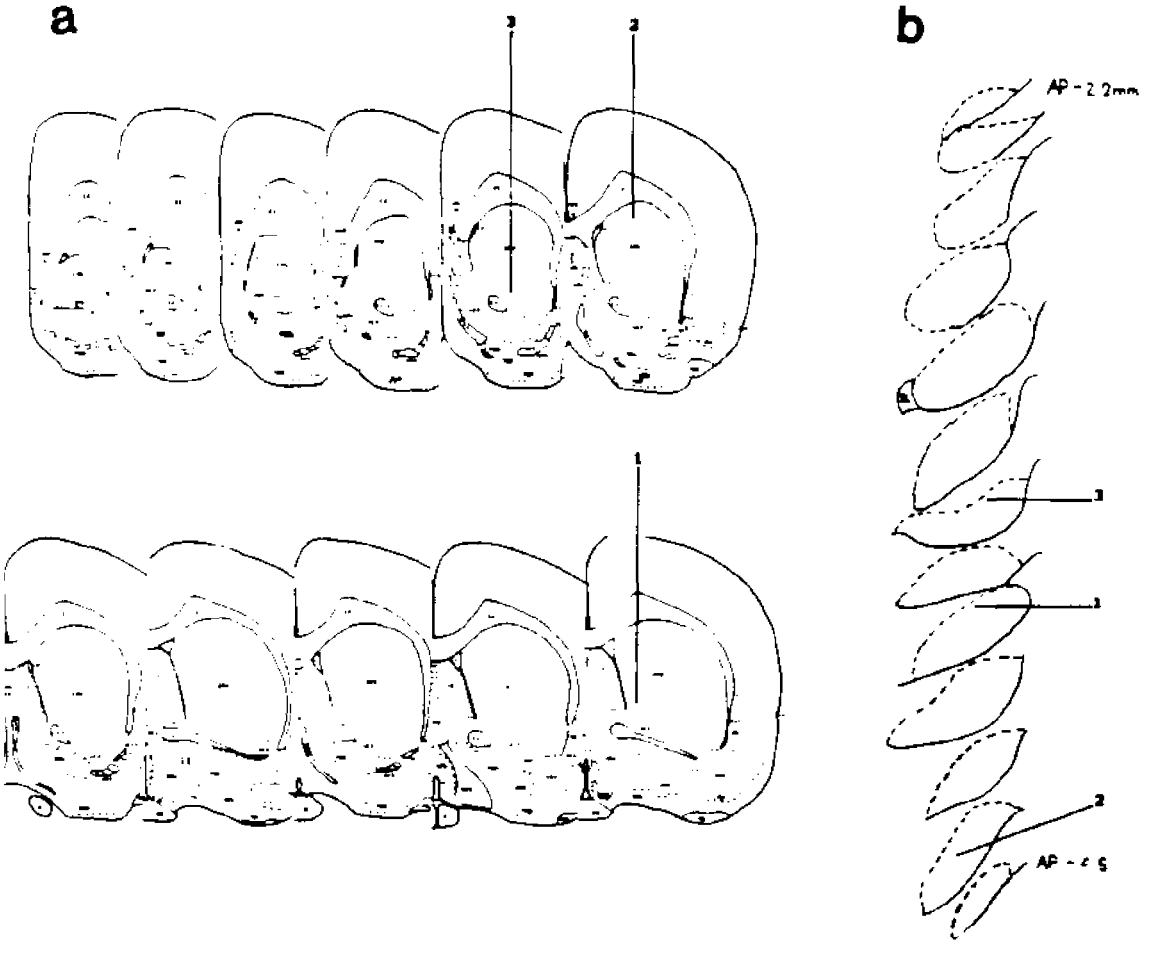


Table 14

Effect on non-dopaminergic cells of direct application of 0.04 umol CPAB into the substantia nigra.

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	96	148	+ 54.2	21.6 +/- 1.2	14.0 +/- 1.1	- 35.2
2	39	46	+ 17.9	25.0 +/- 0.4	25.0 +/- 0.3	0
3	108	137	+ 26.9	20.0 +/- 0.7	33.0 +/- 1.3	+ 65.0
4	41	57	+ 39.0	13.4 +/- 1.0	16.8 +/- 0.8	+ 25.4
5	171	229	+ 33.9	19.0 +/- 1.2	23.0 +/- 1.5	+ 21.1
6	227	309	+ 36.1	27.0 +/- 2.1	31.0 +/- 3.4	+ 14.8
		MEAN	+ 34.7			+ 15.2

* Post-drug significantly different from pre-drug (Paired T-test: $p<0.05$)
** Post-drug not significantly different from pre-drug (Paired T-test)

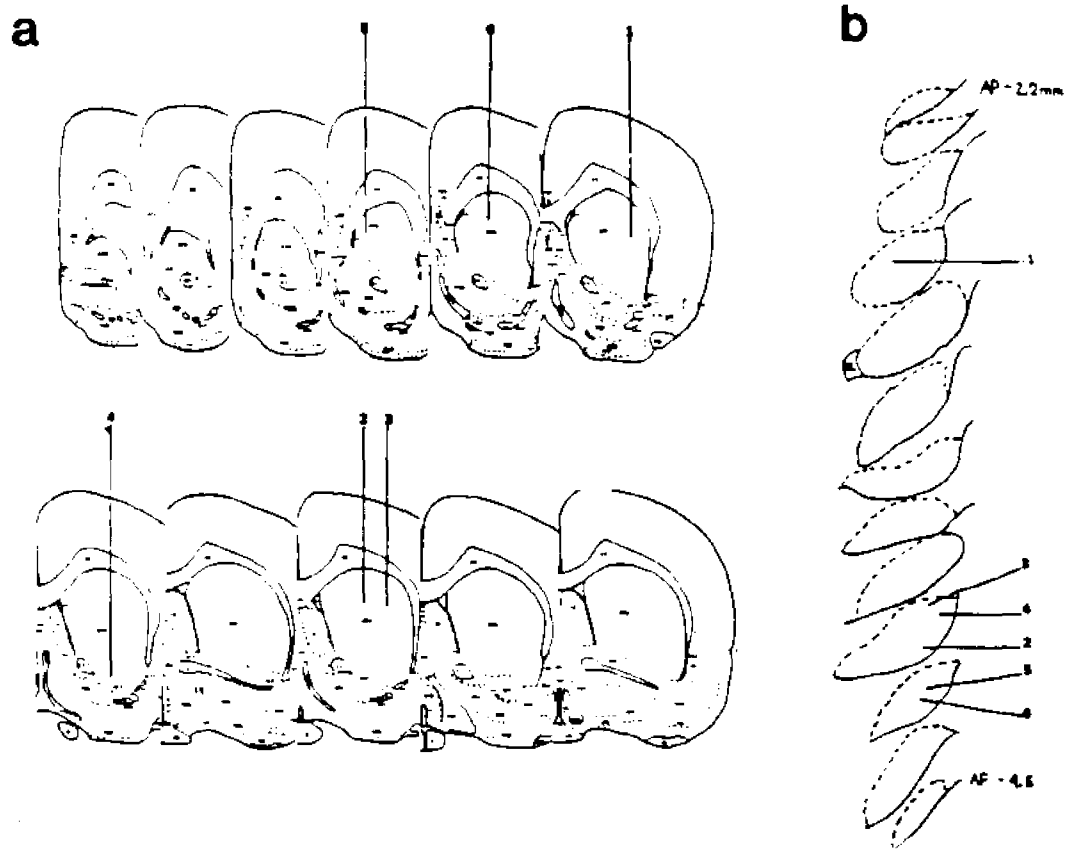


Table 15

Effect on dopaminergic cells of direct application of 0.04 umol CPAB into the substantia nigra.

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	24	32	+ 33.3	4.2 +/- 0.5	6.0 +/- 0.6	+ 42.9
2	73	91	+ 24.7	10.0 +/- 0.2	10.3 +/- 0.1	+ 3.0
3	273	310	+ 13.6	2.5 +/- 0.7	3.5 +/- 0.4	+ 40.0
		MEAN	+ 23.8			+ 28.6

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

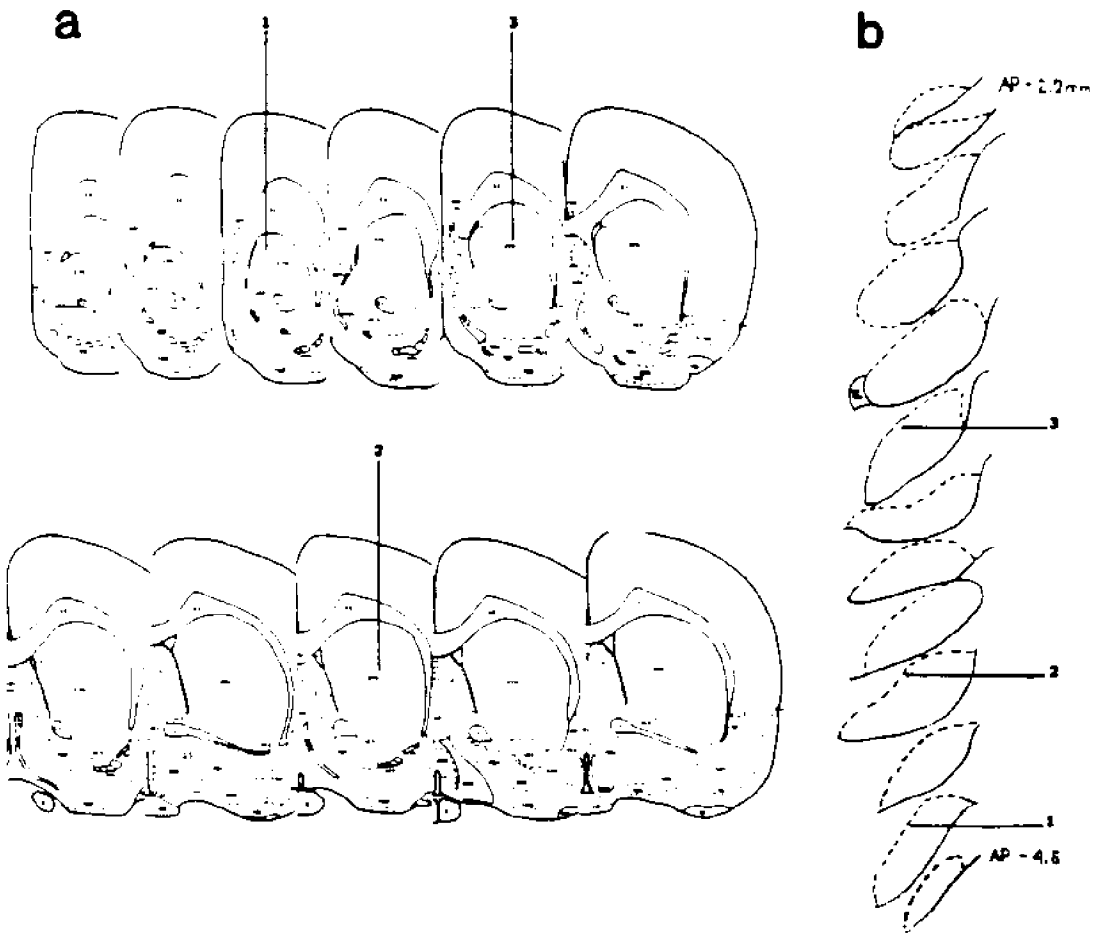


Table 16

Effect on non-dopaminergic cells of direct application of 0.04 umol CPLAB into the substantia nigra.

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	273	261	- 4.4	28 +/- 2.3	31 +/- 1.8	+ 10.7
2	190	184	- 3.2	12 +/- 1.5	15 +/- 1.3	+ 25.0
3	92	84	- 8.7	25 +/- 0.4	25 +/- 0.1	0
		MEAN	- 5.4			+ 11.9

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

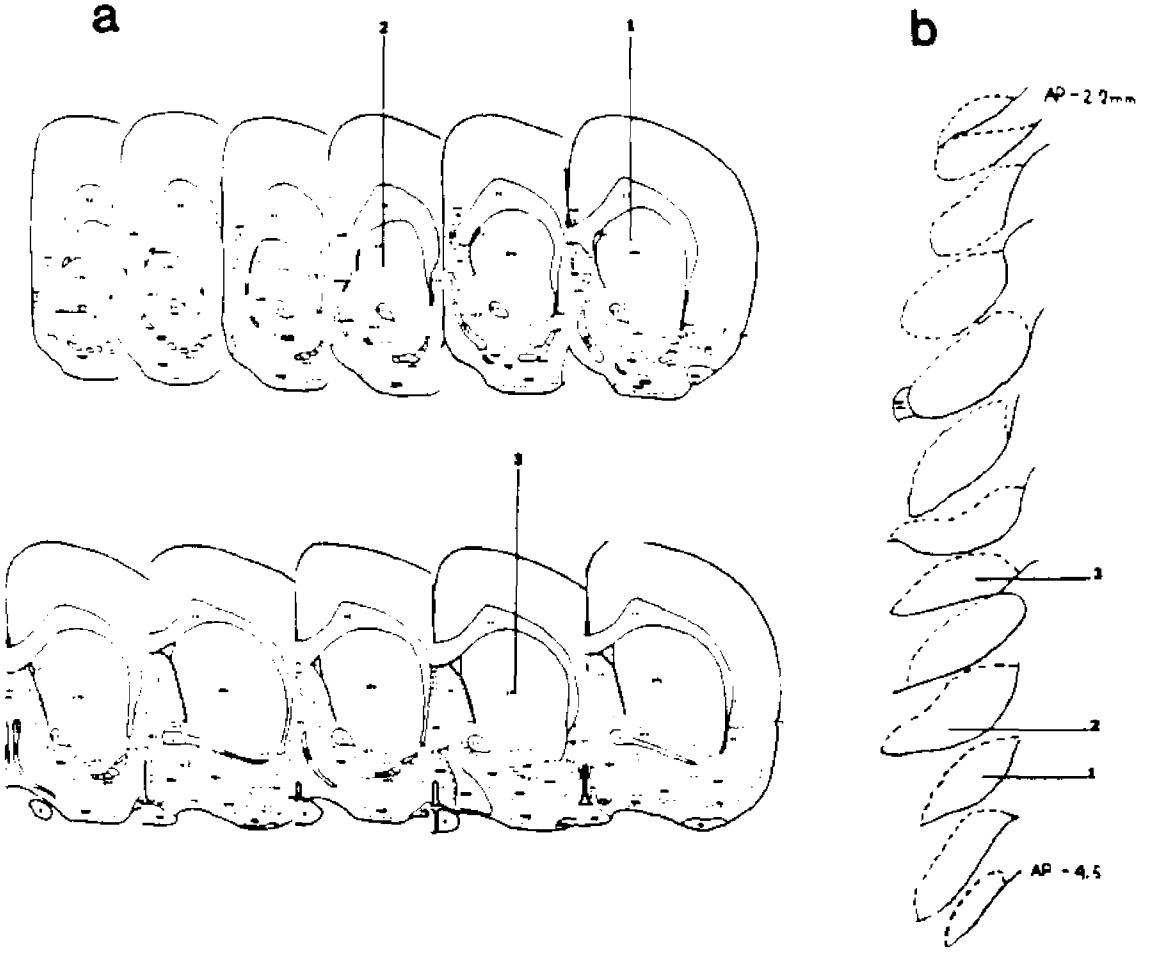


Table 17

Effect on dopaminergic cells of direct application of 0.04 umol CPLAB into the substantia nigra.

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	28	29	+ 3.6	4.2 +/- 0.3	4.8 +/- 0.1	+ 14.3
2	68	72	+ 5.8	5.0 +/- 0.2	5.5 +/- 0.2	+ 10.0
		MEAN	+ 4.7			+ 12.1

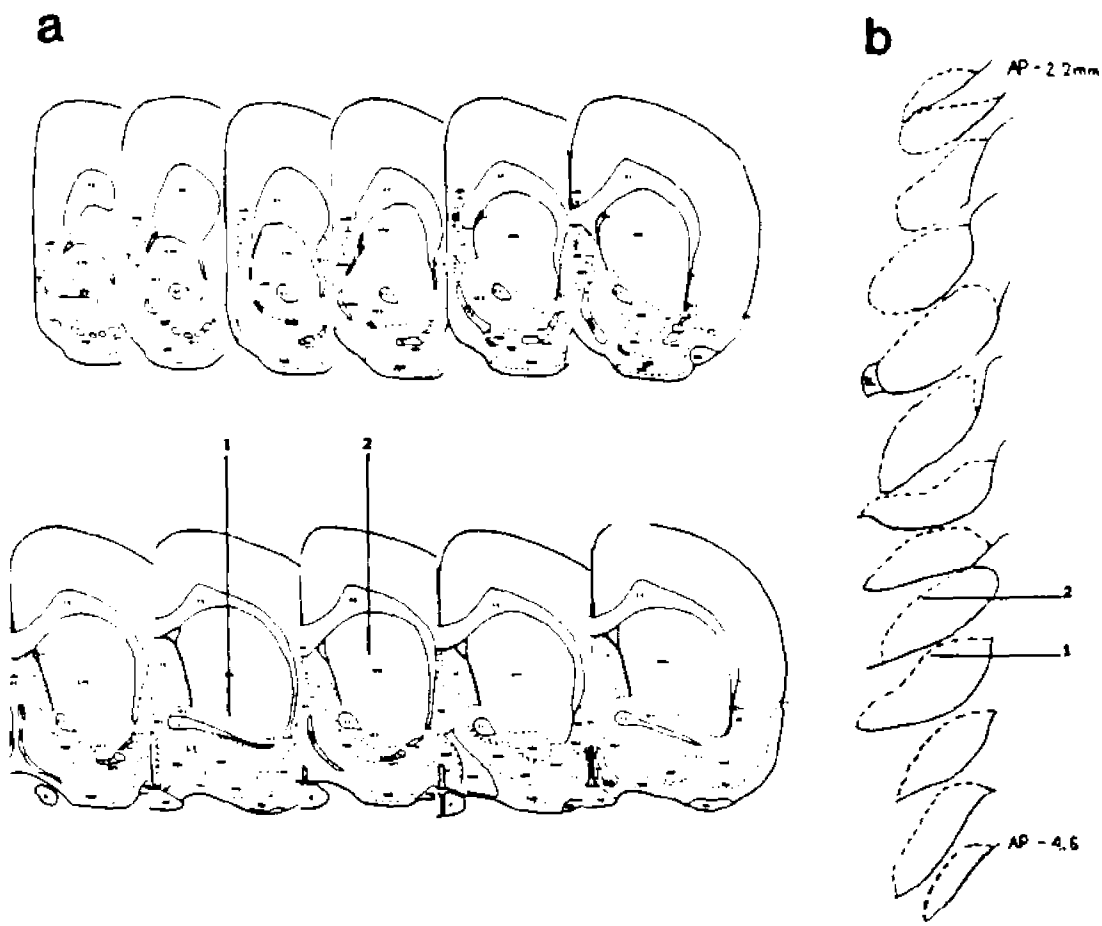


Table 18

Effect on non-dopaminergic cells of direct application of 0.9% saline* or H_2O* into the substantia nigra

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1*	81	83	+ 2.5	30.0 +/- 0.3	30.0 +/- 0.2	0
2*	83	80	- 3.6	37.0 +/- 1.7	32.3 +/- 1.8	- 12.7
3*	101	109	+ 7.9	20.0 +/- 0.5	19.0 +/- 0.8	- 5.0
4*	174	182	+ 4.6	12.0 +/- 1.2	14.5 +/- 1.9	+ 20.8
5*	84	82	- 2.4	25.0 +/- 0.1	24.0 +/- 0.4	- 4.0
		MEAN	+ 1.0			- 0.2

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

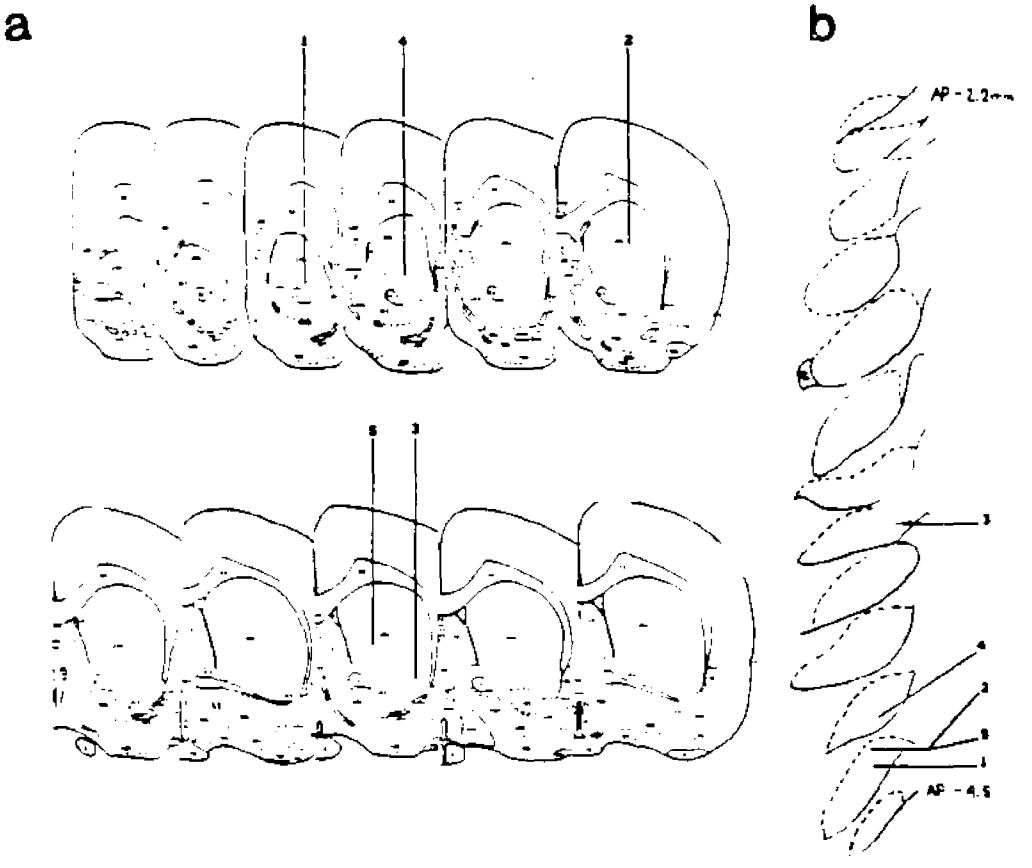


Table 19

Effect on dopaminergic cells of direct application of 0.9% saline* or H_2O* into the substantia nigra

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1*	21	26	+ 19.0	4.2 +/- 0.8	6.0 +/- 1.3	+ 42.9
2*	68	64	- 5.8	5.0 +/- 0.2	5.2 +/- 0.1	+ 4.0
		MEAN	+ 12.4			+ 23.5

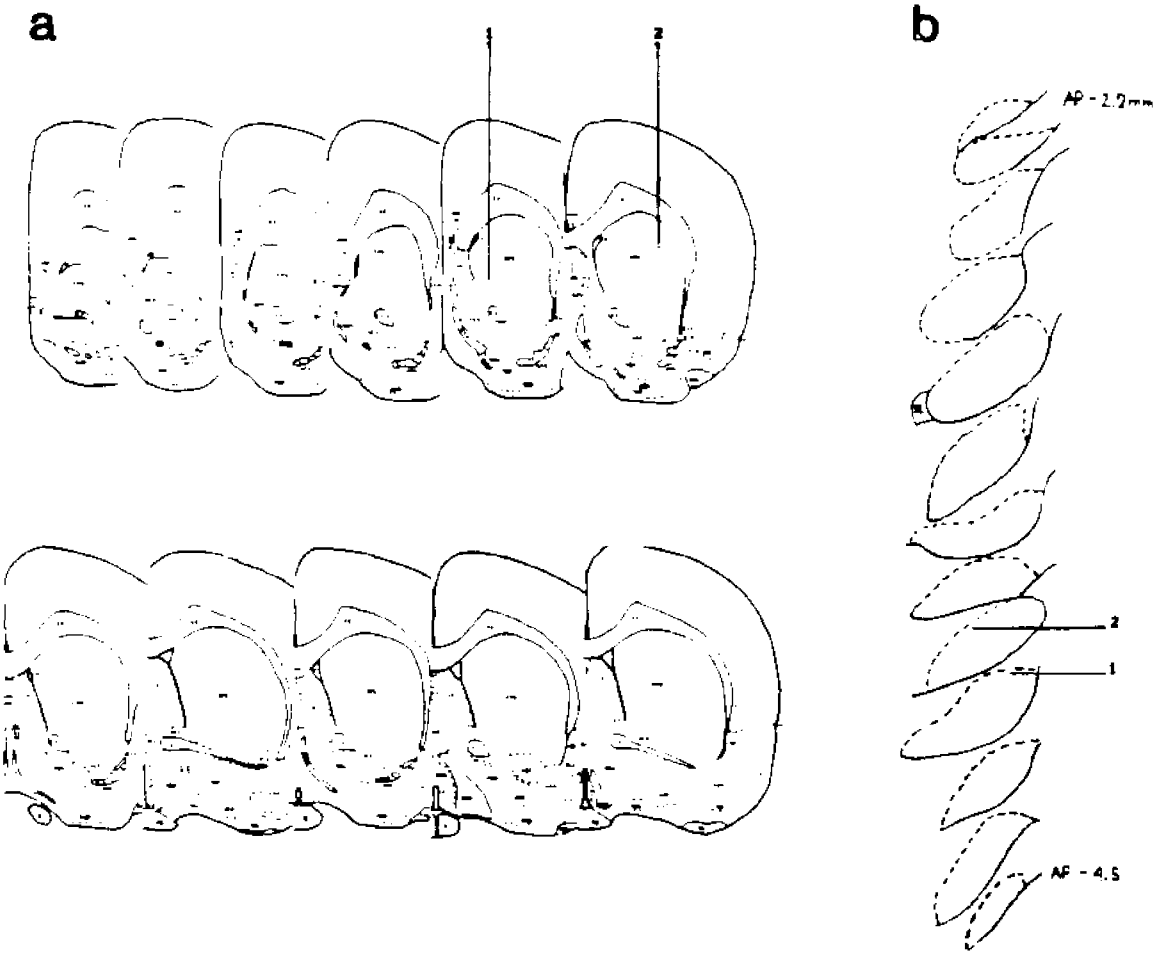


Table 20

Effect on non-dopaminergic cells of direct application of 0.04 umol CPAB into the caudate-putamen

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	63	75	+ 19.0	27.0 +/- 2.4	32.0 +/- 2.9	+ 18.5
2	277	302	+ 9.0	26.0 +/- 1.9	38.0 +/- 1.6	+ 52.0
3	193	198	+ 2.6	17.5 +/- 1.3	23.0 +/- 0.8	+ 31.4
4	68	103	+ 51.5	20.0 +/- 1.7	26.5 +/- 1.4	+ 32.5
		MEAN	+ 20.5			+ 33.5

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug significantly different from pre-drug (Paired T-test: $p<0.05$)

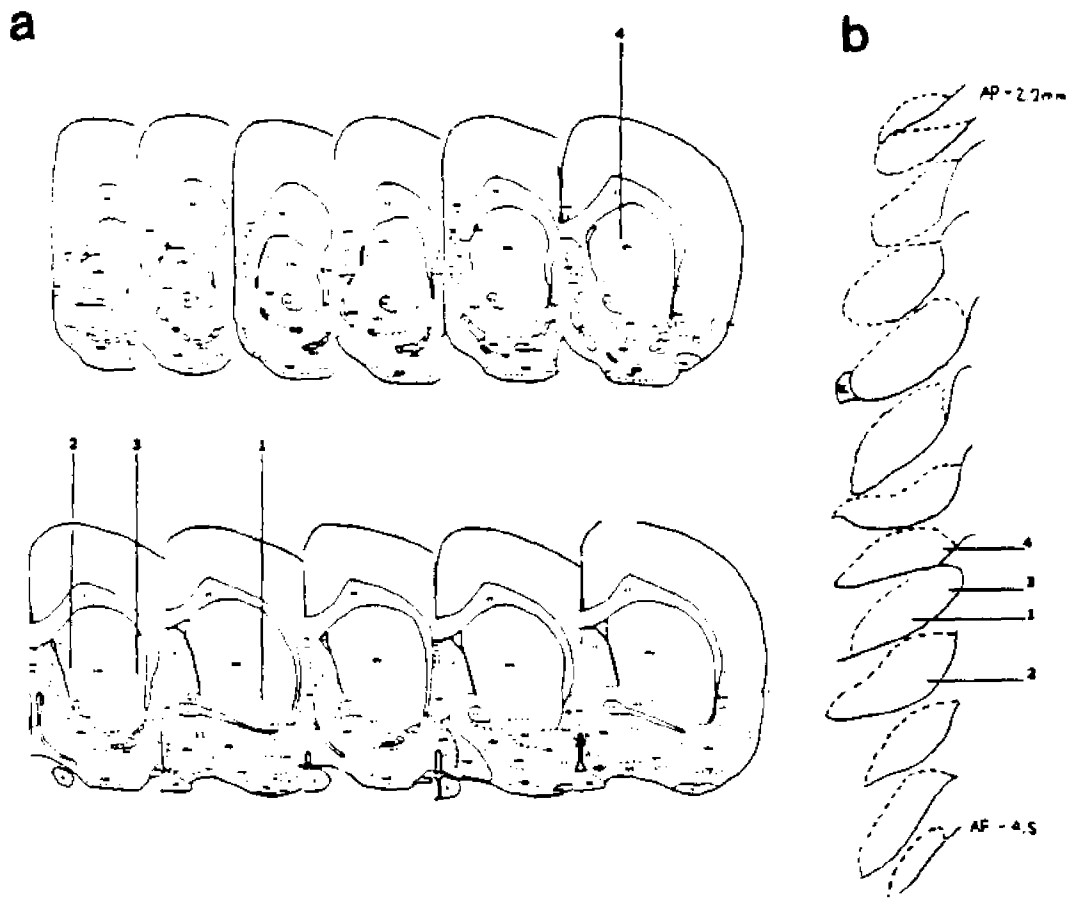


Table 21

Effect on dopaminergic cells of direct application of 0.04 umol CPAB into the caudate-putamen

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	53	92	+ 73.6	10.1 +/- 1.1	14.2 +/- 1.3	+ 40.6
2	189	291	+ 54.0	5.6 +/- 0.8	6.9 +/- 0.5	+ 23.2
3	171	281	+ 64.3	8.2 +/- 0.2	10.5 +/- 0.1	+ 28.0
4	90	168	+ 86.7	7.7 +/- 0.9	12.0 +/- 0.7	+ 55.9
		MEAN	+ 69.6			+ 36.9

* Post-drug significantly different from pre-drug (Paired T-test: $p<0.05$)
** Post-drug significantly different from pre-drug (Paired T-test: $p<0.05$)

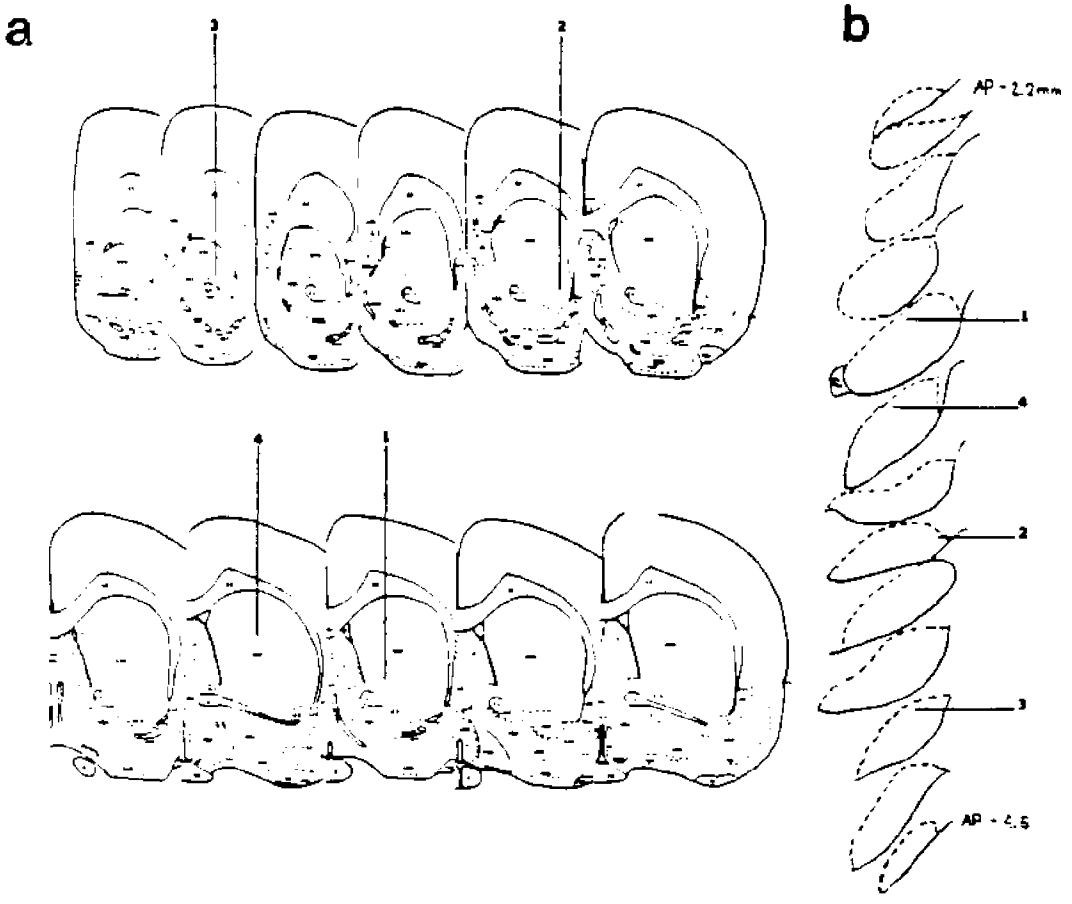


Table 22

Effect on non-dopaminergic cells of direct application of 0.04 umol CPLAB into the caudate-putamen

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	186	192	+ 3.2	18.5 +/- 2.2	20.0 +/- 1.7	+ 8.1
2	110	86	- 21.0	25.0 +/- 0.3	23.0 +/- 0.2	- 8.0
3	245	252	+ 2.9	32.5 +/- 1.4	28.0 +/- 1.6	- 13.9
4	85	84	- 1.2	12.0 +/- 0.1	12.5 +/- 0.3	+ 4.2
		MEAN	- 4.2			- 2.4

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

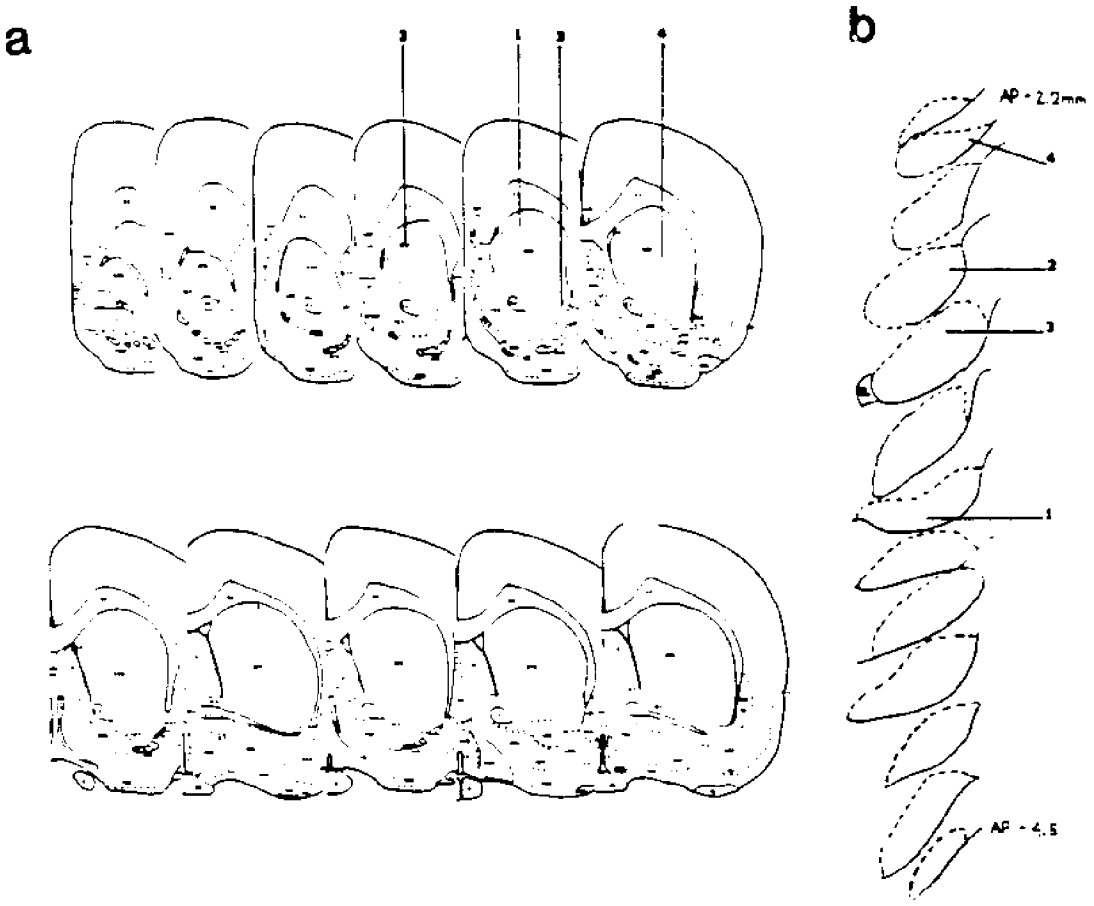


Table 23

Effect on dopaminergic cells of direct application of 0.04 umol CPLAB into the caudate-putamen

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	16.0	20.0	+ 25.0	5.3 +/- 0.3	5.9 +/- 0.4	+ 11.3
2	37.0	34.5	- 6.8	4.5 +/- 0.1	3.9 +/- 0.1	- 13.3
		MEAN	+ 9.1			- 1.0

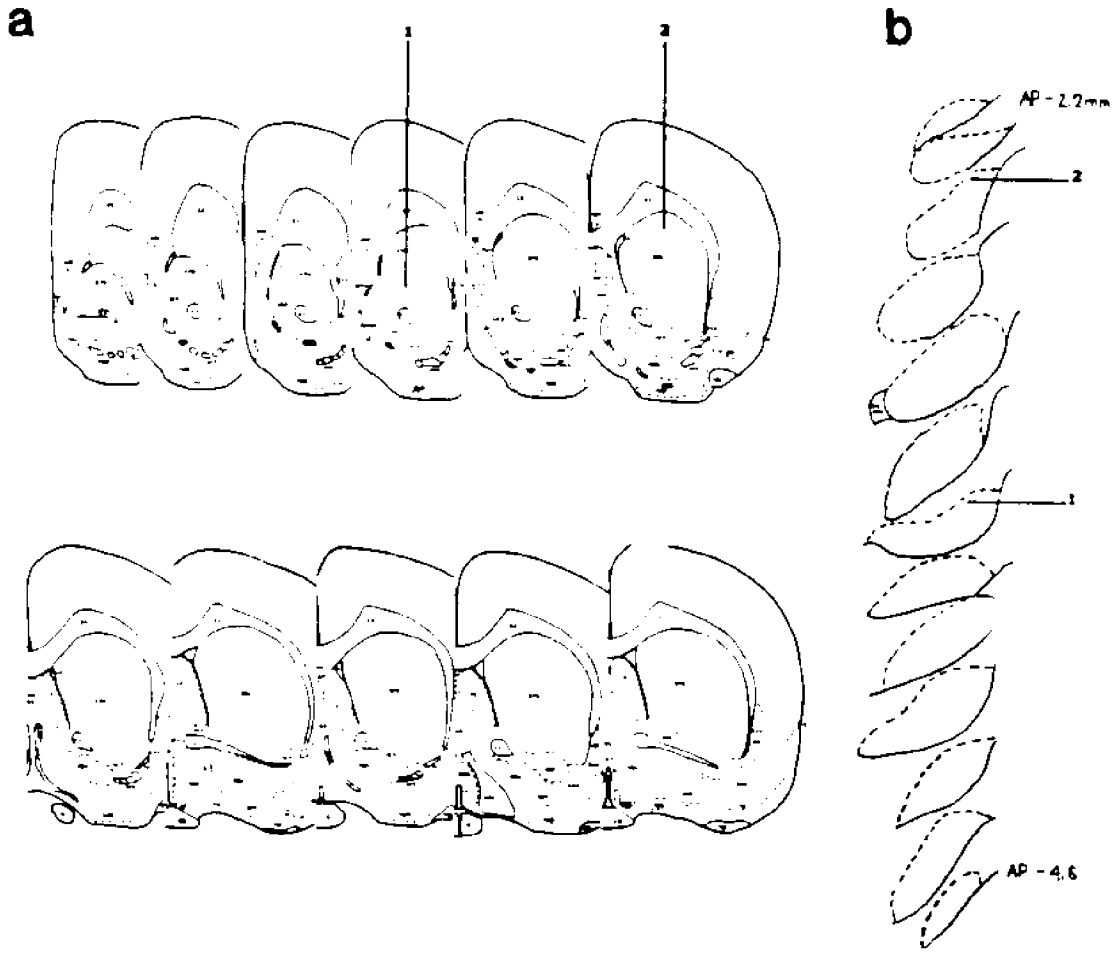


Table 24

Effect on dopaminergic cells of direct application of 0.9% saline* or H_2O** into the caudate-putamen

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1*	23	27	+ 17.4	5.0 +/- 0.2	4.8 +/- 0.4	- 4.0
2*	38	46	+ 21.1	3.5 +/- 0.3	3.5 +/- 0.1	0
3*	248	251	+ 1.2	9.0 +/- 1.2	11.5 +/- 1.7	+ 27.8
		MEAN	+ 13.2			+ 7.9

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

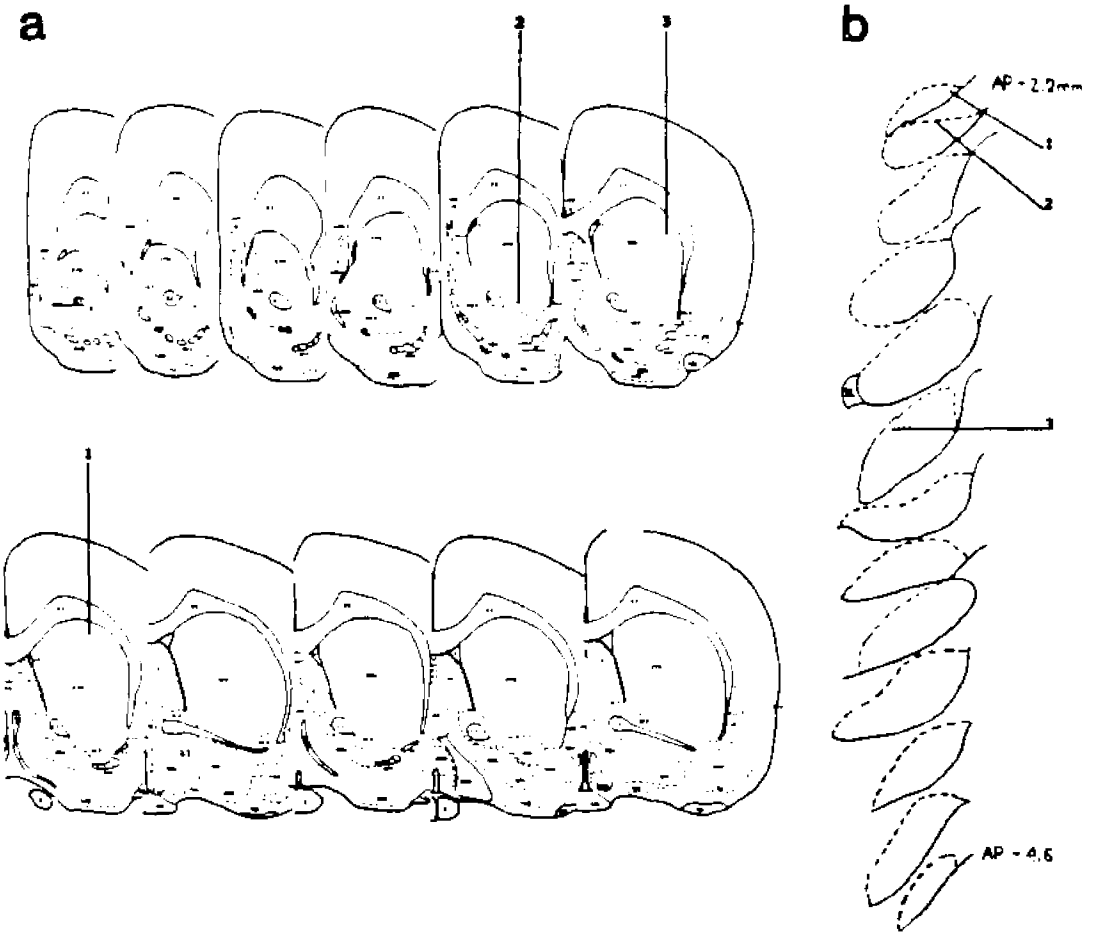


Table 25

Effect on non-dopaminergic cells of direct application of 0.9% saline* or H_2O* into the caudate-putamen

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1*	84	92	+ 9.5	12.5 +/- 0.8	15.0 +/- 0.6	+ 20.0
2*	89	103	+ 15.7	23.5 +/- 0.5	25.0 +/- 0.5	+ 6.4
3*	167	173	+ 3.6	26.0 +/- 1.0	29.0 +/- 1.7	+ 11.5
4*	81	97	+ 19.8	18.0 +/- 2.3	21.5 +/- 1.9	+ 19.4
		MEAN	+ 12.2			+ 14.3

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

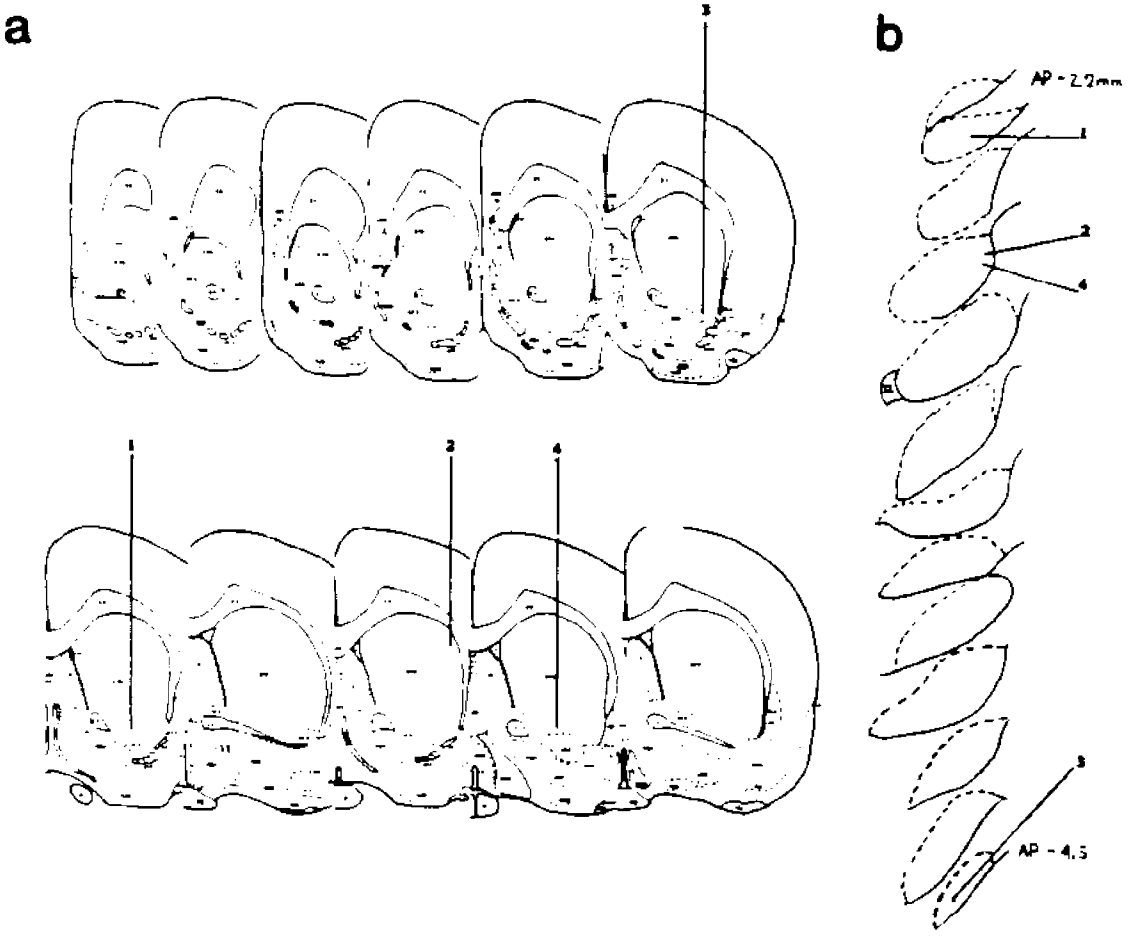


Table 26

Effect on non-dopaminergic cells of naloxone 1 mg/kg, i.v.

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	250	237	- 5.2	19.5 +/- 1.3	19.0 +/- 1.7	- 2.6
2	118	110	- 6.8	32.0 +/- 2.9	32.0 +/- 3.3	0
3	363	321	- 11.6	29.4 +/- 0.5	28.9 +/- 0.2	- 1.7
4	173	135	- 22.0	25.6 +/- 0.2	26.1 +/- 0.1	+ 2.0
		MEAN	- 11.4			- 0.6

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

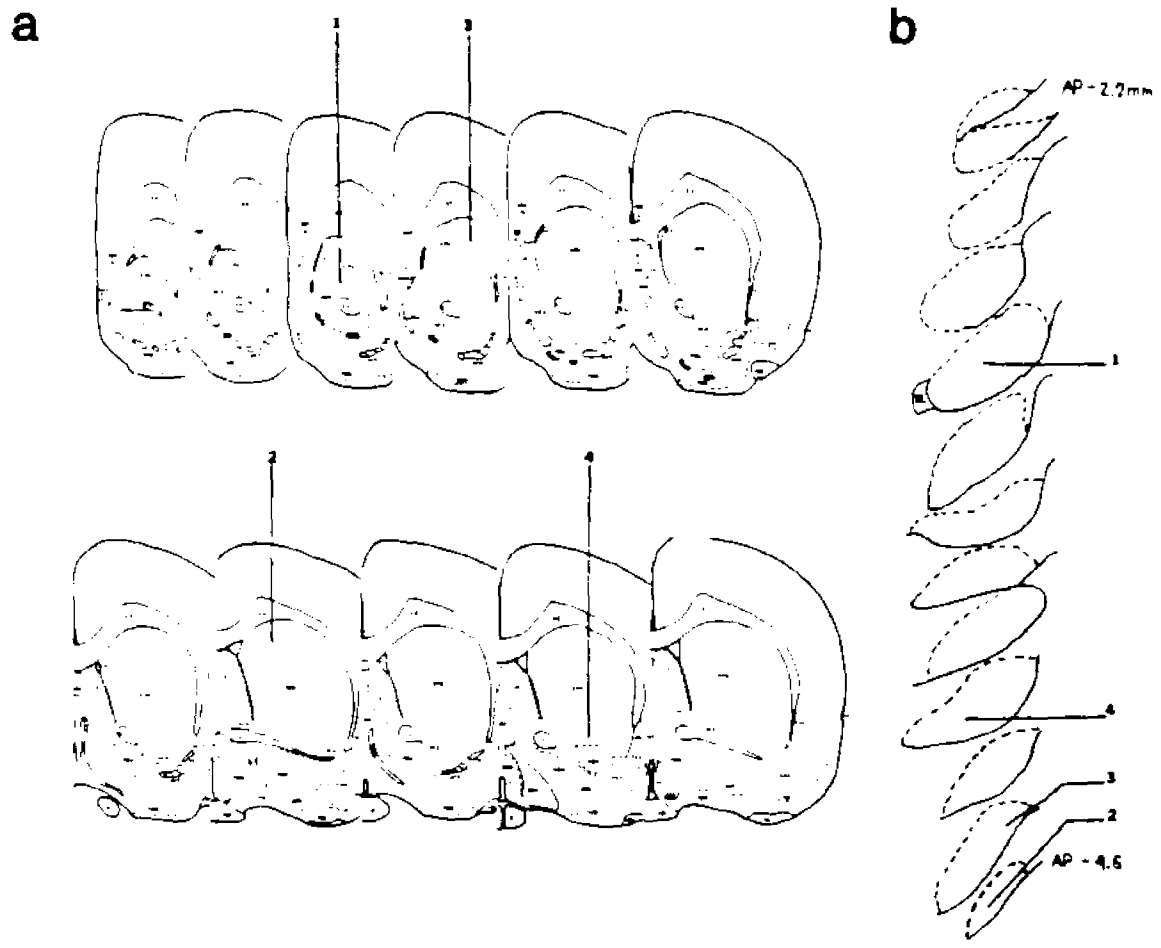


Table 27

Effect on dopaminergic cells of naloxone 1 mg/kg, i.v.

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	20	22.5	+ 12.5	4.3 +/- 0.4	3.8 +/- 1.4	- 11.6
2	76	73.0	- 2.7	12.2 +/- 1.0	12.8 +/- 0.7	+ 4.9
3	38	33.0	- 13.2	9.5 +/- 0.8	9.3 +/- 0.5	- 2.1
4	92	103.0	+ 12.0	5.3 +/- 0.9	5.8 +/- 1.3	+ 9.4
		MEAN	+ 2.2			+ 0.2

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

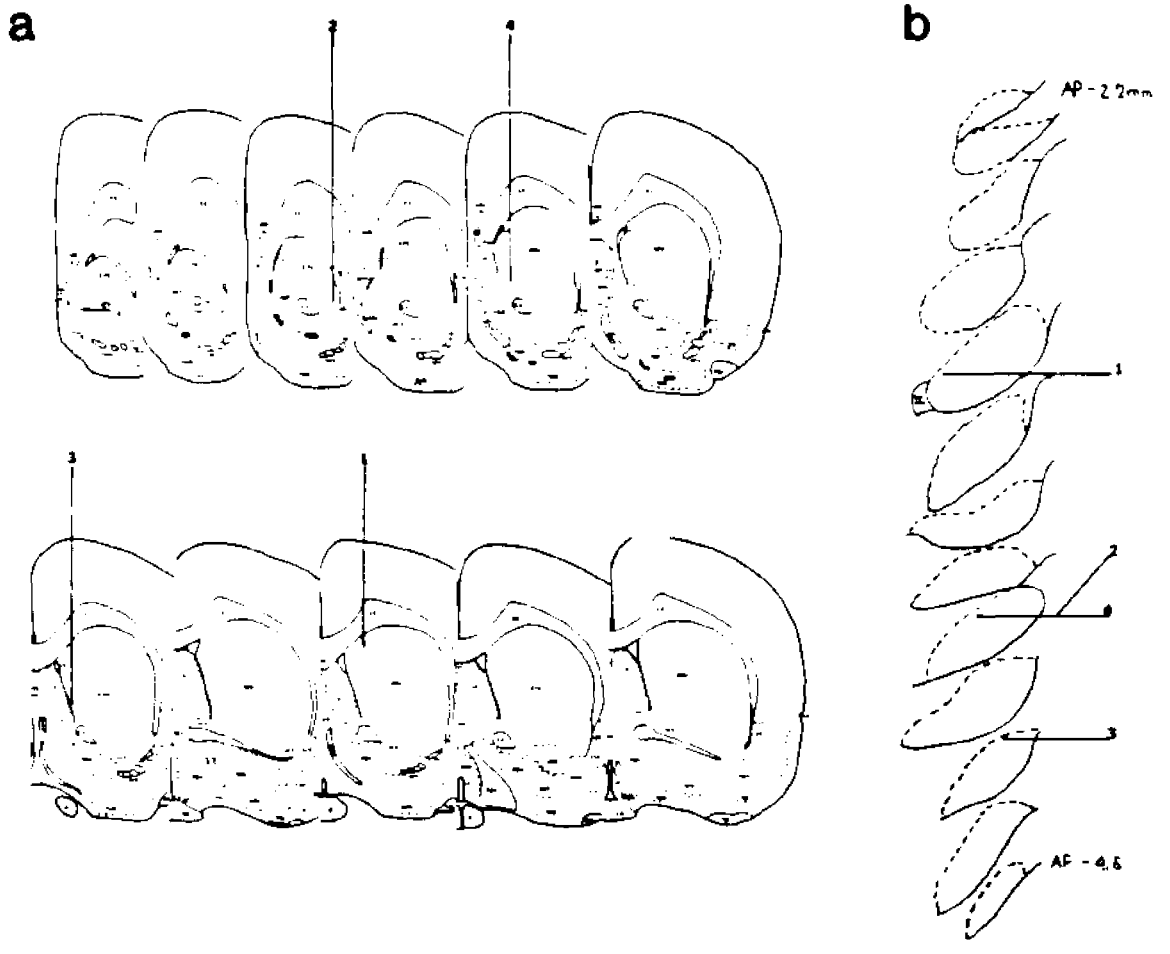


Table 28

Effect on non-dopaminergic cells of intracerebroventricular infusion of 1 umol CPAB and naloxone 1 mg/kg, i.v

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	272	386	+ 41.9	19.5 +/- 1.7	24.0 +/- 2.1	+ 23.1
2	150	211	+ 40.7	31.5 +/- 2.8	40.2 +/- 3.0	+ 27.6
3	387	492	+ 27.1	28.6 +/- 2.3	36.0 +/- 2.9	+ 22.4
4	131	177	+ 35.1	26.5 +/- 1.3	29.5 +/- 0.8	+ 11.3
		MEAN	+ 32.6			+ 21.3

* Post-drug significantly different from pre-drug (Paired T-test: $p<0.05$)
** Post-drug significantly different from pre-drug (Paired T-test: $p<0.05$)

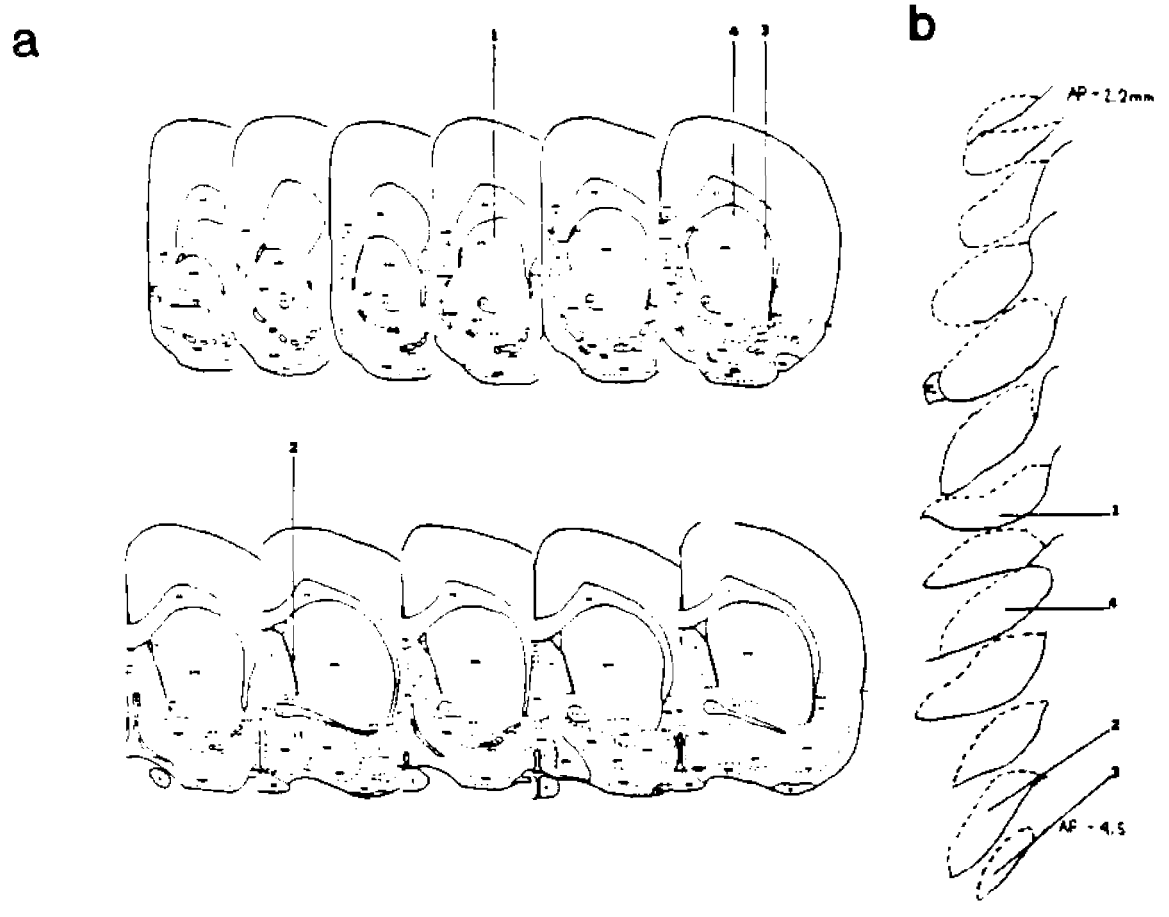


Table 29

Effect on dopaminergic cells of intracerebroventricular infusion of 1 umol CPAB and naloxone 1 mg/kg, i.v

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	23.0	14.4	- 37.4	4.4 +/- 0.6	7.7 +/- 0.4	+ 75.0
2	63.0	28.6	- 54.6	12.6 +/- 1.1	15.2 +/- 1.?	+ 20.6
3	42.5	32.0	- 24.7	9.0 +/- 0.3	18.6 +/- 0.6	+ 106.7
4	91.0	62.0	- 31.9	5.9 +/- 0.7	12.4 +/- 0.7	+ 110.2
		MEAN	- 37.1			+ 78.1

* Post-drug significantly different from pre-drug (Paired T-test: p<0.05)
** Post-drug significantly different from pre-drug (Paired T-test: p<0.05)

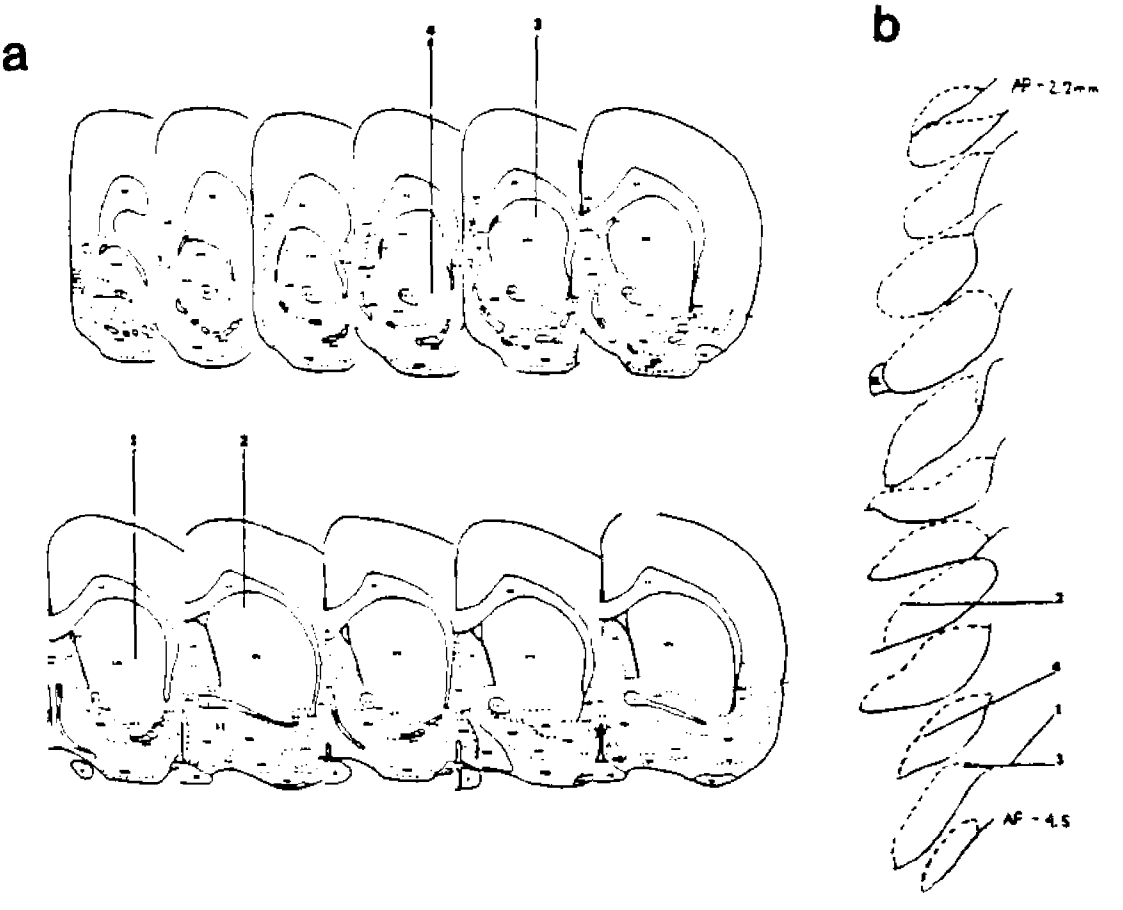


Table 30

Effect on non-dopaminergic cells of direct application of 0.002 nmol naloxone into the substantia nigra

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	147	136	- 7.5	22.0 +/- 1.3	23.5 +/- 1.5	+ 6.8
2	259	251	- 3.1	31.5 +/- 2.5	32.0 +/- 2.6	+ 1.6
3	89	87	- 2.3	19.5 +/- 0.8	18.5 +/- 0.2	- 5.1
		MEAN	- 4.3			+ 1.1

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

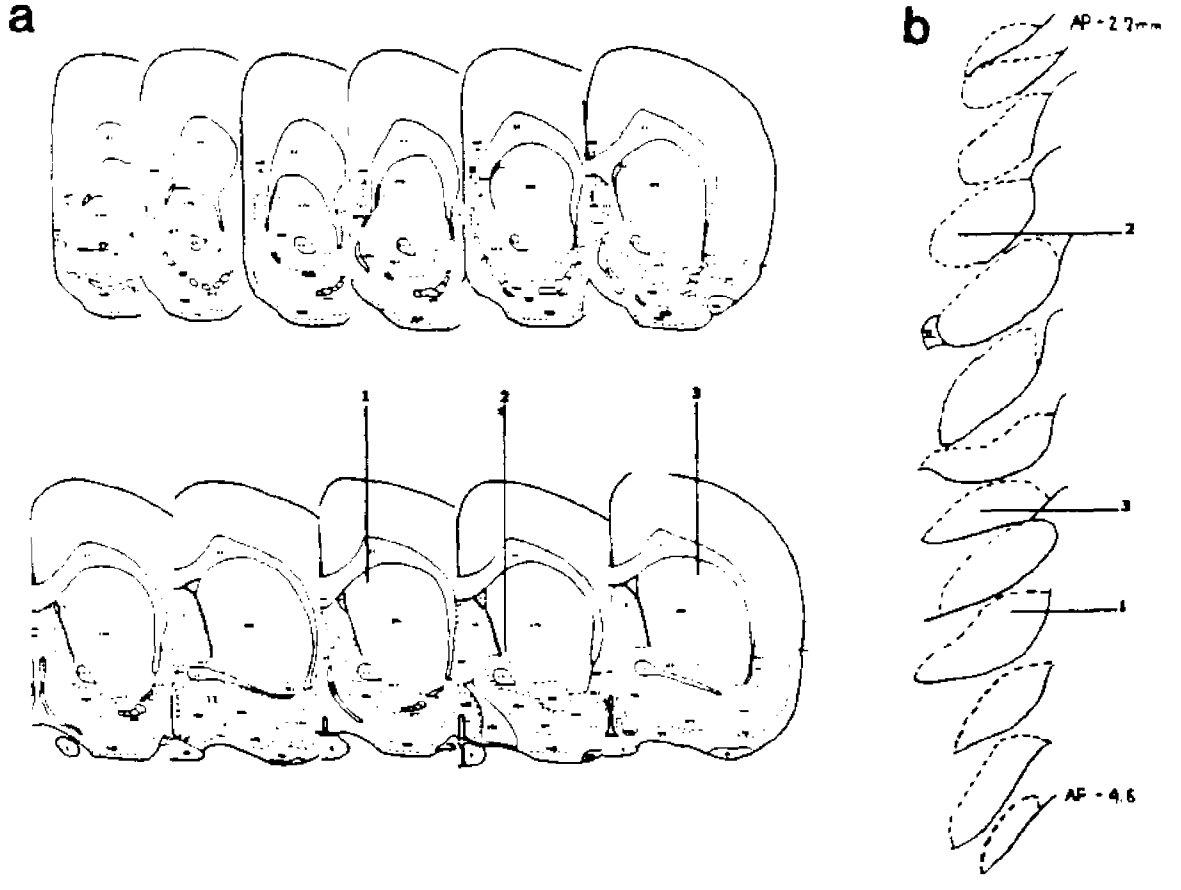


Table 31

Effect on dopaminergic cells of direct application of 0.002 nmol naloxone into the substantia nigra

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	112	110	- 1.8	6.5 +/- 0.2	7.0 +/- 0.2	+ 7.7
2	253	260	+ 2.8	10.0 +/- 1.1	10.5 +/- 0.9	+ 5.0
3	61	58	- 4.9	4.5 +/- 0.6	6.0 +/- 0.1	+ 33.0
		MEAN	- 1.3			+ 15.3

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

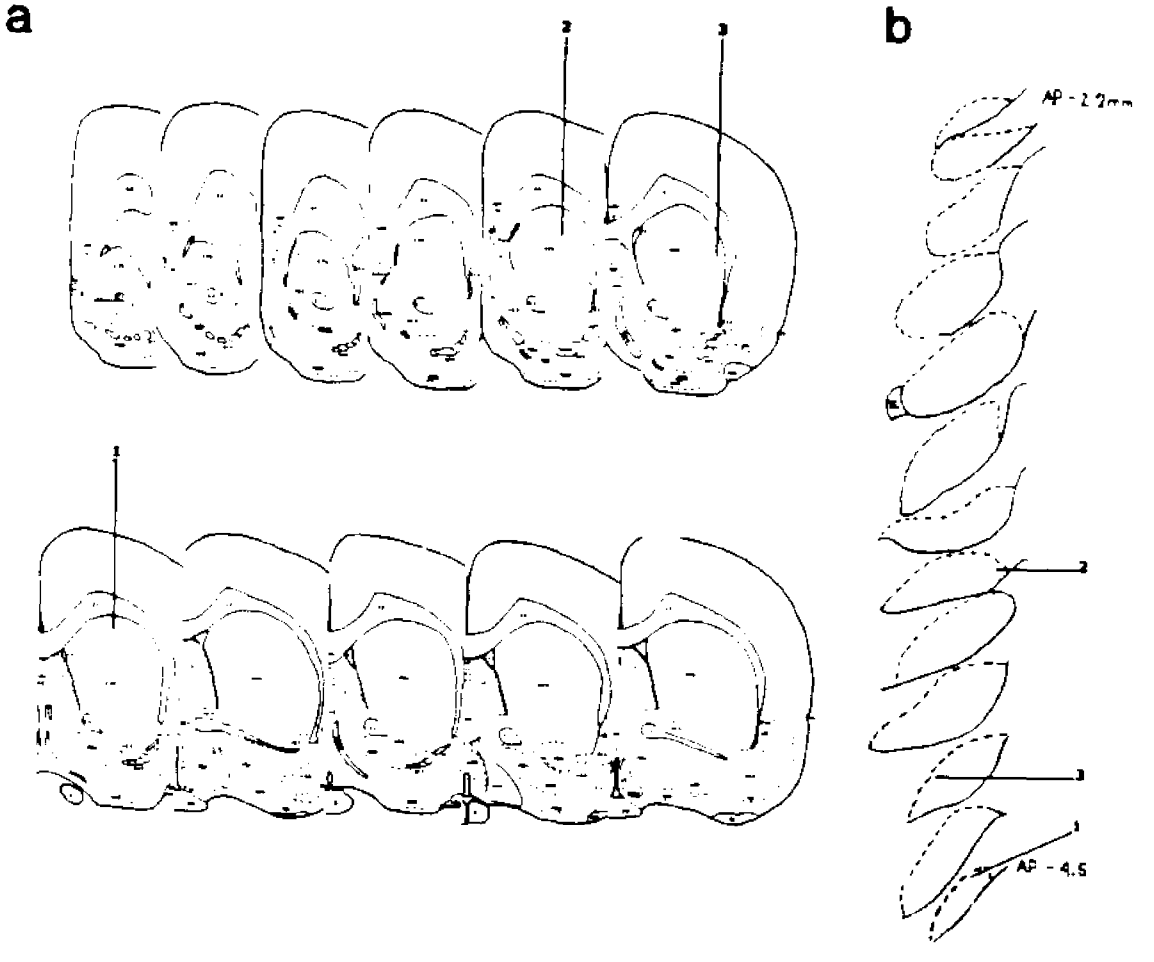


Table 32

Effect on non-dopaminergic cells of direct application of 0.002 nmol naloxone into the caudate-putamen

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	177	227	+ 28.2	24.5 +/- 1.9	27.0 +/- 2.4	+ 10.2
2	91	255	+ 180.2	18.2 +/- 1.2	17.9 +/- 0.9	- 1.6
3	59	151	+ 155.9	32.0 +/- 2.3	31.0 +/- 1.7	- 3.1
4	125	229	+ 83.2	28.0 +/- 0.3	28.0 +/- 0.1	0
		MEAN	+ 111.9			+ 1.4

* Post-drug significantly different from pre-drug (Paired T-test: P<0.05)
** Post-drug not significantly different from pre-drug (Paired T-test)

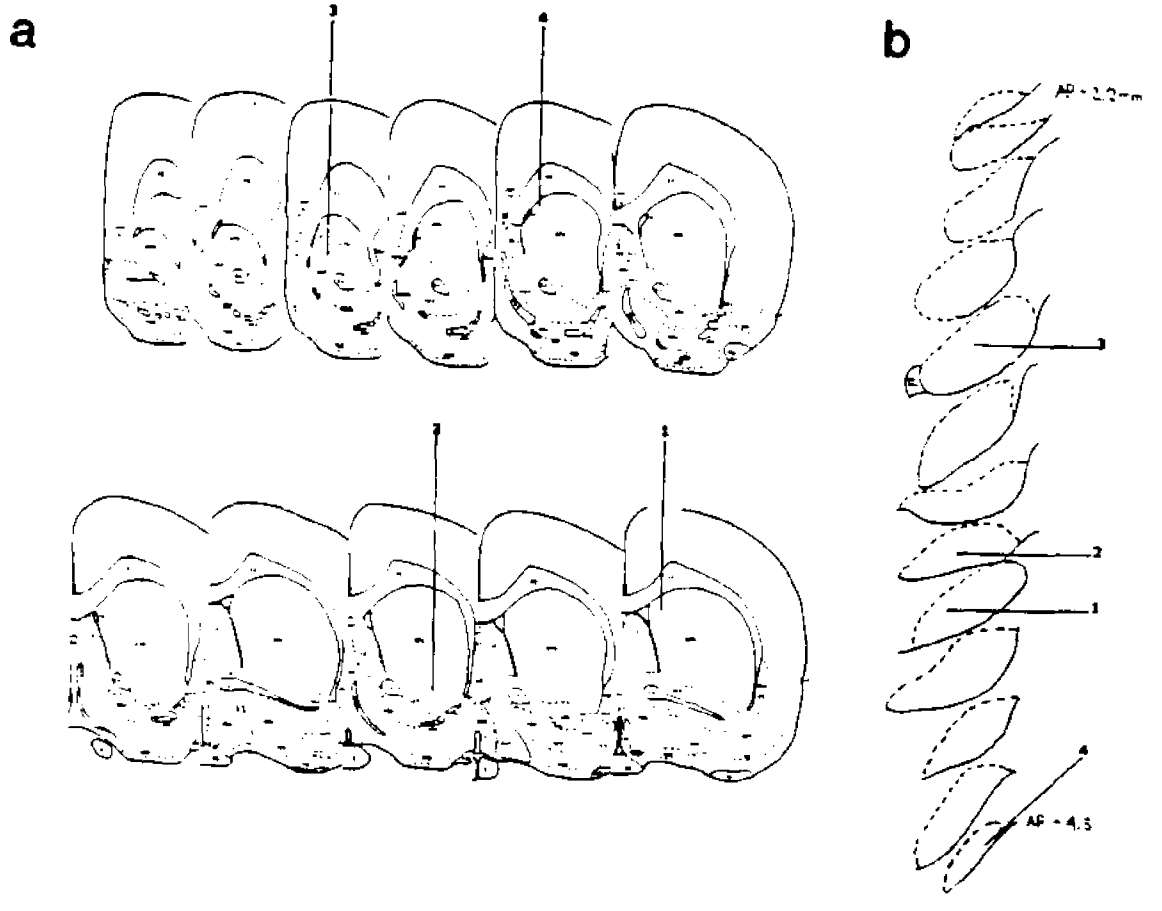


Table 33

Effect on dopaminergic cells of direct application of 0.002 nmol naloxone into the caudate-putamen

EXPT NUMBER	EVOKED RESPONSE PRE-DRUG	EVOKED RESPONSE POST-DRUG*	% CHANGE	SPONTANEOUS RATE (Hz) PRE-DRUG	SPONTANEOUS RATE (Hz) POST-DRUG**	% CHANGE
1	32	76	+ 137.5	10.2 +/- 0.7	9.8 +/- 0.9	- 3.9
2	248	482	+ 94.4	5.3 +/- 0.2	5.5 +/- 0.5	+ 3.7
3	190	295	+ 55.3	7.2 +/- 0.4	8.3 +/- 0.2	+ 15.3
		MEAN	+ 95.7			+ 5.0

* Post-drug not significantly different from pre-drug (Paired T-test)
** Post-drug not significantly different from pre-drug (Paired T-test)

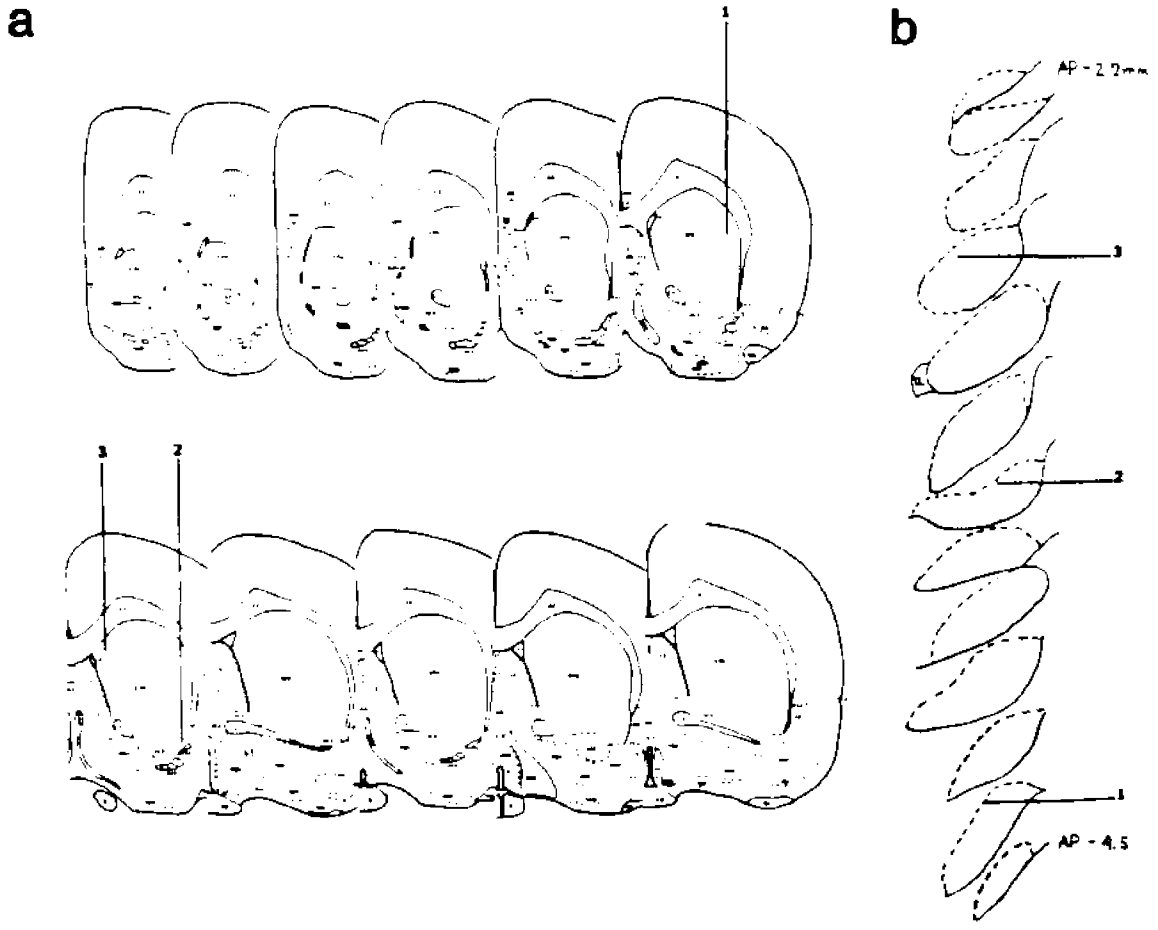


Table 34

Effect on non-dopaminergic cells of intracerebroventricular infusion of 1 umol CPAB and direct application of 0.002 nmol naloxone into the substantia nigra.

EXPT NUMBER	SPONTANEOUS RATE (Hz) PRE-CPAB	SPONTANEOUS RATE (Hz) POST-CPAB	% CHANGE	SPONTANEOUS RATE (HZ) POST-NALOXONE*	% CHANGE
1	23.0 +/- 2.9	40.0 +/- 1.8	+ 73.9	29.0 +/- 1.7	- 27.5
2	33.0 +/- 2.4	51.8 +/- 2.4	+ 57.0	42.5 +/- 5. 1	- 18.0
3	18.0 +/- 1.3	31.6 +/- 1.0	+ 75.6	23.5 +/- 0.8	- 25.6
		MEAN	+ 68.8		- 23.7

* Post-naloxone significantly different from post-CPAB (Paired T-test: p<0.01)

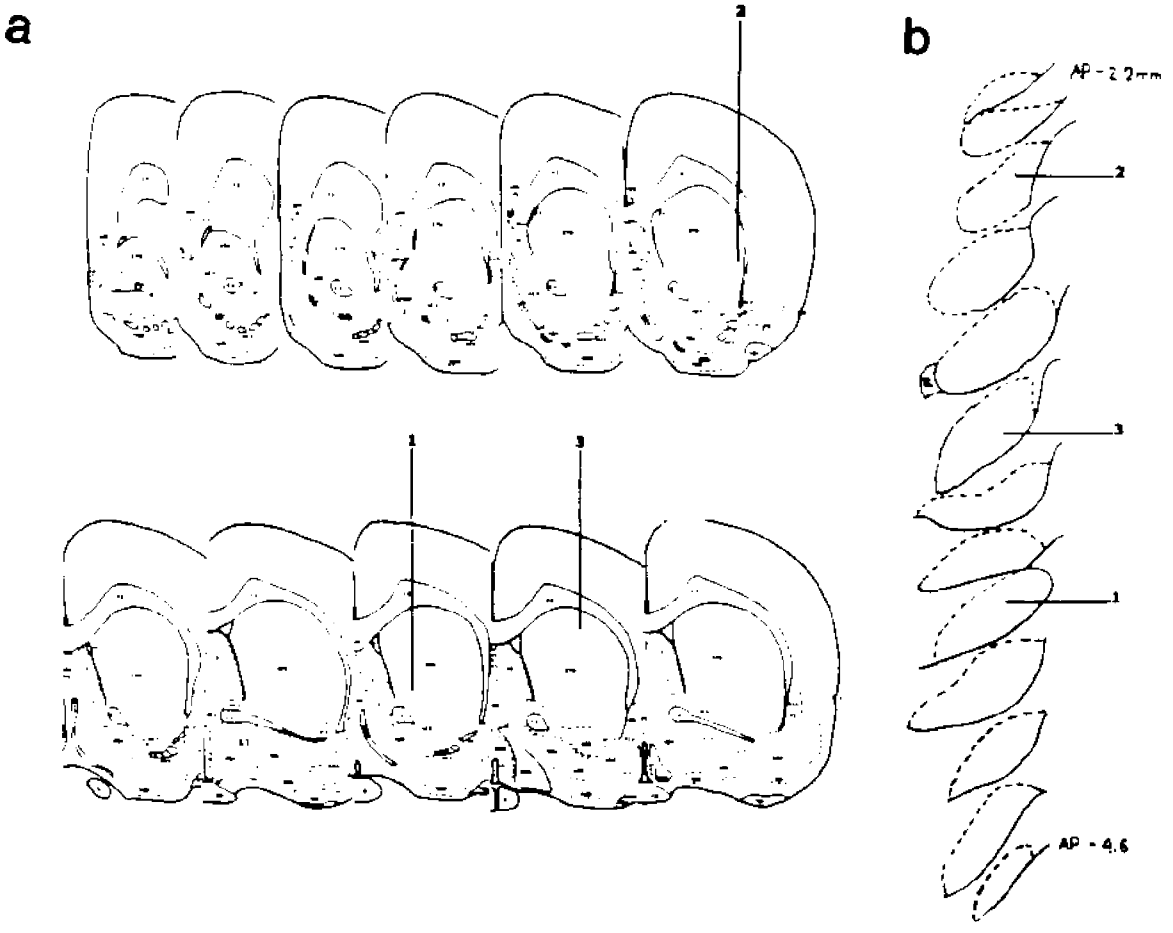


Table 35

Effect on dopaminergic cells of intracerebroventricular infusion of 1 umol CPAB and direct application of 0.002 nmol naloxone into the substantia nigra.

EXPT NUMBER	SPONTANEOUS RATE (Hz) PRE-CPAB	POST-CPAB	% CHANGE	SPONTANEOUS RATE (HZ) POST-NALOXONE*	% CHANGE
1	7.0 +/- 0.3	14.8 +/- 0.9	+ 111.4	12.5 +/- 0.6	- 15.5
2	10.5 +/- 0.6	19.3 +/- 0.9	+ 83.8	14.0 +/- 0.7	- 27.5
3	5.5 +/- 0.4	14.7 +/- 0.6	+ 166.4	12.0 +/- 0.5	- 18.0
		MEAN	+ 120.5		- 20.3

* Post-naloxone not significantly different from post-CPAB (Paired T-test)

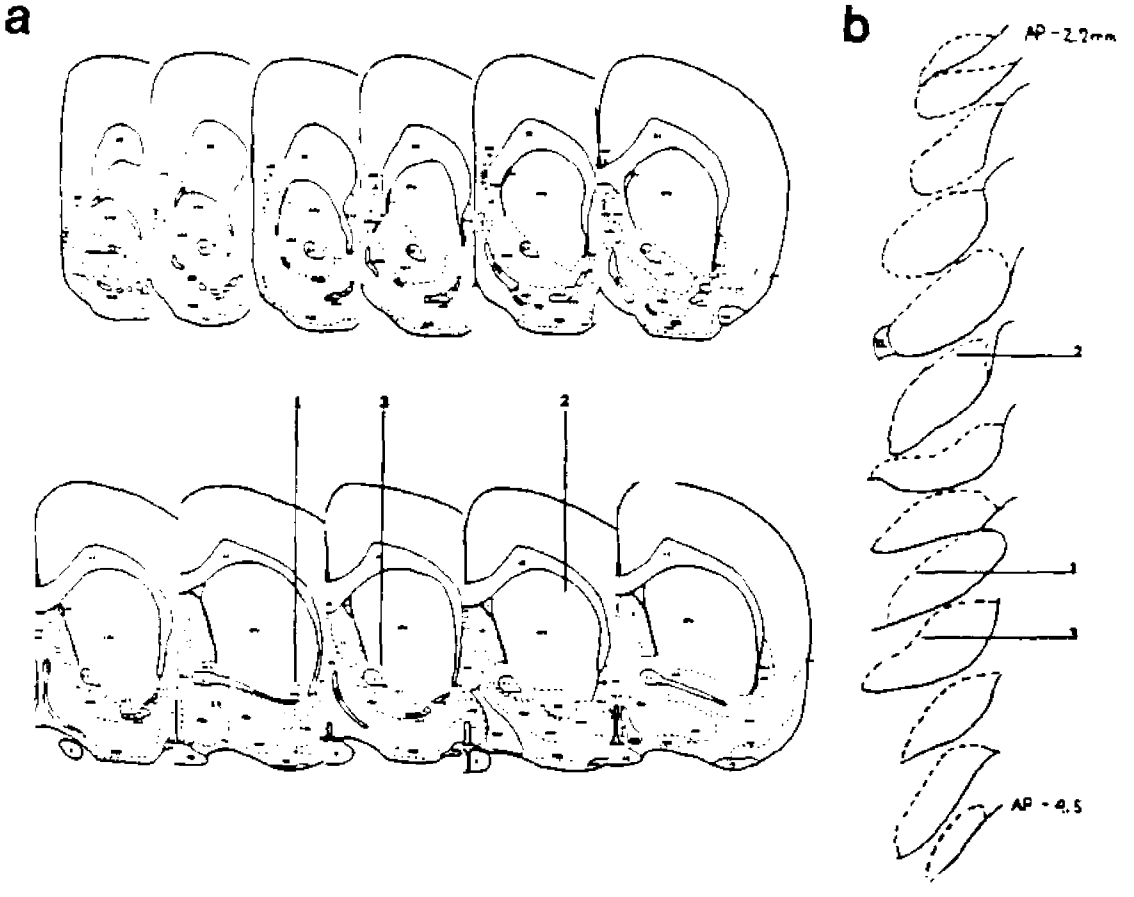


Table 36

Effect on non-dopaminergic cells of intracerebroventricular infusion of 1 umol CPAB and direct application of 0.002 nmol naloxone into the caudate-putamen

EXPT NUMBER	SPONTANEOUS RATE (Hz) PRE-CPAB	POST-CPAB	% CHANGE	SPONTANEOUS RATE (HZ) POST-NALOXONE*	% CHANGE
1	25.0 +/- 1.9	40.3 +/- 2.13	+ 61.0	32.0 +/- 1.5	- 20.5
2	19.0 +/- 1.2	32.2 +/- 0.9	+ 69.5	26.0 +/- 0.6	- 19.3
3	30.0 +/- 0.9	47.7 +/- 0.3	+ 59.0	43.0 +/- 0.5	- 9.9
		MEAN	+ 63.1		- 16.6

* Post-CPAB significantly different from post-naloxone (Paired T-test: $p<0.05$)

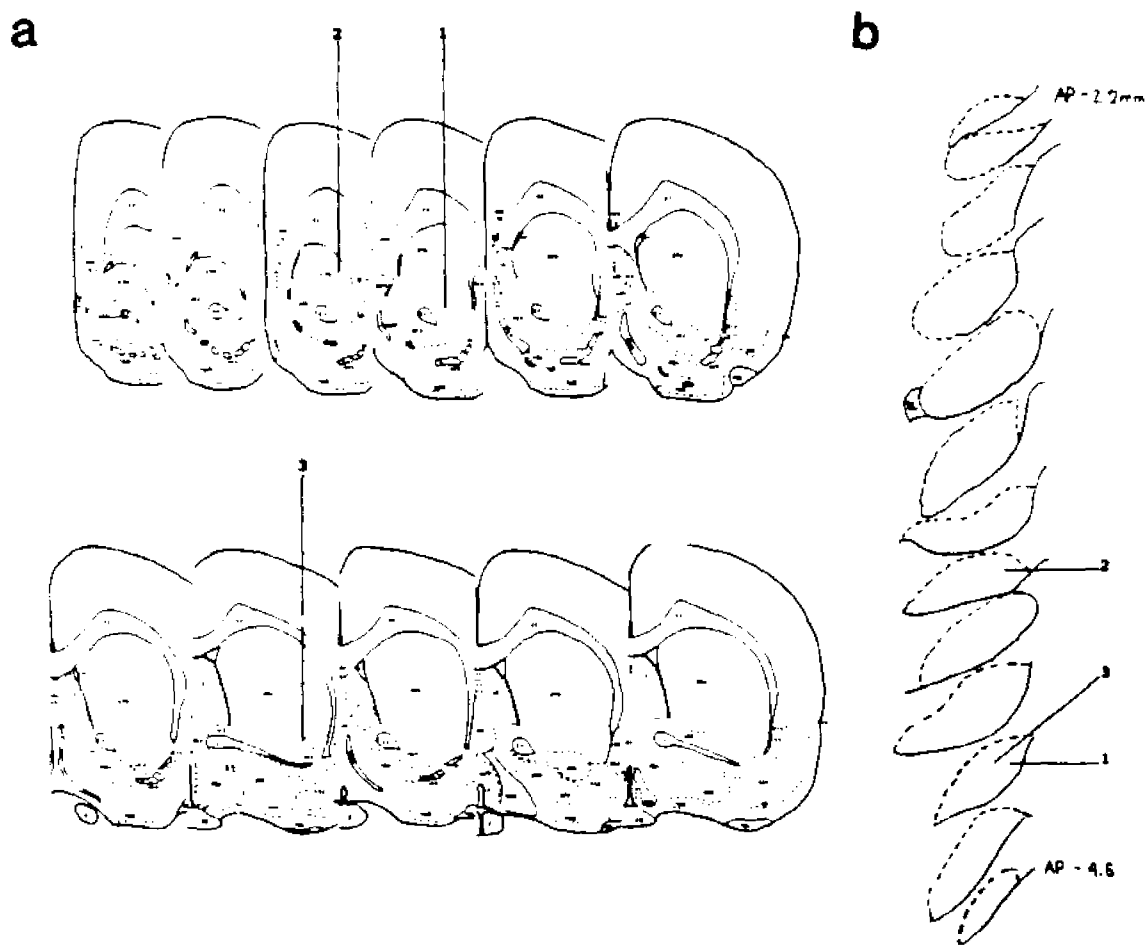


Table 37

Effect on dopaminergic cells of intracerebroventricular infusion of 1 umol CPAB and direct application of 0.002 nmol naloxone into the caudate-putamen

EXPT NUMBER	SPONTANEOUS RATE (Hz) PRE-CPAB	SPONTANEOUS RATE (Hz) POST-CPAB	% CHANGE	SPONTANEOUS RATE (Hz) POST-NALOXONE*	% CHANGE
1	10.4 +/- 0.2	18.7 +/- 0.4	+ 79.4	14.0 +/- 0.7	- 25.1
2	5.2 +/- 0.3	9.0 +/- 0	+ 73.1	8.6 +/- 0.2	- 4.5
3	7.5 +/- 0.8	13.7 +/- 0.6	+ 82.7	10.2 +/- 0.8	- 25.3
		MEAN	+ 78.4		- 18.3

* Post-CPAB not significantly different from post-naloxone (Paired T-test)

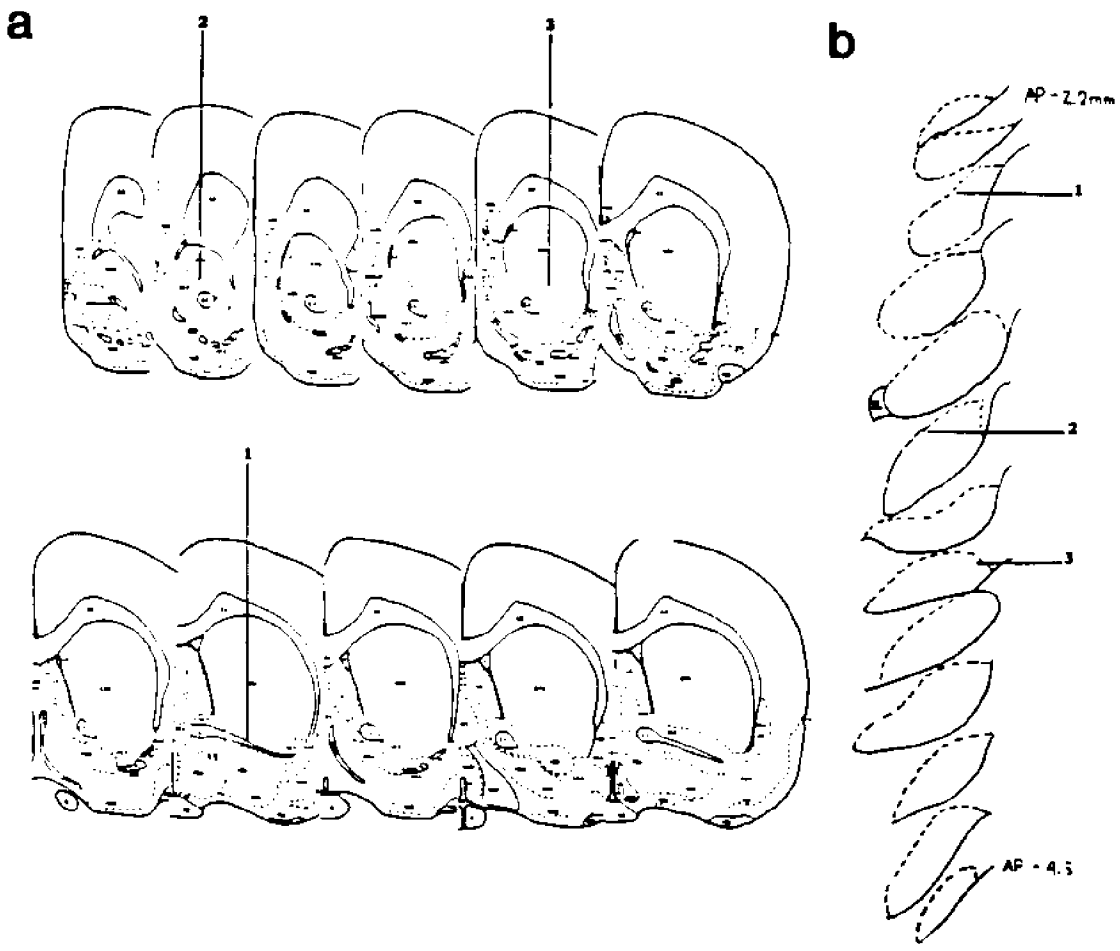


TABLE 38

EFFECT ON NON-DOPAMINERGIC CELLS OF 1 umol CPAB (i.c.v) AND DIRECT INFUSION OF 0.002 nmol NALOXONE INTO THE CAUDATE-PUTAMEN

EXPT NUMBER	EVOKED RESPONSE PRE-CPAB	POST-CPAB/NALOXONE*	% CHANGE
1	52	125	+ 140.4
2	185	322	+ 74.1
3	272	353	+ 29.8
		MEAN	+ 81.4

* Post-drug significantly different from pre-drug (Paired T-test: $p<0.05$)

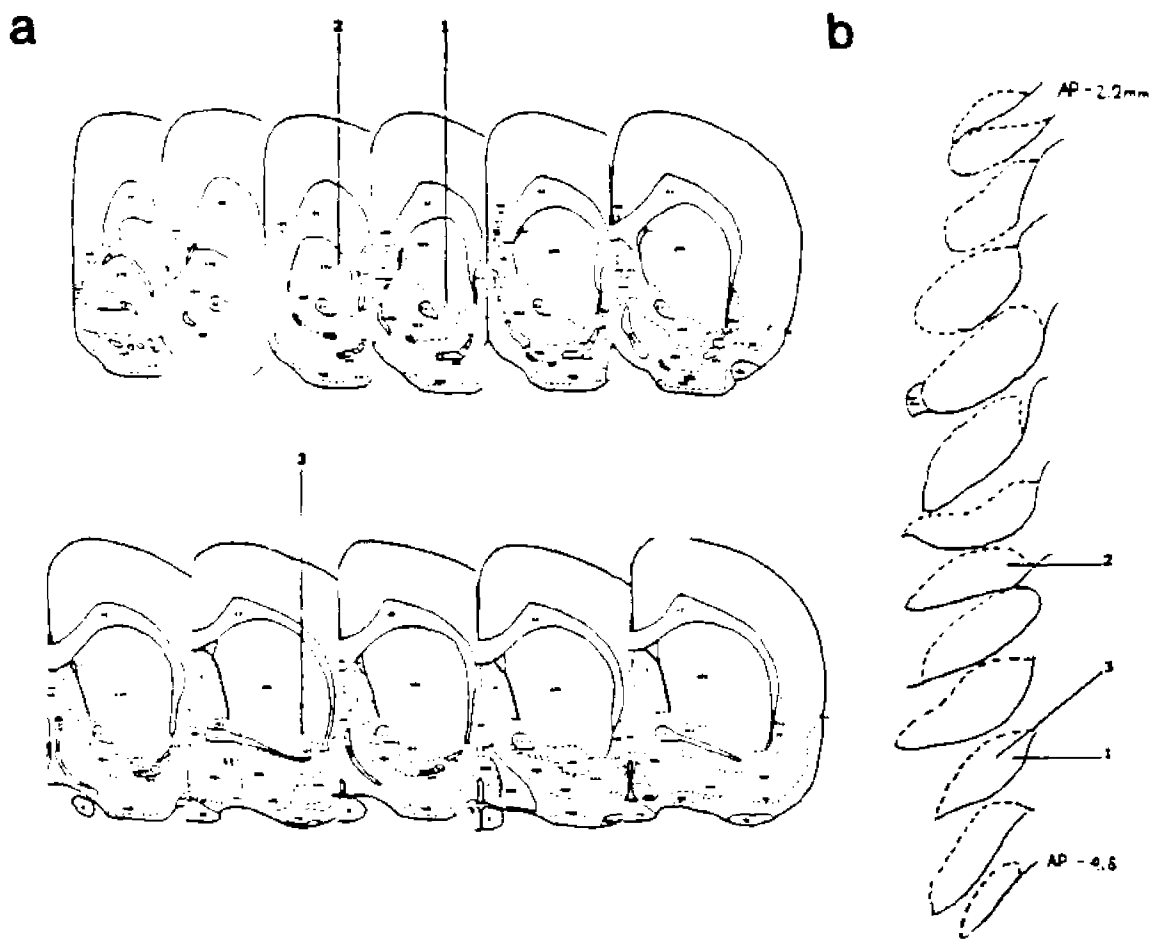


TABLE 39

EFFECT ON DOPAMINERGIC CELLS OF 1 umol CPAB (i.c.v) AND DIRECT INFUSION OF 0.002 nmol NALOXONE INTO THE CAUDATE-PUTAMEN

EXPT NUMBER	EVOKED RESPONSE PRE-CPAB	POST-CPAB/NALOXONE*	% CHANGE
1	190	176	- 7.4
2	304	341	+ 12.2
3	192	201	+ 4.7
		MEAN	+ 3.2

+ Post-drug not significantly different from pre-drug (Paired T-test)

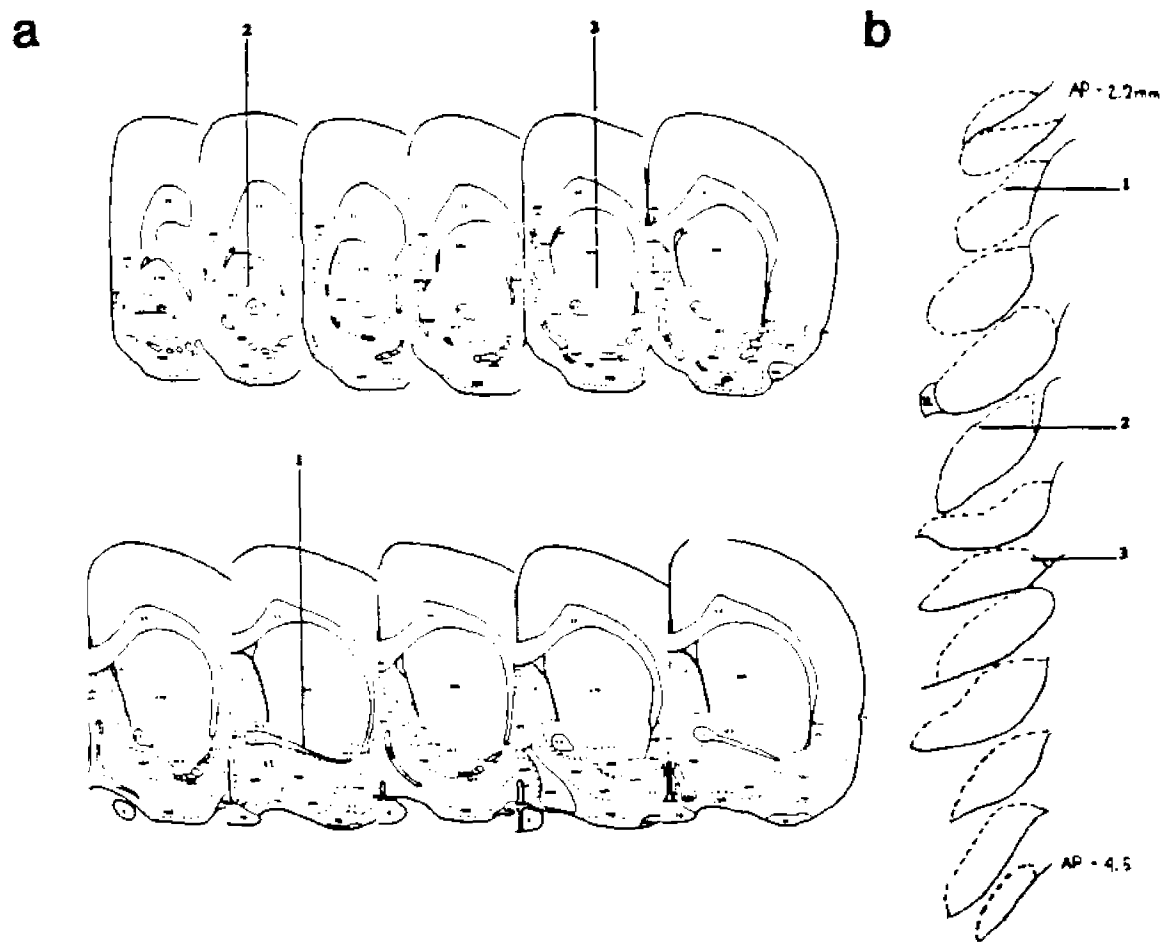


TABLE 40

EFFECT ON NON-DOPAMINERGIC CELLS OF 1 umol CPAB (i.c.v) AND DIRECT INFUSION OF 0.002 nmol NALOXONE INTO THE SUBSTANTIA NIGRA

EXPT NUMBER	EVOKED RESPONSE PRE-CPAB	POST-CPAB/NALOXONE*	% CHANGE
1	139	210	+ 51.1
2	258	378	+ 46.5
3	89	133	+ 49.4
		MEAN	+ 49.0

* Post-drug not significantly different from pre-drug (Paired T-test)

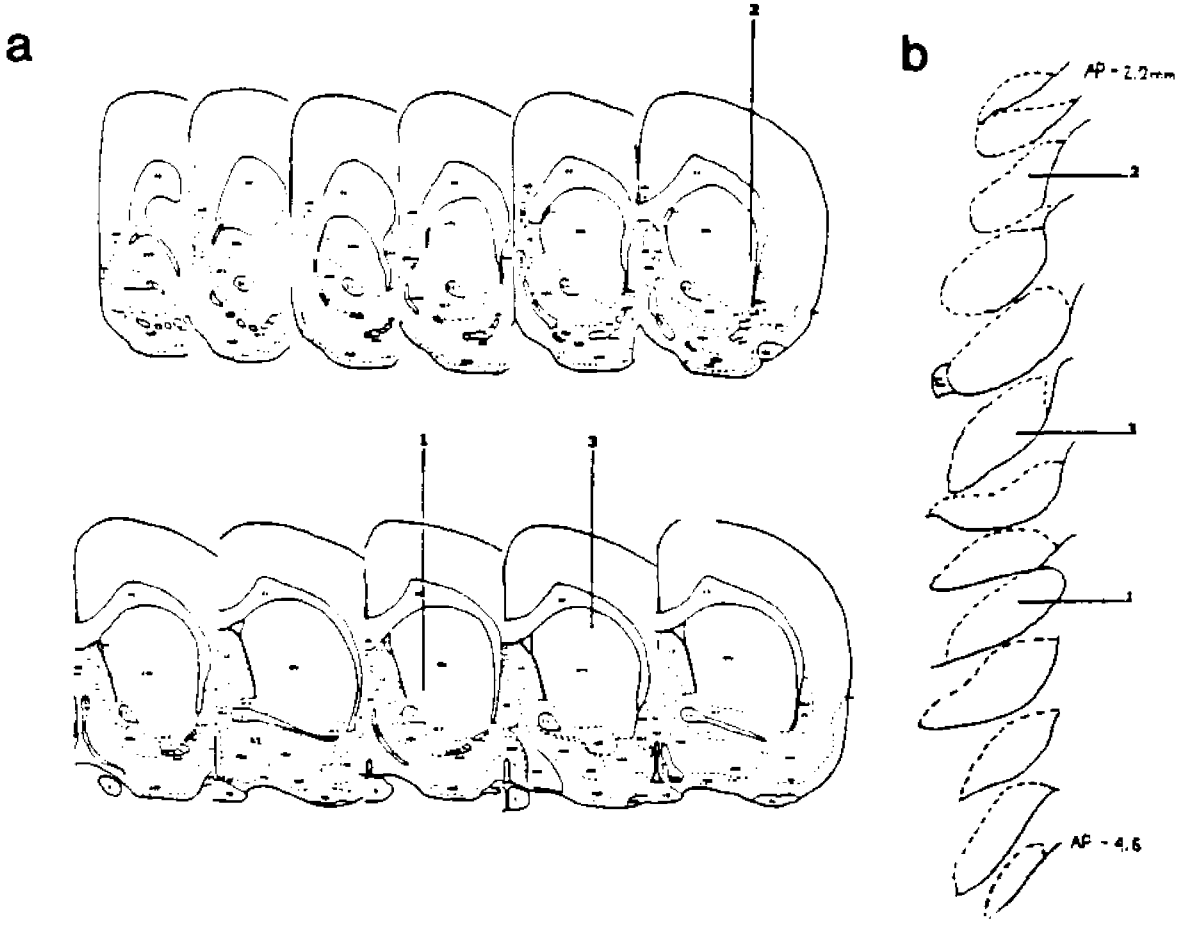
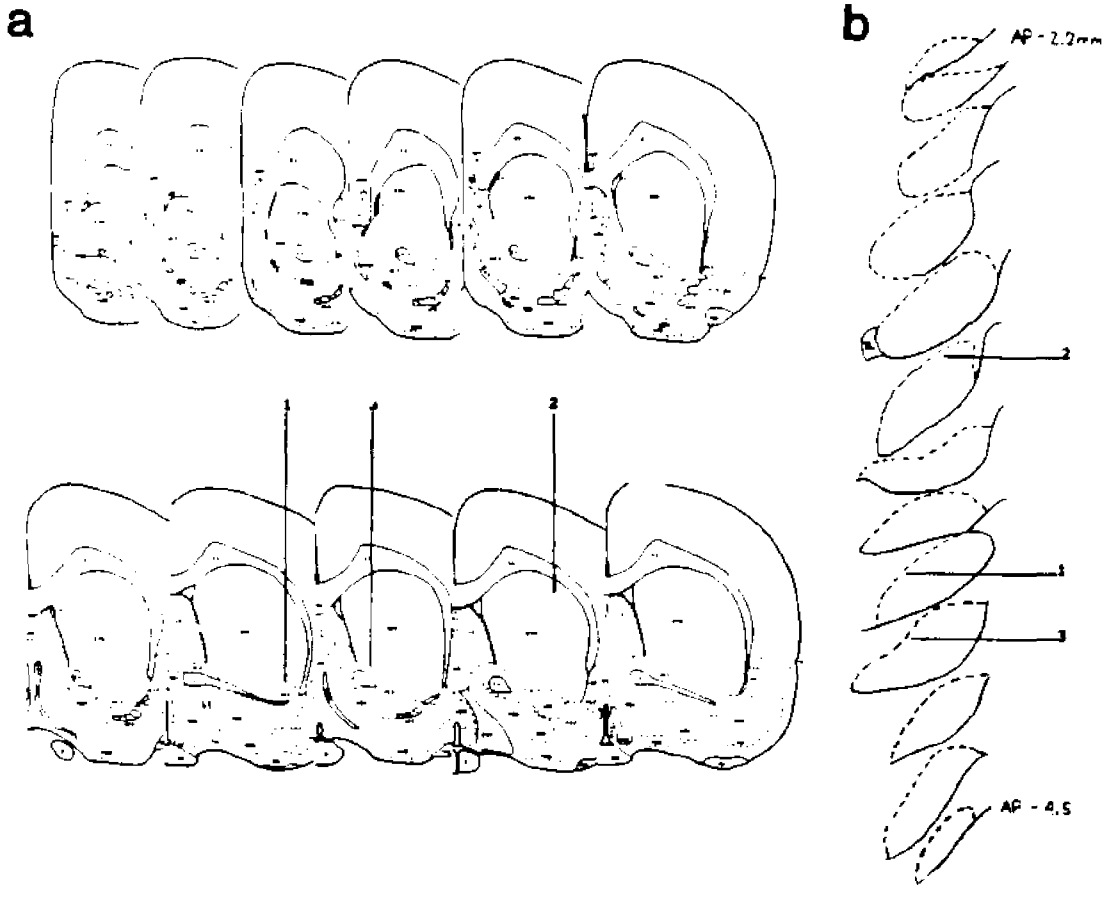


TABLE 41

EFFECT ON DOPAMINERGIC CELLS OF 1 umol CPAB (i.c.v) AND DIRECT INFUSION OF 0.002 nmol NALOXONE INTO THE SUBSTANTIA NIGRA

EXPT NUMBER	EVOKED RESPONSE PRE-CPAB	POST-CPAB/NALOXONE*	% CHANGE
1	82	153	+ 86.6
2	118	194	+ 64.4
3	220	310	+ 40.9
		MEAN	+ 64.0

* Post-drug significantly different from pre-drug (Paired T-test: p<0.01)



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