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Dopamine Release in Vivo from Nigrostriatal, Mesolimbic, and Mesocortical Neurons: Utility of 3-Methoxytyramine Measurements

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I. Introduction

RESEARCH over the last decade has dramatically advanced our understanding of how drugs modify synaptic function. This knowledge encompasses a wide range of mechanisms involved in the production, release, and postsynaptic coupling of received chemical signals. It is the purpose of this review to integrate data from a number of biochemical approaches which are relevant to evaluating the concept that 3-methoxytyramine (3-MT) is a metabolite of dopamine (DA) that is formed after DA release and is therefore a biochemical index of DA release (for abbreviations, see table 1).

Detailed studies of neurotransmitter synthesis and postsynaptic receptor function have been made possible with the introduction of molecular approaches to neuroscience research. However, the intervening process, neurotransmitter release, is still the most difficult process to study, requiring in vivo procedures with which tonic actions of afferent fiber systems to chemically defined pathways can be monitored. Such in vivo release studies require sensitive analytical methods and, in many cases, complex surgical procedures are needed. In the specific case of DA release, a number of approaches have been extensively investigated, all of which use DA overflow as an index of DA release (see ref. 122 for review).

(*a*) With the *push-pull perfusion technique* (26, 126), the push-pull cannula consists of two concentric tubes which are stereotaxically inserted into a defined brain region. A perfusion fluid is pumped into this local brain region via the inner tube ("push") and the perfusate collected from the outer tube ("pull"). This approach, however, suffers from the major problem of local and variable tissue damage at the tip of the cannula (122) and has therefore been used to a greater extent in species larger than the rat where larger brain regions can be perfused. Although never proven, this presumably limits local tissue damage compared to the use of this technique in the rat.

(*b*) With the *cup technique* (26), a brain region of interest is exposed and covered with a small plexiglass cup containing a perfusion fluid. The leakage of transmitters into this fluid bathing the surface of the area under study is then monitored. This technique requires radical surgery to remove overlying cortical areas to obtain access to subcortical structures, such as the striatum, and has mainly been used in species larger than the rat.

(*c*) With the *ventricular perfusion technique* (146), artificial cerebrospinal fluid is perfused into the lateral ventricle and collected from the cisterna magna; however, the site of origin of released neurotransmitters or their metabolites can only be inferred.

(*d*) With *in vivo voltammetry* (111), the oxidation of locally released catecholamines is monitored with a graphite electrode. In vivo voltammetry is unique in that it offers a real-time analysis of neurotransmitter release. However, the identity of the monitored electrochemical signal can only be inferred from general electrochemical responses measured in vitro and is never absolute.

(*e*) With *intracerebral dialysis* (200, 215), the use of a selectively permeable dialysis membrane is involved, and therefore this technique is not susceptible to the degree of local tissue damage observed with push-pull perfusion, while allowing direct confirmation of the dialyzed endogenous compounds. This method is limited by the requirement of surgery, by the growth of a glial coating on the dialysis membrane, by the collection periods of 10 to 20 min, and by the sensitive analytical methods which are required to monitor the very low levels of neurotransmitter which diffuse into the dialysate.

II. Methodology

Since the steady-state levels of 3-MT are in the fmol to pmol per mg protein range, the measurement of this parameter of DA release awaited the development of the following key methodological advances.

A. Microwave Tissue Fixation

After decapitation, there is a postmortem release of DA which is rapidly methylated by catechol-*o*-methyltransferase (COMT; EC 2.1.1.6) to form 3-MT (42). This postmortem accumulation has been shown to be biphasic with an initial exponential phase followed by a linear phase that continues for up to 2 h in the rat (42) and up to 60 h, independent of age (1 to 84 yr), in human caudate-putamen (43, 168). The biphasic curve for early time points in the rat (42) is presumably the result of two processes. At early times following death, there is an initial rapid release of DA which has been determined with push-pull perfusion studies (26) and brain dialysis studies (189). At later times after death, there is a dra-

TABLE 1
Explanation of terms

Abbreviation	Definition
ADTN	2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene
AMPT	Alpha-methylparatyrosine
AOAA	Aminooxyacetic acid
BW 2344	Rimcazole
CCK	Cholecystokinin
CCK-8S	Cholecystokinin-8-sulfate
CGS 10746B	5-(4-Methyl-1-piperazinyl)imidazo[2,1-*b*][1,3,5]benzothiadiazepine maleate
CGS 15855A	*trans*-1,3,4,4α,5,10β-Hexahydro-4-propyl-2*H*-[1]benzopyrano[3,4-*b*]pyridin-9-ol
CGS 15873	(+)-*trans*-1,3,4,4α,5,10β-Hexahydro-4-propyl-2*H*-[1]benzopyrano[3,4-*b*]pyridin-7-ol
CGS 16314A	(−)-Enantiomer of CGS 15855
CL 77-328	*cis*-5,6-Dimethoxy-2-methyl-3-[2-(4-phenyl-1-piperazinyl)ethyl]indoline
COMT	Catechol-*o*-methyltransferase
CPZ	Chlorpromazine
DA	Dopamine
DADLE	D-Ala^2-D-Leu^5-enkephalin
L-DOPA	L-(3,4-Dihydroxyphenyl)alanine
DOPAC	Dihydroxyphenylacetic acid
FG 7142	Methylamide-β-carboline-3-carboxylate
GABA	Gamma-amino butyric acid
GBL	Gamma-butyrolactone
GBR 12909	1-{2-[Bis(4-fluorophenyl)methoxy]ethyl}-4-(3-phenylpropyl)piperazine
GC-MF	Gas chromatography-mass fragmentographic
GC-MS	Gas chromatography-mass spectroscopy
HA-966	1-Hydroxy-3-amino-pyrrolidone-2
HVA	Homovanillic acid
LY 11555	(4α-*R*-*trans*)-4,4α,5,6,7,8,8α,9-Octahydro-5-*n*-propyl-2*H*-pyrazolo-3,4-γ-quinoline
MAO	Monoamine oxidase
MAO-A	Monoamine oxidase (type A)
MAO-B	Monoamine oxidase (type B)
MAOI	Monoamine oxidase inhibitor
MFB	Medial forebrain bundle
MK 801	10,11-Dihydro-5-methyl-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine
MR 2034	(−)-α-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2-(L-tetrahydrofurfuryl)-2′-hydroxy-6,7-benzomorphan
3-MT	3-Methoxytyramine
6-OHDA	6-Hydroxydopamine
PCP	Phencyclidine
PEA	Phenethylamine
PFC	Prefrontal cortex
POB	Phenoxybenzamine
(+)-3-PPP	(+)-N,n-Propyl-3-(hydroxyphenyl)piperidine
RMI 81582	3-(2-Chloro-11*H*-dibenz[*b*,*e*]azepine-11-ylidene)-N,N-dimethyl-1-propanamine
Ro 151788	Flumazenil
R-PIA	N^6-*R*-Phenylisopropyladenosine
SCH 23390	(*S*)-(+)-8-Chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol
SKF 38393	2,3,4,5-Tetrahydro-1-phenyl-1*H*-3-benzazepine-7,8-diol
SKF 83566	7-Bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benazepine-7-ol
THIP	4,5,6,7-Tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol
TL-99	6,7-Dihydroxy-2-dimethylaminotetralin
TTX	Tetrodotoxin
U-50488H	*trans*-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide

matic postmortem increase in 3-MT that results from a loss of monoamine oxidase (MAO) activity (MAO is an oxygen-dependent enzyme) and continued COMT activity (168) which metabolically traps the released DA in the form of 3-MT (fig. 1). These biphasic surges in postmortem DA release emphasize the requirement for rapid (i.e., ms) inactivation of COMT, which has only been achieved with the use of focused microwave irradiation (68, 92). In this case, the postmortem increases in 3-MT are blocked, and the levels of 3-MT are stabilized (207). Microwave irradiation is also more convenient than sacrifice by freeze-blowing, since it is more rapid and allows microdissection of brain regions. The alternate technique of in situ freezing in liquid nitrogen yields 3-MT levels which are intermediate between those obtained by decapitation and microwave fixation (63).

B. Micropunch Methods

Micropunch techniques (136) allow discrete brain regions to be microdissected from 250- to 1000-μm-thick

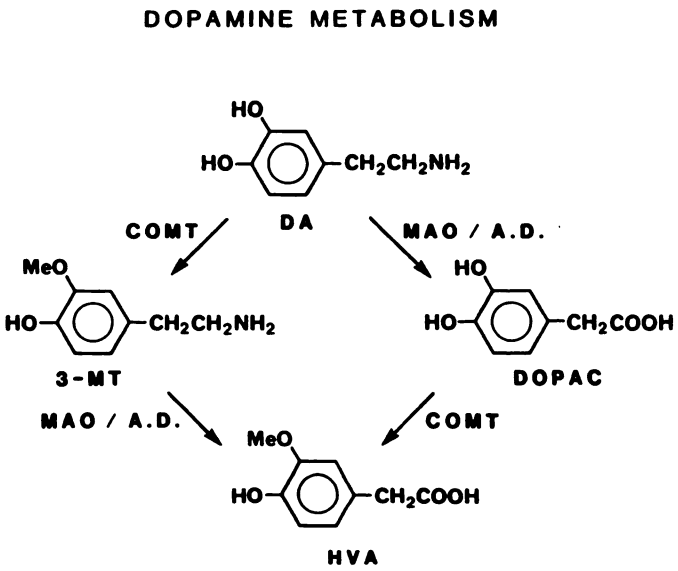

FIG. 1. Metabolic routes for the degradation of DA in the CNS. *A.D.*, aldehyde dehydrogenase (EC 1.2.1.3); *COMT*, catechol-O-methyltransferase (EC 2.1.1.6); *MAO*, monoamine oxidase (EC 1.4.3.4).

brain sections and have greatly increased the anatomical resolution of neurochemical measurements of DA and its metabolites (207).

C. Neuroanatomical Methods

Tyrosine hydroxylase immunohistochemistry (117, 120), in situ hybridization of tyrosine hydroxylase mRNA (30), dopamine receptor binding (196), dopamine fluorescence microscopy (85, 118), quantitative D-1 and D-2 receptor autoradiography (9, 10), and fiber tracing methods (178) have all been used to localize dopamine cell body and terminal areas in the brain. This data base is useful in the definition of brain regions to be microdissected for neurochemical studies.

D. Analytical Methods

The first studies of drug effects on 3-MT dynamics utilized fluorescence spectroscopy. With this technique, baseline levels of 3-MT had to be elevated (99, 101) by inhibition of 3-MT metabolism with monoamine oxidase inhibitors. Subsequently, several laboratories developed a gas chromatography-mass fragmentographic (GC-MF) assay using electron impact ionization (68, 105, 191, 225) which could detect striatal steady-state levels of 3-MT in animals sacrificed by focused microwave irradiation. This GC-MF assay was further improved by the use of negative chemical ionization conditions, which increased by 42-fold the sensitivity of 3-MT measurements (63, 206). Subsequently, high-pressure liquid chromatography methods were established (145, 201) which could measure striatal and olfactory tubercle 3-MT levels but not cortical 3-MT (201). For the measurements of 3-MT in less densely innervated brain regions, such as neocortical areas innervated by mesocortical dopaminerigic projections, refinements of the established GC-MF assay were required (213). These refinements involved the inclusion of a simple organic solvent extraction of dopamine and 3-MT from acidic tissue extracts to reduce background noise (213).

III. Biochemistry

A. 3-MT Steady-State Levels

1. Sources of 3-MT. As summarized in figs. 1 and 2, DA in dopaminergic nerve endings can be metabolized intraneuronally (50, 108, 157, 199) by MAO to generate dihydroxyphenylacetic acid (DOPAC). This MAO pool in the dopaminergic nerve endings of the rat striatum has been shown with 6-hydroxydopamine lesions to be monoamine oxidase (type A) (MAO-A) (55, 123) and via brain dialysis experiments with selective MAO-A inhibitors (100). In contrast, DA released into the synaptic cleft is inactivated both by DA reuptake into the dopaminergic nerve ending and by methylation involving membrane-bound COMT on postsynaptic neuronal elements (96, 98). Released DA is also taken up by glia (81, 138) and possibly by local neurons. Within these compartments, DA is oxidized by MAO to form DOPAC (2, 55, 163, 175) and methylated by soluble COMT (96, 155, 166) to form 3-MT. Importantly, the dopaminergic nerve endings themselves are devoid of COMT as determined by electrolytic (96, 123) and chemical (2, 183) lesions of the nigrostriatal pathway and by observations with enzyme immunohistochemistry (98). These findings, and studies of DA metabolite dynamics, have led to the conclusions that DOPAC is an accurate index of intra-

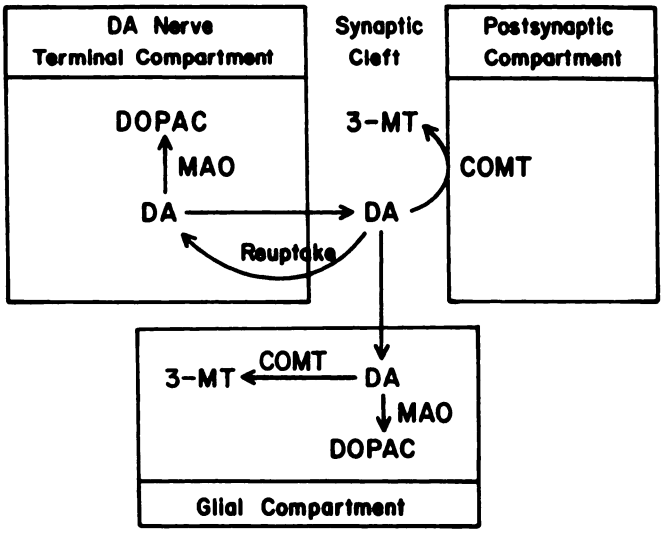

FIG. 2. Simplified working model of the neuronal and extraneuronal metabolism of DA. Intraneuronally, DA free in the cytosol is accessible to MAO and can be oxidized to form DOPAC which in turn can efflux from the nerve ending to be methylated to form the secondary metabolite HVA in both surrounding glia and postsynaptic neurons. In the cleft, reuptake of DA into the dopaminergic nerve ending, methylation by membrane-bound COMT, or uptake into glia and subsequent methylation or oxidation are all possible routes of inactivation for DA. DOPAC formed in the glial compartment can also be methylated to form HVA.

neuronal DA metabolism (157, 199, 207) and that 3-MT is an index of DA release (207, 215), Homovanillic acid (HVA) is a secondary metabolite of both DOPAC *and* 3-MT and is therefore of limited utility in defining the effects of experimental manipulations on dopamine release or metabolism (fig. 1).

In the case of 3-MT, levels of this metabolite will be influenced by two different compartments which contain COMT: the synaptic cleft and glia (96, 155). Importantly, the generation of 3-MT in *either* compartment requires prior DA release and therefore can serve as an index of DA release. Thus, 3-MT is a relative, not a direct, measure of DA release. The very rapid (5 to 10 min) changes in 3-MT following modifications of DA release (reviewed below) demonstrate the temporally close coupling between DA release and 3-MT formation in these postsynaptic compartments (215, 216).

2. Regional levels of 3-MT in the rat. A regional comparison of the steady-state levels of 3-MT in rat brain is presented in table 2. The distribution of 3-MT levels correlates well with the associated regional levels of DA (6). An excellent agreement is obtained from nine laboratories for absolute steady-state concentrations of striatal 3-MT in microwave fixed tissues. The only exception is found in a report (190) in which case basal 3-MT levels were in the typical range of concentrations reported for animals killed by decapitation (68). Clearly, this study suffered from inadequate microwave fixation.

3. Species differences in 3-MT levels. Of the four species in which 3-MT has been measured following microwave irradiation, steady-state 3-MT levels in the mouse, gerbil, and hamster striatum are all significantly greater than those observed in the rat (table 3; ref. 207). In a comparison of the kinetics of 3-MT in the rat and mouse (216), the fractional rate constant (the proportion of the 3-MT pool that is metabolized per unit time) was determined after pargyline treatment. The fractional rate constant was 3 times greater in rat striatum and 6 times greater in rat striatal dialysates than in the mouse. These data suggest either that a greater proportion of released DA in the rat striatum is methylated to form 3-MT or that the clearance of 3-MT differs in these two species.

4. 3-MT conjugation. Virtually all 3-MT is in the free (nonconjugated) form in both the rat (198) and mouse (63) striatum. However, in the cerebrospinal fluid (CSF) of the squirrel monkey, dog, and human, 3-MT exists mainly in the conjugated form (63). Both the human and squirrel monkey CSF contains approximately 1.2 pmol/ml of free 3-MT and 12 pmol/ml of conjugated 3-MT. It has been suggested that 3-MT is not cleared from the rat brain but is further metabolized to HVA, since brain 3-MT levels are stable for more than 3 h after inhibition of DA synthesis with alpha-methylparatyrosine (AMPT) in combination with monoamine oxidase inhibition (79).

B. 3-Methoxytyramine Dynamics

1. Enzyme inhibition. a. 3-MT ACCUMULATION. In most brain regions, the steady-state levels of 3-MT are less than 1% of the DA steady-state levels (table 2). However, when the dynamics of this pool are examined either by monitoring the decline in 3-MT after treatment with the COMT inhibitor, tropolone (191, 201), or by monitoring the accumulation of 3-MT after inhibition of MAO with pargyline (212, 213), fractional rate constants ranging from 7 to 19 h^{-1} are obtained depending upon the brain region examined. A high rate constant of 36 was reported in one study (191), but this was based on the use of only a zero time group and a group sacrificed after 1-min treatment with tropolone. In fig. 3, an example of 3-MT accumulation in the rat nucleus accumbens after inhibition of MAO with pargyline is presented. A comparison of the dynamics of 3-MT compared to the DA pool and other metabolite pools is presented in table 4. These data indicate that, while the levels of 3-MT are low relative

TABLE 2

Regional dopamine and 3-MT levels and 3-MT fractional rate constants for microwave-irradiated rat brain

Region	Dopamine (pmol/mg protein)	3-MT (pmol/mg protein)	3-MT, k (hr^{-1})	Ref.
Striatum	670	2.9		77
		2.3		68
		1.5		78
		2.3		58
		2.4		47
		1.5		121
	642	1.4		225
	607	1.9	36.4	191
	497	1.1		145
	649	1.9		224
	588	1.8		211
	511	1.2	18.1	202
	520	2.0	17.7	203
	660	2.0	12.9	212
	590	2.5		128
	633	1.9		217
	388	2.2	8.9	160
	664	2.2	17.3	213
	597	2.8		94
	416	1.9		8
	456	2.7		6
	937	8.4		190
Nucleus accumbens	429	2.4	10.4*	94
Olfactory tubule		1.7	8†	202
	485	1.7		6
	485	1.7	6.6	214
Prefrontal cortex	18	0.11	9.1	213
Cingulate cortex	11	0.11	15.5	213
	15	0.16		6
Hypothalamus	36	0.26		6
Entorhinal cortex	21	0.40		6
Hippocampus	15	0.25		6

* Unpublished observations.

† Estimate based on one accumulation point after pargyline.

TABLE 3

Dopamine and 3-MT levels in the striatum and olfactory tubercle of the mouse, gerbil, and hamster (euthanasia via microwave irradiation)

Region	Species	DA (pmol/mg protein)	3-MT	Ref.
Striatum	Mouse	672	4.0	225
			13.0	29
		565	4.3	218
		665	4.3	207
		672	4.4	217
		531	7.9	15
		466	6.6	8
		415	6.7	33
	Gerbil	1087	5.0	207
	Hamster	551	4.6	207
Olfactory tubule	Mouse	249	1.9	8
		676	4.4	33

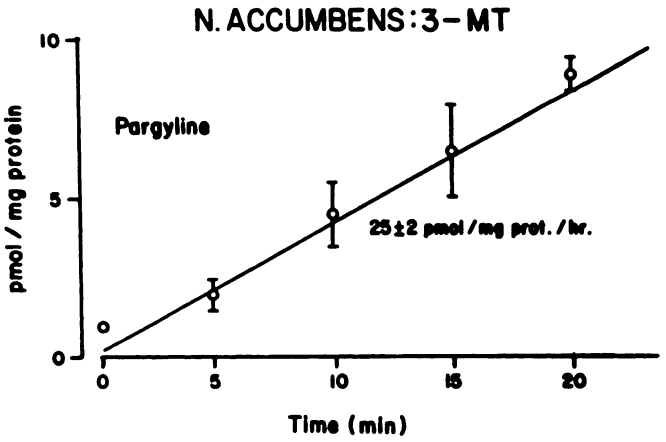

FIG. 3. Linear accumulation of 3-MT in the rat nucleus accumbens after the i.p. administration of pargyline (75 mg/kg). Values are the mean ± SEM for 7 observations.

TABLE 4

Dynamics of the DA, 3-MT, DOPAC, and HVA pools of the rat striatum

Pool	Steady state (pmol/mg protein)	k (h^{-1})	Turnover rate (pmol/mg protein/h)
DA	620 ± 15*	0.21	130 ± 6
3-MT	2 ± 0.1	12.9	26 ± 2
DOPAC	68 ± 3	2.3	156 ± 10
HVA	58 ± 2	1.3	75 ± 4

* Mean ± SEM (n = 7 to 10).

to DA, DOPAC, or HVA, 3-MT is the most dynamic DA metabolite with a fractional rate constant of 12.9 h^{-1}. These rapid dynamics of the 3-MT pool are also observed with precursor labeling studies. For example, after both intraventricular (77) and intravenous (185) administration of [^{3}H]tyrosine, 3-MT is the DA metabolite with the highest specific activity, supporting a preferential flux of released DA through the 3-MT metabolic pool.

b. MULTIPLE DA POOLS. One complication in interpreting DA metabolite changes is the possibility of multiple DA pools which can be mobilized to support transmission. Early studies of DA turnover were inconclusive in resolving the different DA pools available for release (50, 199). However, studies using in vivo voltammetry in the rat striatum along with electrical stimulation of the medial forebrain bundle (MFB) have indicated that the rate constant for the releasable DA pool is 2.76 h^{-1} (130). A similar value of 2.5 was obtained for the extracellular DA pool in the rat striatum after treatment with pargyline (fig. 4) and monitoring DA release with striatal dialysis (215, 216). These values are approximately 10 times the values obtained for the turnover of the total DA pool using precursor labels or inhibition of synthesis (108, 199). These data suggest that the releasable pool of DA is smaller and more dynamic than the total neuronal stores of DA within the striatum (216). Studies of the dynamics of the recovery of DA release in the striatum after 10-Hz electrical stimulation of the MFB have suggested that, acutely (from 0.5 to 2 min), this process involves DA mobilization from another pool and does not involve DA synthesis except in the case of continued longer term stimulation (129). The roles of multiple vesicular and/or cytosolic DA pools remain to be defined. However, in the same paradigm, amfonelic acid was shown to mobilize a DA pool after inhibition of DA synthesis with AMPT (59). These data support the concept of multiple vesicular DA pools (77). Reserpine experiments also support the role of a nonvesicular DA pool in some drug effects (197, 202). This pool contributes to approximately 20% of the 3-MT steady-state levels observed in the rat striatum (216) with the remaining 80% presumably being derived from vesicular DA pools.

c. 3-MT ACCUMULATION VERSUS STEADY-STATE MEASUREMENTS. The accumulation of 3-MT after inhibition of MAO has been used to measure, via 3-MT, the effects of drugs on dopamine release. Pargyline (75 to 100 mg/kg i.p.) elevates striatal 3-MT levels by 10- to 18-fold within 20 min to 3 h postinjection (102, 160) and facilitates detection of this metabolite normally found in trace (1 to 6 pmol/mg of protein) amounts.

An important aspect in monitoring 3-MT dynamics after MAO inhibition with pargyline is the mode of animal sacrifice. Historically, when methods such as microwave fixation were unavailable to measure steady-state 3-MT levels, baseline values were artificially elevated by decapitation and drug effects monitored on this higher baseline (99, 101, 102). This approach was satisfactory in situations where profound drug effects on dopamine release were studied (table 5). However, other effects on steady-state 3-MT levels, such as those obtained with DA uptake blockers (58), were inconclusive using pargyline-dependent 3-MT accumulation in decapitated rats (99). This is likely to have been the result of the rapid postmortem increases in 3-MT which masks such subtle drug effects (202). Clearly, it is advisable even when monitoring pargyline-dependent 3-MT accumulation to use microwave fixed tissues, thereby elimi-

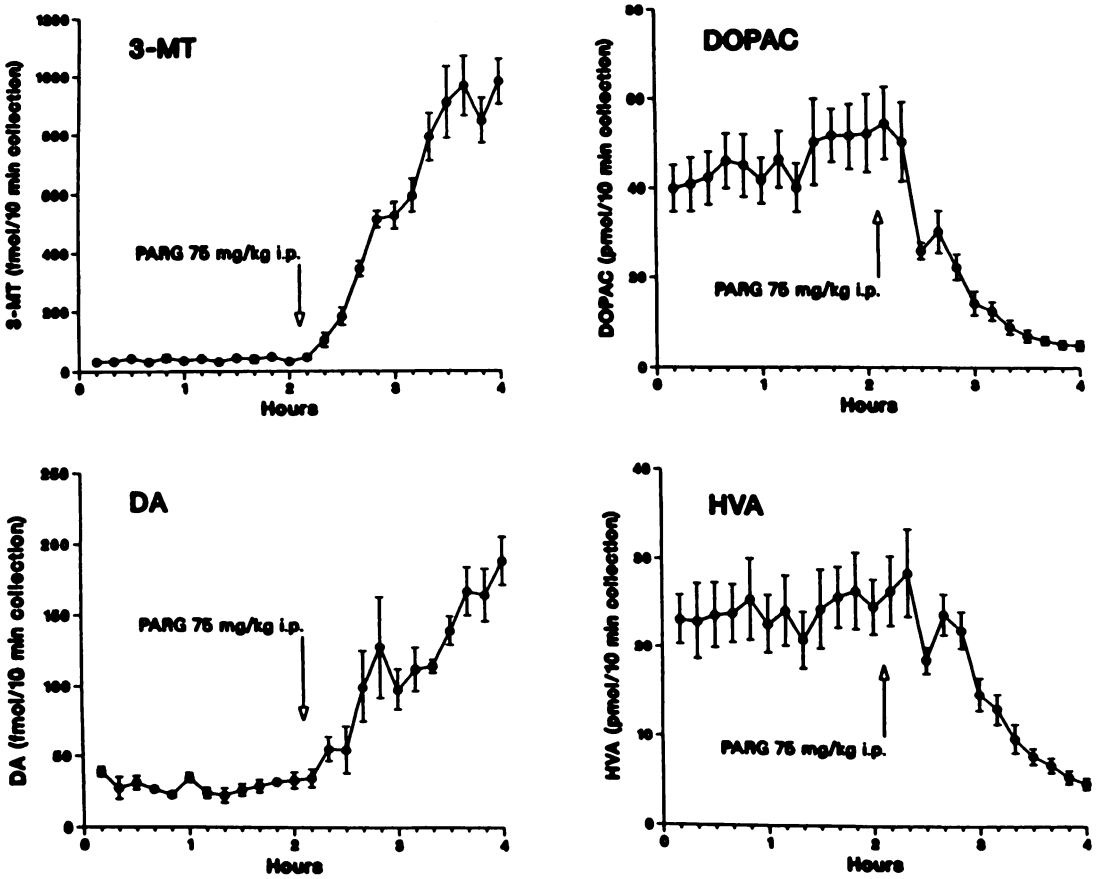

FIG. 4. Actions of pargyline (*PARG*) on the levels of 3-MT, DA, DOPAC, and HVA collected in rat striatal dialysates. Values are the mean ± SEM for 10-min collection periods of 5 animals (215).

nating this potential postmortem artifact. However, compared to 3-MT determinations in the brains of drug-treated animals killed by focused microwave irradiation (15, 185, 203), the pargyline accumulation technique is disadvantageous for several reasons. (*a*) Pargyline pretreatment prevents basal measurements of DOPAC, HVA, and dopamine and thereby precludes inferences of dopamine metabolism based on the acid metabolite levels. (*b*) The pargyline accumulation technique dampens the magnitude of changes in 3-MT following treatment with agents that decrease dopamine neuron impulse conduction, including gamma-butyrolactone (GBL) or apomorphine (6, 160), and drugs like haloperidol and chlorpromazine, which increase impulse conduction. Finally (*c*) MAO inhibition adds pharmacological and physiological complexities to the experimental design and thus weakens interpretations of the actions on release of the drug under study.

2. Electrical stimulation. In studies (219) of electrical stimulation of the substantia nigra of unanesthetized rats, frequency-dependent increases in striatal 3-MT, DOPAC, and HVA were observed with no changes in DA steady-state concentrations. Similar data have been obtained with ventricular perfusates of the anesthetized cat in which increased efflux into the ventricles of radiolabeled DA and 3-MT was observed during nigral stimulation (188).

Importantly, the increases in rat striatal 3-MT levels clearly preceded the increases in DOPAC and HVA (fig. 5), indicating that changes in DA release are detected prior to alterations in intraneuronal synthesis/metabolism of DA. Of interest, when either striatal steady-state 3-MT levels (219) or DA in striatal dialysates (90) are monitored after electrical stimulation of the substantia nigra, both parameters increase with stimulation frequencies between 2 and 5 Hz and plateau at approximately 20 Hz. At 100 Hz, striatal 3-MT levels begin to reverse and decrease (219), indicating that the nigrostriatal fibers cannot sustain transmission at this high frequency rate. Similarly, in vivo voltammetry studies have indicated that, with electrical stimulation of the rat medial forebrain bundle, DA neurons can follow stimulation frequencies between 25 and 50 Hz but that transmission begins to fail between 100 and 200 Hz (171).

3. Attenuation of DA neuronal impulse flow. Striatal 3-MT levels (102) and the amount of dopamine measured by microdialysis (227) are decreased by cessation of nigrostriatal impulse flow following i.p. injections of GBL. The suppression of dopamine release by GBL has been substantiated by electrophysiological (158) and behavioral (14) measures.

In contrast, DOPAC and HVA levels *increase* following cessation of impulse flow (194). The increases in striatal

TABLE 5

Actions of drugs on accumulation of 3-MT after inhibition of MAO

Drug	Dose (mg/kg)	Duration (min)	Route	Species	Tissue	3-MT accumulation (% of control)	Ref.
Stimulants							
Amphetamine	3	60	i.v.	Rabbit	Striatum	440	79
Methamphetamine	7	60	i.p.	Mouse	W.B.*	216	135
	1	90	i.p.	Rat	W.B.	136	99
	3	90	i.p.	Rat	W.B.	174	99
Cocaine	3	90	i.p.	Rat	W.B.	100	99
	10	90	i.p.	Rat	W.B.	123	99
	30	90	i.p.	Rat	W.B.	225	99
	3	60	i.v.	Rabbit	Striatum	100	79
Desipramine	15	90	i.p.	Rat	W.B.	100	99
	45	90	i.p.	Rat	W.B.	149	99
Imipramine	50	2700	s.c.	Rat	W.B.	100	165
Depressants							
Diazepam	3	90	i.p.	Rat	W.B.	85	99
	10	90	i.p.	Rat	W.B.	87	99
	30	90	i.p.	Rat	W.B.	77	99
Ethanol	2360	90	p.o.	Mouse	W.B.	47	116
GBL	750	30	i.p.	Rat	Striatum	46	101
R-PIA	3	60	i.p.	Rat	Striatum	78	133
Adrenergics							
POB	30	90	i.p.	Rat	W.B.	100	99
Propanolol	30	90	i.p.	Rat	W.B.	100	99
Clonidine	10	90	i.p.	Rat	W.B.	100	99
Dopamine agonists							
Apomorphine	3	90	i.p.	Rat	W.B.	100	99
	10	90	i.p.	Rat	W.B.	73	99
Neuroleptics							
Haloperidol	0.3	90	i.p.	Rat	W.B.	224	99
	1	90	i.p.	Rat	W.B.	289	99
	3	90	i.p.	Rat	W.B.	288	99
	0.5	90	i.p.	Rat	Striatum	385	160
	1	45	i.p.	Rat	Striatum	245	160
CPZ	1	90	i.p.	Rat	W.B.	151	99
	3	90	i.p.	Rat	W.B.	205	99
	10	90	i.p.	Rat	W.B.	311	99
	10	120	i.v.	Rabbit	Striatum	672	79
	10	45	i.p.	Rat	Striatum	160	101
	10	2700	s.c.	Rat	W.B.	235	165
Fluphenazine	1	45	i.p.	Rat	Striatum	190	160
Buspirone	2.5	45	i.p.	Rat	Striatum	195	160
	10	45	i.p.	Rat	Striatum	201	160
Molindone	0.5	45	i.p.	Rat	Striatum	175	160
	2.5	45	i.p.	Rat	Striatum	210	160
Clozapine	10	45	i.p.	Rat	Striatum	164	160
	20	45	i.p.	Rat	Striatum	220	160
SCH 23390	0.25	60	i.p.	Rat	Striatum	100	160
	2.5	60	i.p.	Rat	Striatum	120	160
Rimcazole	20	45	i.p.	Rat	Striatum	100	160
	40	45	i.p.	Rat	Striatum	150	160

* W.B., whole brain.

DOPAC in these paradigms cannot be simply explained by a decrease in autoreceptor feedback on dopaminergic nerve endings as a result of decreased DA release. This has been clearly demonstrated by dose-response studies of 1-hydroxy-3-amino-pyrrolidone-2 (HA-966) which decreases nigrostriatal cell firing and DA release in this pathway (132, 219). With this agent, DA release (3-MT levels) can be decreased to 40% of control with no change in DOPAC levels. However, with increasing doses, DOPAC levels are increased, in the absence of any further decreases in 3-MT levels. These data argue against a change in autoreceptor activity as the cause of the dramatic increases in DOPAC after interruption of neuronal firing rates in nigrostriatal neurons.

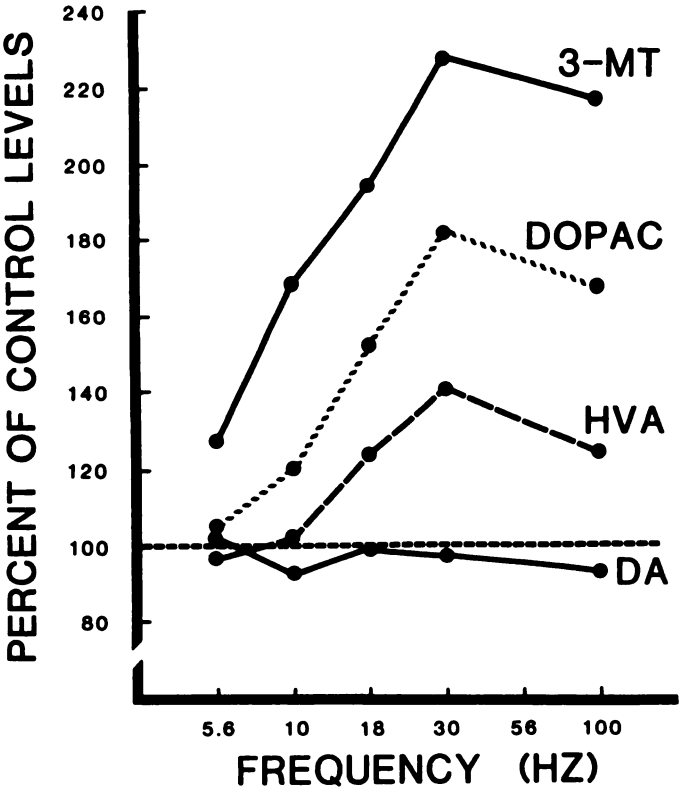

FIG. 5. Effect of stimulation frequency in the substantia nigra on rat striatal DA and its associated metabolites. Rats were restained and were unanesthetized. The stimulation consisted of square wave pulses (1.5 ms, 200 μA) with alternating 20-s "on" and "off" periods for 20 min (219).

4. Brain lesions. a. PARTIAL OR COMPLETE DESTRUCTION OF DA NEURONS. Degeneration of about 80% of the primate mesotelencephalic dopamine projection is associated with the akinesia, rigidity, and tremor of Parkinson's disease (25, 87, 154). A similar degree of nigrostriatal degeneration is required for the appearance of behavioral impairments in rodents (124, 151, 162), suggesting that as few as 20% of striatal dopamine neurons can sustain a variety of sensorimotor capabilities. However, characterization of the biochemical compensations of these surviving neurons has not resolved whether they maintain dopamine release at normal levels or, as proposed by Mortimer and Webster (131), that less than normal amounts of dopamine release may be sufficient for normal behavioral function. Six-fold increases in striatal dopamine release have been measured, either by the in vitro efflux of dopamine (169) or in vivo 3-MT levels (12) relative to the dopamine content of terminals surviving extensive (≥80%) denervations. Striatal dopamine innervation can be estimated by dopamine levels, since they covary with the amount of high-affinity dopamine uptake (12, 125, 230) and tyrosine hydroxylase (80) over the entire range of dopamine denervation. Thus, the change in dopamine metabolism or release per dopamine nerve terminal can be estimated according to the remaining dopamine concentration. The relative increases in dopamine release from surviving nerve terminals far exceed increases in neuronal dopamine metabolism assessed with DOPAC (2-fold) or HVA (3-fold) increases relative to dopamine (12).

We have also measured the 6-hydroxydopamine (6-OHDA)-induced depletion of rat striatal 3-MT, DOPAC, HVA, and dopamine to determine the extent of changes in dopamine release and metabolism following less severe dopamine denervations (11). Unlike DOPAC and HVA, which decrease almost in proportion to the dopamine decreases when dopamine losses are from 30 to 80%, 3-MT concentrations do not decrease unless dopamine losses exceed 90% (fig. 6). Denervations of 90 to 95% and 95 to 99% reduce DOPAC, HVA, and 3-MT to an

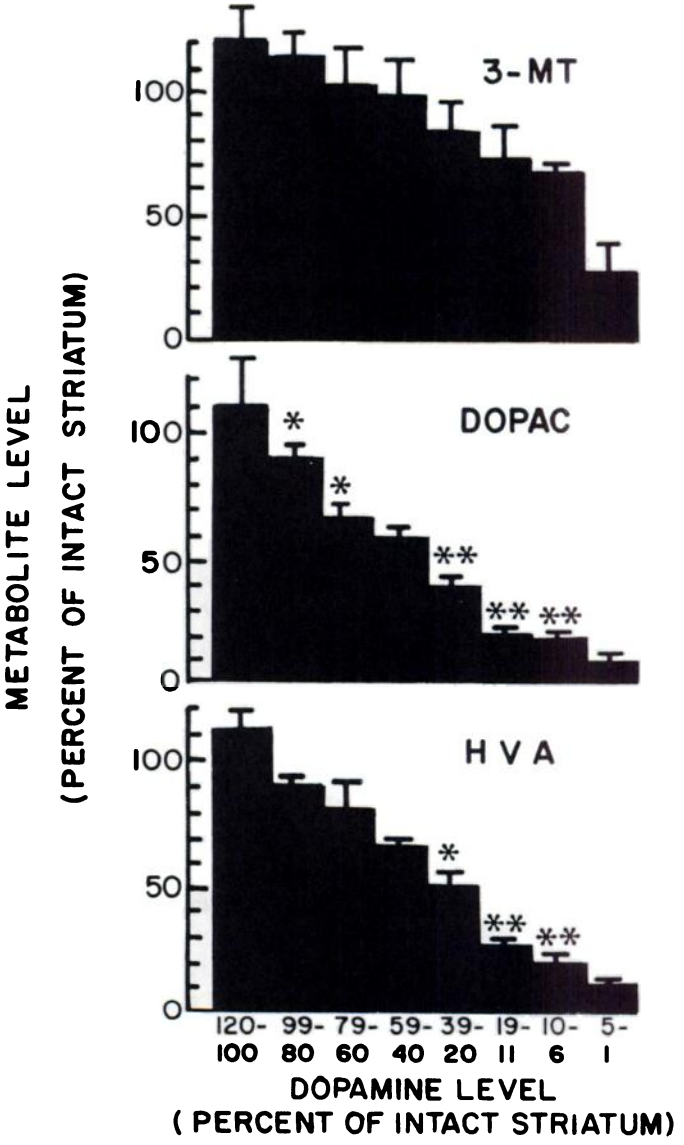

FIG. 6. Extent of striatal dopamine metabolism (*DOPAC, HVA*) and release (*3-MT*) as a function of dopamine denervation (*Dopamine*). *, $P < 0.05$; **, $P < 0.01$ versus 3-MT depletion, Student's *t* test. $n = 3$ to 8 per group. All DOPAC and HVA losses are significant for residual dopamine concentrations of 60% or less; 3-MT losses are significant for residual dopamine concentrations of 20% or less ($P < 0.05$, Student's *t* test) (11).

equal extent. Importantly, 3-MT, as well as DOPAC and HVA, is unmeasureable when dopamine losses are virtually complete (98 to 99%).

The lack of changes in 3-MT over the 0 to 80% denervation range is virtually identical to the results of dopamine release measurements by brain dialysis of the denervated striatum (156a). The preservation of 3-MT levels in neostriata depleted by up to 80% of the dopamine innervation indicates that dopamine release can be maintained at near-normal levels by as few as 20% of the normal dopamine input. Thus, as shown in vitro (169) and in vivo with microdialysis (156a) or 3-MT (refs. 11 and 12; fig. 6), far fewer than the normal number of dopamine nerve terminals can maintain dopamine release and thus normal behaviors.

b. LESIONS OF THE STRIATONIGRAL FEEDBACK PATHWAYS. Lesions of the crus cerebri, which sever most or all of the striatal feedback pathway to the substantia nigra, result in a 56% increase in striatal 3-MT levels 12 days after the lesion in rats (44). These augmentations in DA release presumably reflect the loss of the inhibitory GABAergic feedback loop to the substantia nigra dopamine cell bodies. Indeed, this lesion results in a 69% decrease in the gamma-amino butyric acid (GABA) levels of the ipsilateral substantia nigra, supporting the lesion of striatal GABAergic inputs to the nigra. In the case of an acute hemitransection where the nigrostriatal tract is also severed, there is a 60% decrease in striatal 3-MT levels in the rat (205, 224). This lower 3-MT baseline at 2 to 3 h post lesion represents a new steady state of increased DA synthesis and decreased DA release (205, 224) in the isolated nerve endings, which nonetheless can be pharmacologically modified with amphetamine (34), opiates (224), and GABAergics (205).

In analogy to the physical lesion induced by acute hemitransection, a decrease in impulse conduction of the striatonigral tract can also be established pharmacologically with GBL (71, 90, 101, 184, 209) and HA-966 (36, 132, 184, 219). In dose-response studies of HA-966, clear-cut decrements in DA release can be monitored by in vivo voltammetry (132), by decreases in basal 3-MT levels (219), and by decreases in pargyline-dependent 3-MT accumulation (132). Similarly, GBL also decreases the DA collected in striatal dialysates (90), striatal steady-state 3-MT levels (209), and pargyline-dependent 3-MT accumulation in rat striatum (101). These decreases in DA release precede any compensatory increases in DA synthesis and metabolism (209). Another example of pharmacological axotomy is with local injections of tetrodotoxin (TTX) into the striatum (223). In this case, nigrostriatal as well as all potential presynaptic afferent inputs are inhibited. Under these conditions, the only DA metabolite changes noted are profound (~90%) decreases in striatal 3-MT levels and a slow rise in DA steady-state levels, with no change in DOPAC or HVA (223). Studies with push-pull perfusion (26) and dialysis (200) of the striatum have also demonstrated decreased DA release after local TXX application.

c. LESIONS OF INTRINSIC STRIATAL NEURONS. Kainic acid lesions of the striatum have been reported to not alter striatal 3-MT levels at 3 days (224) but increase these levels to 200% of control at 6 days post lesion (44). A comprehensive time course of this phenomenon remains to be defined in one experiment such that the effects of acute and chronic lesions of striatonigral feedback pathways can be assessed on striatal DA release.

C. Effects of Aging

Dopamine, but not 3-MT, is decreased with age in the basal ganglia of normal humans (43). However, as mentioned above, 3-MT levels increase dramatically after death in human caudate-putamen (168) and in rodents (190), when microwave irradiation is not used (102) or used improperly (190) (section II A). The postmortem increase in dopamine release could therefore mask age-related decreases of 3-MT in the human basal ganglia. Since dopamine levels are also lower in the caudate-putamen of aged rats (13, 125; see ref. 65 for review), we measured dopamine, DOPAC, HVA, and 3-MT in this region and in the olfactory tubercle in aged (28 mo of age) or young (4 mo of age) Fischer 344 rats that received i.p. injections of vehicle or pargyline 10 min before sacrifice by microwave irradiation. Concentrations of dopamine, but neither 3-MT, DOPAC, nor HVA, were decreased in the caudate-putamen (table 6). In contrast, each metabolite was lower in the olfactory tubercle of the aged rats, but the accumulation of 3-MT after pargyline was not, nor was it lower in the caudate-putamen. Thus, caudate-putamen 3-MT and the turnover of dopamine through its releasable pool in either region are not lower in the aged rat. Possible reasons for the persistence of 3-MT in brain regions with partial dopamine denervations have been made based on the unilateral dopamine lesion model (11) (see section IV D).

D. Interpretation of DA Metabolite Changes

In general, the use of DA metabolites to interpret the status of neuronal activity in dopaminergic pathways has been made inappropriately in the literature (reviewed in refs. 50, 54, 108, 157, and 199) despite the early studies of Sharman and co-workers, which clearly showed that DOPAC is the major intraneuronal (i.e., DA nerve ending) DA metabolite with HVA being a secondary metabolite of this DOPAC pool (157). Similarly, the studies of Kehr (101, 102) showed that 3-MT is an accurate index of DA release. Despite these landmark studies, HVA, DOPAC, or DOPAC/DA ratios are still often referred to as indices of DA release. This interpretation has been shown to be inaccurate in many cases (reviewed in table 6) as to preclude the use of these parameters for making inferences concerning DA release.

Therefore, at this point we will define the assumptions upon which the utility of 3-MT as an index of dopamine

TABLE 6

Dopamine and metabolites in the striatum and olfactory tubercle of young and aged rats (n = 9/group). Young (4-mo-old) or aged (29-mo-old) male Fischer 344 rats were given injections of either the saline vehicle (1 ml/g, i.p.) or pargyline (75 mg/kg) 10 min before sacrifice by microwave irradiation. Dopamine and metabolites (206) and protein were measured in the caudate-putamen and olfactory tubercle

Vehicle treatment	3-MT	Dopamine	DOPAC	HVA
Caudate-putamen				
Young	1.2 ± 0.13*	747 ± 84	98 ± 9	44 ± 4
Old	1.0 ± 0.15	533 ± 42† (−28)‡	71 ± 7	40 ± 6
Olfactory tubercle				
Young	0.71 ± 0.09	411 ± 36	64 ± 2	26 ± 1
Old	0.55 ± 0.04§ (−23)	331 ± 31	46 ± 4§ (−28)	18 ± 2§ (−31)
Pargyline treatment‖	**3-MT**	**Dopamine**	**DOPAC**	**HVA**
Caudate-putamen				
Young	6.9 ± 0.89	693 ± 20	62 ± 6	40 ± 4
Old	4.8 ± 1.00	609 ± 80† (−28)	58 ± 7	42 ± 6
Olfactory tubercle				
Young	2.7 ± 0.36	480 ± 31	42 ± 4	22 ± 2
Old	2.4 ± 0.25	365 ± 29†	35 ± 2 (−24)	18 ± 1

* Mean ± SEM.
† $P < 0.05$.
‡ Numbers in parentheses, percentage.
§ $P < 0.01$ versus young animals.
‖ Pargyline singificantly ($P < 0.05$) and consistently altered 3-MT values compared to those of vehicle-treated, age-matched cohorts.

release is based, and then attempt to validate these assumptions with studies of the pharmacological modulation of DA synthesis, metabolism, and release.

In the presence of *unaltered, steady-state concentrations of DA*, the following assumptions are made.

(*a*) Changes in DOPAC levels are an index of intraneuronal DA synthesis/metabolism in the cytoplasmic pool. For example, if DA levels are unchanged after a drug but DOPAC levels are increased, then DA metabolism is increased in the DA nerve ending in conjuntion with enhanced DA synthesis which maintains the unaltered DA steady state. This situation exemplifies the homeostatic mechanisms which maintain the steady-state concentrations of DA after a variety of experimental manipulations.

(*b*) Changes in HVA are secondary to the efflux of DOPAC from the nerve ending and/or changes in the efflux of this metabolite from the brain. Changes in 3-MT concentrations have never been shown to significantly alter HVA concentrations, except following injections of the COMT inhibitor, tropolone (section IV A).

(*c*) Changes in 3-MT levels are indicative of DA release and its subsequent methylation in the cleft and glial cells surrounding the synapse.

This working model is presented in fig. 2. A key feature of this conceptual framework is that DA, DOPAC, HVA, and 3-MT must be measured in order to fully evaluate the functional status of dopaminergic neurons. This is best realized by table 7, in which a number of examples present a clear-cut *uncoupling* of DA synthesis/metabolism (DOPAC) and release (3-MT). These examples are in sharp contrast to the examples where DA metabolism and release are *coupled* processes (table 8) and demonstrate that false conclusions would be derived if HVA or DOPAC were used as indices of DA release and correlated with behavioral or postsynaptic changes after drug treatment. Therefore, in contrast to studies of cholinergic (221) or amino acid-utilizing (210) pathways, where measurements of transmitter turnover are tightly coupled with transmitter synthesis and release, measurements of DA turnover allow only limited interpretations of changes in dopaminergic transmission. *There is no logical framework for the use of such measurements as markers for DA release.* It should thus be clear that measurements of DA turnover utilizing precursor labeling (77, 185), measurements of L-(3,4-dihydroxyphenyl)alanine (L-DOPA) accumulation after inhibition of DOPA decarboxylase, or monitoring the decline in DA levels after inhibition of tyrosine hydroxylase are only indices of DA synthesis rates and not release. However, as in cholinergic and amino acidergic pathways, DA neurons possess the ability to maintain the steady-state concentration of DA during changes in neuronal activity. Therefore, in the case of altered DA synthesis rates, changes in the steady-state levels of DA metabolites allow an investigator to determine if such changes in synthetic activity are coupled to release.

Caution must be used in the interpretation of metabolite concentration data when DA steady-state levels change. Indeed, in such cases it is imperative to determine the time course and characteristics for the development of a new steady-state level of DA. The use of these precautions and the aquisition of key indices of DA synthesis, DA metabolism, and DA release will be of the

TABLE 7

Examples of treatments in which DA metabolism and release are uncoupled processes

Drug	Preparation	DOPAC	3-MT	Release*	Ref.
GABA-A agonists	Rat striatum	↑	↓	↓	161, 205
TTX	Rat striatum	↔	↓	↓	26, 223
GBL-type compounds	Rat striatum	↑	↓	↓	90, 102, 132, 209, 219, 227
Acute hemisection	Rat striatum	↑	↓	↓	3, 26
Partial nigrostriatal lesion	Rat striatum	↓	↑	↑	11, 12, 169
Muscarinic agonists	Rat striatum	↑	↓		201
Amphetamine	Rat striatum	↓	↑	↑	47, 58, 90, 215, 225, 229
Dibutyryl cyclic AMP	Rat striatal slices	↑		↔	137
Mu opiate agonists	Rat striatum	↑	↔	↔	110, 225
Delta opiate agonists	Rat striatum	↑	↔		225, 226
Atypical neuroleptics	Mouse striatum	↑	↓		14a, 15, 218

* DA release as assessed by brain dialysis, voltammetry, or push-pull perfusion.

TABLE 8

Examples of pharmacological treatments in which DA metabolism and release are coupled processes

Drug	Preparation	DOPAC	HVA	Release*	Ref.
DA autoreceptor agonists	Rat and mouse striatum, PFC, and O.T.†	↓	↓	↓	4, 8, 101, 102, 111, 229
Mu and delta opiate agonists	Rat nucleus accumbens, mouse striatum, rat cortex	↑	↑		94, 105a, 222, 225
PCP receptor agonists	Rat PFC and pyriform cortex	↑	↑		31, 56, 82, 152
Classical neuroleptics	Rat and mouse striatum	↑	↑	↑	14a, 119, 218
Non-amphetamine stimulants	Rat striatum	↑	↑	↑	48, 58, 104, 145, 191
CCK-8S	Rat striatum, rat PFC, mouse striatum	↓	↓	↓	5, 7, 28, 51, 113

* DA release as assessed by brain dialysis, voltammetry, or push-pull perfusion.
† O.T., olfactory tubercule.

utmost importance in the design of studies to evaluate the more complex roles of polysynaptic circuits and cotransmitters in the regulation of dopaminergic transmission.

IV. Pharmacology

In the following discussion of pharmacological modulation of 3-MT levels (table 9), unless otherwise specified, all data are concerned with the rat striatum. In this section, we will cover species differences whenever they have been encountered. Whenever possible, changes in 3-MT levels will be compared with changes in DA collected in push-pull perfusates and brain dialysates.

A. Enzyme Inhibitors

The tyrosine hydroxylase inhibitor, AMPT, inhibits DA synthesis, resulting in rapid decreases in striatal steady-state levels of DA, DOPAC, HVA, and 3-MT (58). Using trans-striatal dialysis, identical actions have been observed for DA, DOPAC, and HVA collected in the dialysates (90); unfortunately 3-MT was not measured in this study.

The monoamine oxidase inhibitor (MAOI), pargyline, increases striatal steady-state levels of 3-MT and decreases DOPAC and HVA levels (41, 101, 203, 211, 216). Similiarly, striatal dialysates, after pargyline administration, contain increased DA and 3-MT along with decreased HVA and DOPAC (90, 106, 215; fig. 4). With both the tissue steady-state studies and the brain dialysis measurements, the changes in DOPAC and HVA lag behind the rapid changes in DA release by 15 to 45 min. The MAO-A inhibitor, clorgyline, also increases striatal steady-state 3-MT levels (191) and DA collected in striatal dialysates (100). In contrast, the manoamine oxidase (type B) (MAO-B) inhibitor, deprenyl, does not increase DA collected in striatal dialysates (100). The reversible MAOI, minaprine (97), also increases striatal 3-MT levels and decreases DOPAC with these actions reversing between 2 and 3 h (64).

The COMT inhibitor, tropolone, rapidly decreases striatal 3-MT levels (191, 201). A parallel but slower decline in HVA levels has also been monitored (201), supporting the more rapid turnover of 3-MT as assessed after inhibition of monoamine oxidase (212, 213).

B. D-1 Agonists and Antagonists

The initial in vitro studies of Farnebo and Hamberger (62) and others (115, 173) used apomorphine to show

TABLE 9

Summary table of drug effects on rat striatal DOPAC and 3-MT steady-state levels after fixation with microwave irradiation

Drug (mg/kg, route)		Time (min)	DOPAC (% of control)	3-MT (% of control)	Ref.
Enzyme inhibitors					
AMPT	(200, i.p.)	60	44	44	58
Pargyline	(50, i.p.)	60	11	727	225
Chlorgyline	(0.1, s.c.)	10		100*	191
	(0.3, s.c.)	10		150	191
	(1, s.c.)	10		200	191
Tropolone	(50, i.v.)	3		42	201
Minaprine	(15, i.p.)	15	72	289	64
		60	52	317	64
		120	67	122	64
Dopamine agonists					
Apomorphine	(0.5, i.v.)	10	66	20	58
	(0.5, i.v.)	10		50	191
	(2, i.p.)	4		50	201
		8		40	201
		12		45	201
		16		50	201
		60		100	201
	(0.1, i.p.)	30	72	63	6
CGS 15855A	(0.5, i.p.)	30	100	68	6
	(2, i.p.)	30	60	53	6
Dipropyl-ADTN	(0.03, i.v.)	10		62	191
Piribedil	(10, i.v.)	10		50	191
	(120, p.o.)	60	43	42	145
Ergocornine	(1, i.v.)	10		61	191
Bromocriptine	(1, i.v.)	10		100	191
Dopamine antagonists					
Haloperidol	(1, i.p.)	60	424	191	78
	(2, p.o.)	120		100	191
	(0.5, i.p.)	4		100	201
		8		160	201
		12		130	201
		16		120	201
		60		100	201
	(1, i.p.)	60	325	145	201
	(0.5, i.p.)	15	210	158	145
		30	336	167	145
		60	385	100	145
	(1, i.p.)	60	456	152	218
CPZ	(20, i.p.)	60	294	100	201
Clozapine	(20, i.p.)	60	395	100	78
	(20, i.p.)	15	156	100	145
		30	213	100	145
		60	308	100	145
	(20, i.p.)	60	221	100	218
Sulpiride	(60, i.p.)	60	221	136	218
Buspirone	(0.5 i.p.)	60	132	100	218
	(2, i.p.)	60	177	120	218
	(5, i.p.)	60	254	131	49, 218
	(10, i.p.)	60	294	146	218
	(20, i.p.)	60	346	139	218
Ciclindole	(5, i.p.)	60	182	153	217
CNS stimulants					
Amphetamine	(1, i.v.)	10	60	163	58
	(5, i.v.)	10		254	191
	(5, i.p.)	60	41	423	225
	(5, i.p.)	30		250	201
		60		400	201
		120		190	201
Methylphenidate	(5, i.v.)	10		100	191
	(10, i.p.)	60	100	150	225
	(50, i.p.)	60	125	243	225
Nomifensine	(10, i.p.)	60	87	137	58
	(5, i.v.)	10		149	191
	(30, i.p.)	25	142	285	145
		60	147	227	145
	(25, i.p.)	20	100	185	202
		60	128	205	202
Cocaine	(3, i.v.)	10	100	168	58
Benztropine	(25, i.p.)	20	100	100	201
		45	100	100	201
Quipazine	(20, i.p.)	25	100	329	145
		60	75	171	145
PEA	(12.5, i.p.)	10	100	191	127
		15	124	251	127
		30	146	328	127
		60	100	100	127
Amphenizole	(25, i.p.)	60	130	238	Unpublished
CNS depressants					
GBL	(400, i.p.)	60	182	78	58
	(750, i.p.)	30		30	201
		60		30	201
		150		30	201
HA-966	(20, i.p.)	60	100	50	219
	(100, i.p.)	60	212	41	219
Reserpine	(5, i.p.)	15		400	201
		30		280	201
		60		80	201
		24 h		40	201
GABAergics					
Muscimol	(2, i.p.)	60	100	79	205
	(4, i.p.)	60	192	68	205
Kojic amine	(5, i.p.)	60	139	100	205
	(20, i.p.)	60	200	53	205
THIP	(10, i.p.)	60	133	74	205
	(20, i.p.)	60	183	79	205
Progabide	(100, i.p.)	60	100	100	205
	(500, i.p.)	60	100	68	205
AOAA	(25, i.p.)	60	100	53	205
Diazepam	(2, i.p.)	60	100	100	205
	(10, i.p.)	60	100	44	205
	(20, i.p.)	60	100	37	205
Clonazepam	(5, i.p.)	60	75	58	212
Nitrazepam	(25, i.p.)	60	71	63	212
Baclofen	(2, i.p.)	60		67	191
	(5, i.p.)	60		57	191
	(10, i.p.)	60		44	191
	(20, i.p.)	60		50	191
Flumazenil	(10, i.p.)	60	100	100	211
Opiates					
Morphine	(2, i.p.)	60	158	100	225
	(32, i.p.)	60	185	100	225
	(30, i.p.)	60	160	100	221
	(10, i.p.)	60	173	139	201
Etorphine	(0.015, i.p.)	60	186	100	225
Methadone	(5, i.p.)	60	199	100	225
Phenazocine	(8, i.p.)	60	232	100	225

* One hundred %, no statistically significant change.

TABLE 9—Continued

Drug (mg/kg, route)		Time (min)	DOPAC (% of control)	3-MT (% of control)	Ref.
MR 2034	(2, i.p.)	60	100	100	225
	(8, i.p.)	60	100	100	225
	(32, i.p.)	60	100	100	225
Ethylketazocine	(4, i.p.)	60	100	100	225
	(16, i.p.)	60	100	100	225
Trifluadom	(2, i.p.)	60	100	100	207
U-50488H	(8, i.p.)	60	100	100	207
Butorphanol	(2, i.p.)	60	129	100	207
	(16, i.p.)	60	153	100	207
	(64, i.p.)	60	100	100	207
Pentazocine	(32, i.p.)	60	164	100	225
	(64, i.p.)	60	100	100	225
Cyclazocine	(8, i.p.)	60	148	100	225
	(32, i.p.)	60	100	100	225
DADLE	(0.002, ivt.)†	60	147	100	225
	(0.01, ivt.)	60	176	100	225
	(0.003, ivt.)	60	125	100	226
Naloxone	(5, i.p.)	60	100	100	226
Kyotorphan	(0.8, ivt.)	60	229	100	148
Muscarinics					
Oxotremorine	(1, i.p.)	60	220	73	201

† ivt., intraventricular.

that dopamine autoreceptors modulate the release of dopamine in the striatum. The advent of selective D-1 and D-2 receptor agonists and antagonists allowed the D-2 nature of the autoreceptor to be unequivocally identified. Thus, unlike D-2 stimulation, D-1 receptor stimulation with 2,3,4,5-tetrahydro-1-phenyl-1*H*-3-benzazepine-7,8-diol (SKF 38393) fails to decrease the potassium-induced (176) or electrical stimulation-induced (114, 174) release of [^{3}H]dopamine from neostriatal slices. The ability of D-2 agonists to lower the release of [^{3}H]dopamine is blocked by D-2, but not D-1, selective antagonists. Similar in vitro findings have been obtained for the guinea pig spinal cord (107) and rat prefrontal and cingulate cortices (143, 144, 179).

Changes in in vivo striatal 3-MT levels following the administration of D-1 or D-2-selective compounds parallel the patterns obtained with these in vitro studies. 3-MT levels in mouse striatum are increased by the D-2 antagonists haloperidol and metoclopramide but not by (*S*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol (SCH 23390) or 7-bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benazepine-7-ol (SKF 83566) (15, 33). Similar effects on accumulated 3-MT levels after pargyline have also been observed for haloperidol and SCH 23390 (160). The partial D-2 agonists *trans*-1,3,4,4α,5,10β-hexahydro-4-propyl-2*H*-[1]benzopyrano[3,4-*b*]pyridin-9-ol (CGS 15855A) and the full D-2 agonist (4α-*R*-*trans*)-4,4α,5,6,7,8,8α,9-octahydro-5-*n*-propyl-2*H*-pyrazolo-3,4-γ-guinoline (LY 171555) decrease 3-MT, while the D-1 agonist SKF 38393 does not lower 3-MT unless high doses (50 to 100 mg/kg) are used, and even then only small (18 to 22%) decreases in 3-MT are found (4, 6, 33).

These in vitro and in vivo studies confirm the D-2 nature of the autoreceptor that controls dopamine release and rule out the D-1 receptor in the autoreceptor control of DA release. While this conclusion is consistent with the absence of D-1 receptors on the terminals and cell bodies of nigrostriatal neurons (9), studies with striatal microdialysis present inconsistent results for the D-1 receptor control of release. Imperato et al (91) observed, and Zetterstrom et al. (228) failed to observe, increases in the amount of striatal dopamine recovered into microdialysis probes following similar doses of the D-1 antagonist, SCH 23390. In contrast, Zetterstrom et al. (228), but not Imperato et al. (91), reported suppressions of dialyzed dopamine following similar doses of the D-1 agonist SKF 38393. The use of general anesthesia by Zetterstrom et al. but not Imperato et al. might account for some of these discrepancies.

C. D-2 Antagonists

D-2 receptor antagonists that ameliorate psychosis but without inducing extrapyramidal side effects are considered "atypical" antipsychotics, whereas D-2 antagonists that produce both effects are termed "typical" antipsychotics. Potencies of these drugs in several behavioral tests can predict their antipsychotic efficacy and their propensity to induce extrapyramidal side effects. These tests are, respectively, inhibition by the drug of either apomorphine-induced cage-climbing behavior and apomorphine-induced stereotypic behavior (52, 70). When drug potencies in these tests have been calculated, in vivo neurochemical measurements can be made at the effective dose or at a multiple of the effective dose to discern a neurochemical mechanism that might differentiate these two groups of drugs. Clozapine and thioridazine have been clearly distinguished in this way from the typical neuroleptics haloperidol, chlorpromazine, and metoclopramide by their diminished capacity to alter striatal dopamine release. This has been demonstrated with push-pull cannulae (22), in vivo microdialysis (227), and in vivo voltammetry (89, 112). Not surprisingly, these findings have been corroborated with 3-MT measurements using gas chromatography-mass spectroscopy (GC-MS) methods (described in section II, A and B). Clozapine and thioridazine leave unaltered, or even decrease, striatal 3-MT but like typical neuroleptics frequently elevate DOPAC and HVA (15, 78, 218).

In a more comprehensive study (14a), we have measured 3-MT, DOPAC, HVA, and DA in the caudate-putamen following p.o. administration to mice of ten atypical antipsychotic drugs or candidates and six typical antipsychotics. The first pattern, obtained with the typical neuroleptics (setoperone, perlapine, haloperidol, chlorpromazine, and metoclopramide), was characterized by large (37 to 79%) increases in dopamine release (3-MT levels) and even larger increases in dopamine metabolism, as measured by DOPAC (97 to 297% increases) and HVA (118 to 228% increases) (table 10). This neu-

TABLE 10

Actions of classical neuroleptic agents on mouse striatal DOPAC and 3-MT levels at behaviorally relevant doses. Drugs were injected p.o. at 1 (top row) or 6 (bottom row) times the 50% effective dose for the inhibition of apomorphine-induced climbing. Mice were sacrificed 60 min later. BW 234U was injected i.p. at the 50% effective dose in the behavioral test, and mice were killed 30 min later

Typical compound	Dose (mg/kg)	3-MT (% of control)*	DOPAC (% of control)*
Setoperone	0.2	110	160
	1.2	158†	343†
Perlapine	11	144‡	300†
	66	140‡	349†
Pimozide	0.3	103	197‡
	1.8	113	397†
Haloperidol	0.12	137‡	368†
	0.72	169‡	393†
Chlorpromazine	2.3	128‡	180
	14	140†	335†
Metoclopramide	2	144†	373†
	12	137‡	466†

* Mean for 6 to 8 per group, expressed as the percentage of control values of the vehicle-injected group.

† $P < 0.01$ greater than control.

‡ $P < 0.05$ (Dunnett's test).

TABLE 11

Actions of atypical neuroleptics on mouse striatal DOPAC and 3-MT at behaviorally relevant doses (legend as in table 10)

Atypical compound	Dose (mg/kg)	3-MT (% of control)*	DOPAC (% of control)*
CGS 10746B	25	73†	95
	150	89	114
CL 77-328	3	79	100
	18	75	215‡
BW 234U	25	93	63‡
Clozapine	8	94	143
	48	81	238§
RMI 81582	2	118	117
	12	116	151§
Fluperlapine	34	108	265‡
	190	82	421‡
Melperone	5	179‡	318‡
	30	134	310‡
Flumezapine	1	131§	239‡
(striatum)	5	92	307‡
	20	79	333‡
Flumezapine (olfactory tubercle)	1	122	147
	5	122	253‡
	20	100	325‡
Mesoridazine	1	90	115
	5	107	130
	20	131§	262‡
Thioridazine	5	115	280§
	30	125§	395‡

* Mean ± SEM for 6 to 8 per group, expressed as the percentage of control values of the vehicle-injected group.

† $P < 0.05$ less than control (Dunnett's test).

‡ $P < 0.01$ greater than control.

§ $P < 0.05$ greater than control.

rochemical profile has also been observed in the rat or mouse striatum following the administration of the typical antipsychotics, chlorpromazine, haloperidol, metoclopramide, perlapine, and to a lesser extent, thioridazine for striatal dopamine release (3-MT) and metabolism (DOPAC) (15, 78, 218, 220, 227) or metabolism only (37, 38, 172, 192). The one typical neuroleptic that failed to increase dopamine release while markedly elevating metabolism was pimozide. However, pimozide, unlike the other typical neuroleptics tested, is a potent calcium channel receptor blocker in the brain (75). This action of pimozide may prevent calcium-dependent dopamine release during concomitant D-2 receptor blockade, as described for the calcium channel antagonist nimodipine (142).

The second pattern, obtained with the atypical compounds, was characterized by no change or a *decrease* in 3-MT at either dose (table 11). In only 4 of the 24 groups receiving an atypical compound were 3-MT levels increased. 5-(4-Methyl-1-piperazinyl)imidazo[2,1-*b*][1,3,5] benzothiadiazepine maleate (CGS 10746B), flumezapine, *cis*-5,6-dimethoxy-2-methyl-3-[2-(4-phenyl-1-piperazinyl)ethyl]indoline (CL 77-328), rimcazole (BW 234U), clozapine, 3-(2-chloro-11*H*-dibenz[*b*,*e*]azepine-11-ylidene)-N,N-dimethyl-1-propanamine (RMI 81582), and fluperlapine did not increase dopamine release and produced variable increases in dopamine metabolism. Melperone increased dopamine release at one dose while thioridazine and mesoridazine increased dopamine release at relatively high doses but increased dopamine metabolism at most doses. Importantly, 3-MT levels were lowered or remained unchanged even after doses that produce marked *increases* in DOPAC (26 to 321% above control) or HVA (26 to 129% above control). This separation between effects on release and metabolism has been observed for clozapine (218) and CGS 10746B, a clozapine analog (15, 209). In the case of CGS 10746B, suppressions of 3-MT following p.o. or i.p. administration occur at doses 4- to 6-fold lower than those required to increase DOPAC and HVA, which unlike 3-MT were not lowered by any dose of CGS 10746B. The changes in 3-MT, unlike those obtained for DOPAC and HVA, correspond with decreases in dopamine neuron firing rates and behavioral indices of nigrostriatal suppression (15).

Overall, the resemblance of these minimal effects on dopamine release by atypical antipsychotics with those described in section IV B for the D-1 compounds SCH 23390 and SKF 83566 (33, 59) suggests that D-1 receptor antagonism may contribute to the antipsychotic mechanism of atypical antipsychotics (14a, 17–19).

A very large number of experiments, reviewed above and in tabular form (table 9), corroborate the utility of 3-MT measurements for assessing dopamine release in vivo. However, some controversy concerning changes in 3-MT in the rat striatum after haloperidol has appeared in the literature. As expected from its blockade of D-2 autoreceptors (62, 114, 115), this potent neuroleptic reliably augments striatal 3-MT levels for at least 80 min in the mouse (15, 218). Similarly, increased DA in the

push-pull perfusates of cat caudate has been reported for a number of neuroleptics including haloperidol (119). In the rat, however, haloperidol elevates DOPAC and HVA, but not 3-MT, at 1 h (145, 197, 202) or 2 h (191) after even very high doses of 3 mg/kg. Haloperidol-induced increases in 3-MT are obtained in the rat only in combination with MAO inhibition with clorgyline (191), nialamide (41), or pargyline (102, 160). When sacrifice is at 8 to 16 min postadministration, however, haloperidol does increase rat striatal 3-MT, by about 60% (145, 202). Similarly, only small and transient increases of DA release from rat striatum have been measured with striatal dialysis (106, 227) and push-pull perfusion (149) after haloperidol. Because striatal dopamine release is only transiently increased after haloperidol treatment, it is likely that an early induction of depolarization block of dopamine neurons by this potent neuroleptic (76) prevents subsequent dopamine release. This is consistent with the ability of the MAO inhibition technique to show an increase in 3-MT, since the early increase in release would contribute to the accumulated pool of 3-MT. Thus, rather than invalidating the usefulness of 3-MT as an index of dopamine release, these data reveal a species difference in the actions of haloperidol on rat striatal dopamine release.

D. DA Autoreceptor Agonists

1. Pharmacology. When administered in doses high enough to stimulate postsynaptic D-2 receptors in the nucleus accumbens and caudate-putamen, full dopamine agonists such as apomorphine increase locomotor behavior in rats (52, 150). However, lower doses of dopamine agonists and administration of dopamine autoreceptor agonists (partial agonists) decrease locomotor behavior and striatal dopamine release (57, 83, 84, 177). The locomotor suppression and decrease in dopamine release occur because of the selective activation of presynaptic dopamine autoreceptors. This lowers the release and synaptic concentrations of dopamine. The lessened stimulation of postsynaptic D-2 receptors attenuates locomotion. These low autoreceptor-selective doses of dopamine agonists also lower 3-MT levels in the rat and mouse striatum and olfactory tubercle (4, 6, 8) and the accumulation of 3-MT after pargyline (102).

The subtle, and physiologically relevant, modulation of dopamine neuron activity by autoreceptors can be achieved with the D-2 receptor agonists apomorphine, N-propylnorapomorphine, bromocriptine, and lisuride, and the partial D-2 agonists CGS 15855A and (+)-*trans*-1,3,4,4α,5,10β-hexahydro-4-propyl-2*H*-[1]benzopyrano [3,4-*b*]pyridin-7-ol (CGS 15873) (4, 8, 72) which have at least a 5-fold autoreceptor selectivity (93). Stimulation of the dopamine autoreceptor attenuates dopamine synthesis (103), turnover (41, 57), nigrostriatal cell firing (1), and the depolarization-evoked release of tritium-labeled dopamine from striatal slices (114, 115, 173, 231). Using brain dialysis (229), or in situ voltammetry (111), systemic apomorphine administration decreases by up to 100% the release of dopamine in the striatum. These autoreceptor-mediated decreases in dopamine release are corroborated by 3-MT measurements. Apomorphine (101), lisuride, and bromocriptine (191) decrease 3-MT accumulation after pargyline. Apomorphine also lowers 3-MT levels of otherwise untreated rats (4, 53, 191, 202), rabbits (173), and mice (8, 33). Suppressions of striatal 3-MT following dipropyl-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN), piribedil, ergocornine (191), LY 171555, CGS 15855A, the (−)-enantiomer of CGS 15855 (CGS 16314A), (+)-N,n-propyl-3-(hydroxyphenyl)piperidine [(+)-3-PPP] 6,7-dihydroxy-2-dimethylaminotetralin (TL-99), and (−)N,n-propylnorapomorphine (8, 33) are consistent with the suppression of dopamine release by these compounds.

Basal and pargyline-accumulated levels of 3-MT in the rat frontal cortex and cingulate cortex are also lowered by CGS 15855A or apomorphine (9). This is in contrast to the inability of cortical dopamine autoreceptors to directly modulate neocortical dopamine synthesis or metabolism (20, 21), although DA agonists have been postulated to indirectly modulate these aspects of dopamine activity through changes in dopamine release (69, 204). That dopamine autoreceptor modulation of dopamine release is present in neocortical areas is also supported by in vitro studies of the frontal and piriform cortices (143, 144, 179).

2. Tolerance studies. Repeated administration for 2 h (8) or constant delivery for 2 days (4) of CGS 15855A or apomorphine lowers striatal and olfactory tubercle 3-MT concentrations. After 14 days of administration, however, 3-MT is no longer suppressed by sustained agonist delivery via Alzet minipumps of daily doses that proved maximally effective at 2 days. In addition, tolerance after 14 days to the release-suppressing properties extends to the inability of large, acute injections of either agonist to lower 3-MT, even with the contribution of the pump-delivered drug (4). Tolerance to the synthesis-suppressing (16), firing rate-suppressing (73), and antipsychotic (181, 182) properties of apomorphine also occurs after about 2 days of chronic administration.

E. CNS Stimulants

A wide variety of central nervous system (CNS) stimulants appear to act via increasing DA release and/or inhibiting DA uptake. In the case of amphetamine-type stimulants, inhibition of MAO may also contribute to their pharmacology.

1. Uptake blockers. The DA uptake blockers, nomifensine, amfonelic acid, 1-{2-[bis(4-fluorophenyl)methoxy] ethyl}-4-(3-phenylpropyl)piperazine(GBR 12909), and cocaine, have been shown to increase extraneuronal DA, as assessed by in vivo voltammetry (104), striatal dialysis (48), and steady-state 3-MT measurements (58, 145, 191, 202). The uptake blocker, benztropine, has also been shown to increase synaptic DA concentrations with vol-

tammetry (170) and striatal dialysis (48). However, in the one study of 3-MT steady-state levels, this compound was inactive. This finding is the one published discrepancy for the relationship between basal 3-MT concentrations and DA release, and it clearly requires further study. By monitoring 3-MT accumulation after MAO inhibition, desipramine was found to increase this process in whole rat brain, as does cocaine (99). In the rabbit striatum, however, cocaine did not enhance 3-MT accumulation after MAOI (79).

The physiological importance of high affinity DA uptake by dopaminergic nerve endings during normal synaptic transmission has been a controversial issue. High affinity uptake has been demonstrated to limit the diffusive entry of DA into striatal slices (13, 169). Similarly, inhibition of uptake into brain slices by cocaine and nomifensine increases DA overflow as assessed by voltammetry (104). In contrast, in vivo stimulation of the MFB, in the presence of DA uptake blockers, has been unable to reveal any differences in DA clearance monitored by voltammetry (60). Studies of DA metabolism in rat striatal slices after inhibition of high affinity uptake by either decreasing sodium concentrations in the medium or by the addition of nomifensine have indicated that, while these slices do not accumulate DA, as compared to normal slices, the metabolism of the labeled DA to DOPAC and HVA is only minimally affected (164). The conclusions from these studies were that high affinity DA uptake is only a minor route of DA inactivation and that the bulk of DA metabolism is secondary to glial uptake. In fact, studies of the diffusional distance of released DA indicate that this is less than 100,000 nm (60); however, this value is much greater than the distance of the synaptic cleft (20 to 30 nm; 140). In addition, this diffusional distance was unaffected by inhibition of high affinity DA uptake, by MAO inhibition, and by COMT inhibition (60), again indicating that metabolism of released DA is mainly secondary to glial uptake. This conclusion would be compatible with the astrocytic sheets which surround synapses within the CNS (140).

The degree of activity of a given dopaminergic pathway may also affect the net effect of drug treatments on DA release. In a study of drug effects on DA release in the rat striatum (voltammetry) after either 1 s or 10 s of 50-Hz stimulation of the MFB, benztropine only potentiated the 1-s and not the 10-s period of electrical stimulation (170, 171). These data clearly indicate that drug effects on DA release can be affected by the level of activity of dopaminergic neurons prior to drug administration. In this regard, previous studies of striatal synaptosomes had demonstrated that high affinity uptake of DA is inhibited under depolarizing conditions (86). These data suggest that under low levels of activity, high affinity DA uptake is a significant process for the inactivation of DA but that under high rates of activity glial metabolism of DA may predominate.

2. Precursor supply. Using the paradigm of MAOI in combination with L-DOPA administration, a correlation between increased motor activity and both striatal and nucleus accumbens 3-MT levels was noted (53). Increased 3-MT levels have also been noted in the brainstem and hypothalamus with the combination of MAOI and L-DOPA administration (23, 39).

Using brain dialysis, direct evidence for increased DA release has been obtained after precursor loading with tyrosine (58a).

3. DA releasers. The early studies of Braestrup (34) clearly indicated that amphetamine-like stimulants (amphetamine, methamphetamine, and phenmetrazine) were unique in that they decreased whole brain DOPAC in the rat, while other stimulants increased this DA metabolite (methylphenidate, nomifensine, amfonelic acid, and pipradol). Subsequent studies indicated that all of these stimulants, independent of class, elevated striatal steady-state 3-MT levels (58, 127, 145, 191, 201, 225) and 3-MT accumulation after MAOI (77, 99). One negative study for the effects of methylphenidate on striatal 3-MT was reported (191). The elevated striatal 3-MT levels were also shown to correlate with the appearance of elevated CSF DA levels after amphetamine administration in the rat (47). Amphetamine treatment has been shown to increase DA release collected in striatal dialysates by a number of laboratories (90, 215, 229) and to elevate 3-MT in striatal dialysates (215). The endogenous stimulant, phenethylamine, which appears to be contained within the rat nigrostriatal dopaminergic pathway (95), also releases DA, as indicated by elevated 3-MT levels (127). The possible modulatory or cotransmitter role of this trace amine in dopaminergic transmission warrants further study.

The site of action of these CNS stimulants is presumably at the dopaminergic nerve ending. In the case of amphetamine, the DOPAC lowering effect is also obtained in DA nerve endings in the rat striatum after an acute hemitransection, demonstrating the presynaptic site of action for this drug (34).

4. PCP receptor agonists. Agonists of the phencyclidine (PCP) receptor demonstrate a stereospecific motor activation in rats which is accompanied by elevated DOPAC and HVA in mesolimbic and mesocortical dopaminergic projections, but not in the nigrostriatal pathway (31, 56). In recent studies of steady-state 3-MT levels and 3-MT accumulation after pargyline, the PCP receptor agonists, PCP, dexoxadrol, ketamine, and 10,11-dihydro-5-methyl-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK 801), were found to increase these parameters in mesocortical dopaminergic pathways (152). These data indicate that PCP receptor stimulation leads to activation of mesocortical and mesolimbic DA pathways (66, 85) and are consistent with the early reports of increased [^{14}C]3-MT formation from [^{14}C]tyrosine in whole mouse brain (82). Small decreases in striatal HVA and DOPAC (56)

were observed after PCP which correlated with decreased striatal DA release as assessed by in vivo voltammetry (88).

5. Lithium. Acute lithium has been reported to decrease mouse whole brain 3-MT (135), while chronic treatment for 11 days increases rat striatal 3-MT levels (121). Similarly, 20 days of lithium treatment increase rat striatal and nucleus accumbens DOPAC levels (61). These data indicate that chronic lithium treatment results in both increased DA synthesis and release.

F. CNS Depressants

Alcohol decreases the accumulation of 3-MT after pargyline treatment (116). Stable adenosine analogs have also been shown to decrease the postmortem accumulation of striatal 3-MT in decapitated rats (133), to decrease basal striatal 3-MT levels (214), and to antagonize pargyline-dependent 3-MT accumulation in the rat striatum (214). The use of selected adenosine agonists and antagonists has suggested that these actions are A-1 receptor mediated.

G. GABAergics

GABAergic modulation of the nigrostriatal pathway is extremely complex in that GABA-A receptors have been demonstrated on dopaminergic nerve endings within the striatum (40, 45), on dopaminergic cell bodies in the substantia nigra (109), and on nerve endings of afferents to the substantia nigra (153). GABA-B receptors are also present within both the striatum and substantia nigra (32).

1. GABA-A agonists. The GABA-A agonists muscimol, kojic amine, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP), and progabide all dose dependently decrease the levels of striatal 3-MT (205). The depressant action of progabide on striatal DA release has also been demonstrated directly with push-pull perfusion studies in the cat caudate (161). The effects of these agents on striatal 3-MT levels were antagonized by the GABA antagonist, picrotoxin, and lasted longer than 3 h (205). GABA-A effects on HVA are more complex. While muscimol, THIP, and kojic amine elevate DOPAC (205), THIP and muscimol increase striatal HVA, while kojic amine and progabide decrease HVA (205). The only consistent changes in DA metabolites with these inhibitory agents were the decreases in 3-MT levels.

Using the paradigm of parenteral injections in acutely hemitransected rats and using local drug injections into the striatum, both THIP and kojic amine were shown to act on GABA-A receptors within the striatum (205). In marked contrast, muscimol decreases striatal 3-MT in hemitransected rats but increases 3-MT after local injections into the striatum (205). This effect in the striatum agrees with studies of the effects of muscimol on DA release in striatal slices in vitro (176a), but its potential role in vivo is unknown since this drug effect is not observed after parenteral administration. This unique action of intrastriatal muscimol is antagonized by bicuculline and by prior kainate lesions of striatal neurons (205). In acutely hemitransected rats, parenteral muscimol does not change striatal 3-MT levels (205). These data argue for a modulation by muscimol of an undefined feedback pathway with cell bodies in the striatum and nerve terminals in the substantia nigra.

Local injections of both muscimol and kojic amine into the substantia nigra were also investigated (205). In these experiments, either compound decreased striatal 3-MT levels in a bicuculline-reversible manner. In summary, GABA-A agonists decrease striatal DA release via actions within both the striatum and substantia nigra.

2. GABA-B agonists. The GABA-B agonist baclofen, like GABA-A agonists, decreases striatal 3-MT in conjunction with dramatic elevations in DOPAC and HVA (191). This uncoupling of DA synthesis and release is similar to that observed with GBL (58, 201). In addition, drug effects on DA synthesis express cross-tolerance for GBL and baclofen, suggesting a common locus of action (35, 71).

3. Indirect GABAergics. The benzodiazepine, diazepam, decreases steady-state 3-MT levels (205, 211, 212) and the accumulation of 3-MT after pargyline treatment (99, 211). Push-pull perfusion studies have also shown that diazepam can antagonize the increased striatal DA release from cat caudate after local perfusion with picrotoxin (46).

The actions of diazepam on striatal 3-MT are observed at doses of 5 mg/kg or greater (218) and are reversed both by the GABA antagonist, picrotoxin, and the benzodiazepine receptor antagonist, flumazenil (Ro 151788; 205, 211). This decrease in striatal 3-MT lasts for more than 8 h and is accompanied by decreases in HVA but with no change in DOPAC (218, 219). In a 3-wk chronic treatment study, the actions of diazepam on striatal 3-MT were found not to express tolerance (212).

The benzodiazepines clonazepam and nitrazepam, like diazepam, decrease striatal 3-MT and HVA; however, these drugs also decrease DOPAC (212). The significance of these differences between various benzodiazepines remains to be defined.

The benzodiazepine antagonist, flumazenil, does not alter any striatal DA metabolite but does antagonize the actions of benzodiazepine agonists (211). The inverse benzodiazepine agonist, methylamide-β-carboline-3-carboxylate (FG 7142), which is anxiogenic, increases DOPAC levels in the rat prefrontal cortex (PFC) (180). Recent studies using brain dialysis, have also detected increased DA release in the PFC after FG 7142 (74).

4. GABA transaminase inhibitors. The GABA transaminase inhibitor, aminooxyacetic acid (AOAA), decreases striatal 3-MT levels with a parallel increase in DA steady-state levels (124% of control) but does not change DOPAC or HVA (205). In rats with acute hemitransections, AOAA still decreases striatal 3-MT (205),

indicating an action within the striatum, possibly at the level of the dopaminergic nerve ending. Intraventricular administration of the GABA transaminase inhibitor, ethanolamine-O-sulfate, also has been reported to decrease striatal 3-MT levels (44).

5. Tolerance studies. The use of DA metabolite measurements to assess drug tolerance is extremely complicated since, in many cases, there is a dissociation between changes in indices of DA metabolism and indices of DA release (table 7). In the case of chronic (3 wk) benzodiazepine treatment, this dissociation is clear in that the drug effects on HVA tolerated, while the decrease in 3-MT did not tolerate (212). Studies of subchronic (1 wk) GABA-A (THIP and kojic amine) and GABA-B (baclofen) agonist treatment have indicated tolerance to the drug effects on DOPAC and HVA (24) as well as DA steady-state levels (71). Cross-tolerance between baclofen, GBL, and HA-966 was also monitored with regard to enhanced DA synthesis (35, 71). However, no study of DA release after chronic GABA-A or GABA-B agonist treatment has been conducted, thereby limiting speculations on the mechanisms or sites of action involved in the development of tolerance to GABAergic modulation of dopaminergic neurons.

H. Opiates and Opioid Peptides

1. Striatal DA metabolism. Early studies of the effects of morphine on striatal DOPAC and HVA (reviewed in ref. 207 and 208) on the incorporation of radioactive precursors into the DA pool (27) and on L-DOPA accumulation (139) indicated that DA metabolism was dramatically enhanced in both the rat and mouse striatum. These data, however, did not explain the differences of acute morphine (110) on motor behavior in these 2 species (i.e., motor activation in the mouse and motor depression in the rat). In 1978, this issue was addressed in more depth when it was reported that, while the incorporation of [^{3}H]tyrosine into labeled rat striatal DA and DOPAC occurred to a greater extent in D-Ala-Met-enkephalin amide-treated animals, no change in the labeling of striatal 3-MT was detected (3). At the same time, enhanced striatal 3-MT levels were measured in mouse but not rat striatum after morphine (147, 224). A comparison of mu and delta analgesics revealed increases in striatal DOPAC and HVA in both the rat and mouse but an increase in 3-MT levels only in the mouse. In the mouse, however, strain differences have been described, with strains lacking the motor stimulant effects of morphine also lacking the increase in striatal 3-MT (147). Local morphine injections indicated that the mouse striatal 3-MT increases after morphine were the result of activation of mu receptors in the substantia nigra (147, 224), while the lack of effect of morphine on rat striatal 3-MT appeared to be the result of a "presynaptic clamping" action within the striatum (27, 224).

The agonist/antagonist analgesics also elevate striatal DOPAC but with a bell-shaped dose-response curve which has been attributed to receptor dualism with this class of opiates (94, 207, 208, 225). As with mu and delta agonists, the agonist/antagonist agents do not increase striatal 3-MT levels (207).

In contrast, kappa agonists do not alter the levels of any of the DA metabolites but stereospecifically antagonize the actions of mu and delta and agonist/antagonist analgesics on nigrostriatal DA metabolism in both the rat (225) and the mouse (207). This action has been interpreted to involve a specific mu-2 isoreceptor antagonist action of these kappa agonists (207).

2. Mesolimbic and mesocortical DA metabolism in the rat. Regional studies of opiate effects on DA metabolism have demonstrated that mu (222) and agonist/antagonist (94) analgesics increase both DA synthesis/metabolism (DOPAC) and release (3-MT) in the rat nucleus accumbens. In contrast, synthesis/metabolism is increased in the septum, while release is unaltered (207). Studies of DA release into rat brain dialysates have also demonstrated that mu agonists dramatically increase DA collected in nucleus accumbens dialysates with no effect or small increases in striatal dialysate DA levels (57a).

Analysis of the actions of morphine on mesocortical DA metabolism (table 12) have demonstrated increased DOPAC (105a, 105b) and 3-MT (222) in the prefrontal, piriform, and cingulate cortices. In contrast, neither DA metabolite was elevated in the entorhinal cortex. The actions of morphine were also reversed by (−)-α-(1*R*,5*R*,9*R*)-5,9-dimethyl-2-(L-tetrahydrofurfuryl)-2′-hydroxy-6,7-benzomorphan (MR-2034) indicating that, as in the striatum (section IV H 1), these opiate effects are mu-2 receptor mediated (105b). These data indicate that the mesocortical dopaminergic pathways, except for the entorhinal cortex (105a), receive potent opioid inputs which can increase the activity of mesocortical DA neurons.

The actions of agonist/antagonist analgesics on mesolimbic and mesocortical DA cells are also complex. In the case of butorphanol, both DOPAC and 3-MT are

TABLE 12

Profile of the actions of morphine on regional DOPAC and 3-MT levels in the rat brain (94, 105a, 105b, 207)

Brain region	DOPAC	3-MT
Nigrostriatal		
Striatum	↑	↔
Mesolimbic		
Nucleus accumbens	↑	↑
Olfactory tubercle	↑	↑
Septum	↑*	↔
Mesocortical		
Prefrontal cortex		
Pyriform cortex	↑	↑
Cingulate cortex		
Entorhinal cortex	↔	↔

* Small increase at "high" doses.

increased in the nucleus accumbens (94); however, no effect was observed in the olfactory tubercle or the prefrontal, piriform, or cingulate cortices (94).

I. Cholecystokinin

Interest in the role of cholecystokinin (CCK) in the nervous system has developed in part because of the ability of this peptide to modulate the release of dopamine in several forebrain areas. Peripheral administration of the sulfated octapeptide of CCK (CCK-8S) decreases the basal release of dopamine from mesoaccumbens dopamine neurons as determined with in vivo voltammetry (28, 113). However, increases in dopamine release have also been measured, with microdialysis, in the striatum and accumbens following peripheral administration of CCK-8S (159). We thus determined with 3-MT measurements whether CCK-8S attenuates basal dopamine release in the frontal cortex, olfactory tubercle, or caudate-putamen and whether CCK-8S can reverse the increase in striatal and limbic dopamine release induced by pharmacological means (*d*-amphetamine or haloperidol). These studies have been performed in both the mouse (5, 7) and the rat (51) and indicate that CCK-8S decreases basal 3-MT levels in the striatum and frontal cortex, and the olfactory tubercle at high doses of the peptide. These effects were both dose and time dependent. Additionally, CCK-8S was found to dose dependently antagonize the increases in striatal and olfactory tubercle 3-MT after amphetamine (15 mg/kg) treatment.

Prior work with CCK has also shown that the sulfated octapeptide decreases spontaneous dopamine release in the nucleus accumbens (28, 113). However, the CCK-8S effects on 3-MT are even more apparent when dopamine release is augmented by *d*-amphetamine or haloperidol. The reversals by CCK-8S of elevations in 3-MT following haloperidol or *d*-amphetamine were not simply additive effects of the two drugs. Rather, the magnitude of the CCK attenuation of elevated dopamine release following either drug greatly exceeded the extent of 3-MT decreases obtained with CCK-8S alone. This was especially true in the olfactory tubercle, where CCK-8S had little or no effect on basal dopamine release but reversed the 200% increase in release induced by *d*-amphetamine. Thus, CCK-8S appears to more greatly suppress dopamine release when it has been augmented, either by membrane depolarization (haloperidol) or by impulse-independent release (*d*-amphetamine). This conclusion is supported by the ability of CCK to block potassium-evoked, but not basal, dopamine release from nucleus accumbens slices in vitro (186, 187). These findings are consistent with the proposed role of endogenous CCK as a suppressor of forebrain dopamine neurons (67, 195) and that antipsychotic effects of CCK (134) may be attributable to an inhibition, by CCK, of hyperactive mesolimbic dopamine neurons (141, 156).

V. Summary of Known Limitations of 3-MT Measurements

The main tenet of the present review is that 3-MT is a DA metabolite which is only generated subsequent to DA release by COMT, an enzyme which is not present within dopaminergic neurons. This hypothesis is supported by the excellent agreement between 3-MT measurements and the levels of DA collected in push-pull perfusates and brain dialysates. Additionally, these data correlate well with behavioral data where the direct comparisons have been made. However, as presented above, several potential pitfalls must be considered in the evaluation of any new pharmacological manipulation of dopaminergic neurons.

(*a*) Monoamine oxidase inhibitors will increase 3-MT levels; therefore, any drug under study should be devoid of this action. An example of this problem was an early study of N,N-dimethyltryptamine in which increased 3-MT levels were hypothesized to indicate a DA releasing action for this agent (167). However, subsequent studies clearly demonstrated that this effect was mainly the result of monoamine oxidase inhibition (193).

(*b*) COMT inhibitors will decrease 3-MT levels (191, 201).

(*c*) Dietary factors which alter the activity of MAO or COMT can also complicate the interpretation of pharmacological studies. For example, COMT is a Mg-dependent enzyme, such that in Mg-deficient rats striatal 3-MT levels are decreased (unpublished observations).

(*d*) Species and pathway differences, with regard to drug effects, should always be tested and not extrapolated.

(*e*) Although there are no published drug effects on the clearance of 3-MT, this should be considered as a possible site of action for some drugs, especially since the dynamics of this metabolite pool demonstrate significant species differences (216).

(*f*) Inability to measure 3-MT associated with the incertohypothalamic dopaminergic pathway, since the nerve endings are juxtaposed to blood vessels and do not form classical synapses (130a).

When these potential pitfalls are all taken into consideration, reliable interpretation of the effects of drugs on DA release should be possible.

VI. Conclusions

A thorough review of the literature indicates that 3-MT is a DA metabolite generated subsequent to DA release and that 3-MT measurements are a useful index of DA release in vivo. Furthermore, the simplicity of 3-MT measurements, as compared to brain dialysis or push-pull perfusion methods, will lead to an increase in the use of this metabolite to monitor DA release from dopaminergic pathways in the CNS.

REFERENCES

1. AGHAJANIAN, G. K., AND BUNNEY, B. S.: Dopamine autoreceptors: pharmacological characterization by microiontophoretic single cell recording studies. Naunyn-Schmiedeberg's Arch. Pharmacol. **297:** 1–10, 1977.
2. AGID, Y., JAVOY, F., AND YOUDIM, M. B. H.: Monoamine oxidase and aldehyde dehydrogenase activity in the striatum of rats after 6-hydroxydopamine lesion of the nigrostriatal pathway. Br. J. Pharmacol. **48:** 175–178, 1973.
3. ALGERI, S., BRUNELLO, N., CALDERINI, A., AND CONSOLAZIONE, A.: Effect of enkephalins on catecholamine metabolism in rat CNS. Adv. Biochem. Psychopharmacol. **18:** 199–210, 1978.
4. ALTAR, C. A., BERNER, B., BEALL, R., CARLSEN, S. F., AND BOYAR, W. C.: Dopamine release and metabolism after chronic delivery of selective or non-selective dopamine autoreceptor agonists. Mol. Pharmacol. **33:** 690–695, 1988.
5. ALTAR, C. A., AND BOYAR, W. C.: Brain CCK-B receptors mediate the suppression of dopamine release by cholecystokinin. Brain Res. (in press), 1988.
6. ALTAR, C. A., BOYAR, W. C., OEI, E., AND WOOD, P. L.: Dopamine autoreceptors modulate the in vivo release of dopamine in the frontal, cingulate and entorhinal cortices. J. Pharmacol. Exp. Ther. **242:** 115–120, 1987.
7. ALTAR, C. A., BOYAR, W. C., OEI, E., AND WOOD, P. L.: Cholecystokinin attenuates basal and drug-induced increases of limbic and striatal dopamine release. Brain Res. **460:**76–82, 1988.
8. ALTAR, C. A., BOYAR, W. C., AND WOOD, P. L.: Dopamine autoreceptor agonists including CGS 15855A decrease the in vivo release and metabolism of dopamine in mouse brain. Eur. J. Pharmacol. **134:** 303–311, 1987.
9. ALTAR, C. A., AND HAUSER, K.: Topography of substantia nigra innervation by D1 receptor-containing striatal neurons. Brain Res. **410:** 1–11, 1987.
10. ALTAR, C. A., JOYCE, J. N., AND MARSHALL, J. F.: Functional organization of dopamine and serotonin receptors in the rat forebrain. *In* Quantitative Receptor Autoradiography, ed. by C. A. Boast, E. W. Snowhill, and C. A. Altar, pp. 53–78, Alan R. Liss, New York, 1986.
11. ALTAR, C. A., AND MARIEN, M. R.: Preservation of dopamine release in the denervated striatum: implications for Parkinson's disease. Neurosci. Lett. (in press), 1988.
12. ALTAR, C. A., MARIEN, M. R., AND MARSHALL, J. F.: Time course of adaptations in dopamine synthesis, metabolism, and release following nigrostriatal lesions: implications for behavioral recovery from brain injury. J. Neurochem. **48:** 390–399, 1987.
13. ALTAR, C. A., AND MARSHALL, J. F.: Neostriatal dopamine uptake and reversal of age-related movement disorders with dopamine uptake inhibitors. *In* Central Determinants of Age-related Declines in Motor Function, ed. by J. Joseph, pp. 343–354, New York Academy, New York, 1987.
14. ALTAR, C. A., O'NEIL, S., AND MARSHALL, J. F.: Sensorimotor impairments and neostriatal dopamine metabolite elevations result from intranigral injection of 6-hydroxydopamine or gamma-hydroxybutyrate in awake rats. Neuropharmacology **23:** 309–318, 1987.

14a. ALTAR, C. A., WASLEY, A. M., BOYAR, W. C., LIEBMAN, J., GERHARDT, S., AND WOOD, P. L.: Dopamine neurochemical profile of atypical antipsychotics resembles that of D-1 antagonists. Naunyn-Schmiedeberg's Arch. Pharmacol. **338:** 162–168, 1988.

15. ALTAR, C. A., WASLEY, A. M., LIEBMAN, J., GERHARDT, S., KIM, H., WELCH, J. J., AND WOOD, P. L.: CGS 10746B: an atypical antipsychotic candidate that selectively decreases dopamine release at behaviorally effective doses. Life Sci. **39:** 699–705, 1986.
16. ANDEN, N.-E., GRABOWSKA-ANDEN, E., AND LILJENBERG, B.: Demonstration of autoreceptors on dopamine neurons in different brain regions of rats treated with gammabutyrolactone. J. Neural Trans. **58:** 143–152, 1983.
17. ANDERSEN, P. H., AND BRAESTRUP, C.: Evidence for different states of the dopamine D1 receptor: clozapine and fluperlapine may preferentially label an adenylate cyclase-coupled state of the D1 receptor. J. Neurochem. **47:** 1822–1831, 1986.
18. ANDERSEN, P. H., GRONVALD, F. C., AND JANSEN, J. A.: A comparison between dopamine-stimulated adenylate cyclase and ^{3}H-SCH 23390 binding in the rat striatum. Life Sci. **37:** 1971–1983, 1985.
19. ANDERSEN, P. H., NIELSEN, E. B., GRONVALD, F. C., AND BRAESTRUP, C.: Some atypical neuroleptics inhibit [^{3}H]SCH 23390 binding in vivo. Eur. J. Pharmacol. **120:** 143–144, 1986.
20. BANNON, M. J., AND ROTH, R. H.: Pharmacology of mesocortical dopamine neurons. Pharmacol. Rev. **35:** 53–68, 1983.
21. BANNON, M. J., WOLF, M. E., AND ROTH, R. H.: Pharmacology of dopamine neurons innervating the prefrontal, cingulate, and piriform cortices. Eur. J. Pharmacol. **92:** 119–125, 1983.
22. BARTHOLINI, G.: Differential effect of neuroleptic drugs on dopamine turnover in the extrapyramidal and limbic system. J. Pharm. Pharmacol. **28:** 429–433, 1976.
23. BAU, N. T., ANGERS, M., DUHAIME, J., AND KUCHELE, O.: Modification of dopamine and norepinephrine metabolism in the rat brain by monoamine oxidase inhibitors. J. Neural Trans. **70:** 39–50, 1987.
24. BEART, P. M., SCATTON, B., AND LLOYD, K. G.: Subchronic administration of GABAergic agonists elevates [^{3}H]GABA binding and produces tolerance in striatal dopamine catabolism. Brain Res. **335:** 169–173, 1985.
25. BERNHEIMER, H., BIRKMAYER, W., HORNYKIEWICZ, O., JELLINGER, K., AND SEITELBERGER, F.: Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological, and neurochemical correlations. J. Neurol. Sci. **20:** 415–455, 1973.
26. BESSON, M. J., CHERAMY, A., FELTZ, P., AND GLOWINSKI, J.: Dopamine: spontaneous and drug induced release from the caudate nucleus in the cat. Brain Res. **32:** 407–424, 1971.
27. BIGGIO, G., CASA, M., CORDA, M. G., DIBELLO, C., AND GESSA, G. L.: Stimulation of dopamine synthesis in caudate nucleus by intrastriatal enkephalins and antagonism by naloxone. Science (Wash. DC) **200:** 552–554, 1978.
28. BLAHA, C. D., PHILLIPS, A. G., AND LANE, R. F.: Reversal by cholecystokinin of apomorphine-induced inhibition of dopamine release in the nucleus accumbens of the rat. Regul. Pept. **17:** 301–310, 1987.
29. BLANK, C. L., SASA, S., ISERNHAGEN, R., MEYERSON, L. R., WASSIL, D., WONG, P., MODAK, A. T., AND STAVINOHA, W. B.: Levels of norepinephrine and dopamine in mouse brain regions following microwave irradiation—rapid post-mortem degradation of striatal DA in decapitated animals. J. Neurochem. **33:** 213–219, 1979.
30. BLUM, M., MCEWEN, B. S., AND ROBERTS, J. L.: Transcriptional analysis of tyrosine hydroxylase gene expression in the tuberinfundibular dopaminergic neurons of the rat arcuate nucleus after estrogen treatment. J. Biol. Chem. **262:** 817–821, 1987.
31. BOWERS, M. B., AND HOFFMAN, F. J.: Homovanillic acid in rat caudate and prefrontal cortex following phencyclidine and amphetamine. Psychopharmacology **84:** 136–137, 1984.
32. BOWERY, N. G., HUDSON, A. L., AND PRICE, G. W.: GABA-A and GABA-B receptor site distribution in the rat central nervous system. Neuroscience **20:** 365–383, 1987.
33. BOYAR, W. C., AND ALTAR, C. A.: Modulation of dopamine release by D2 but not D1 receptor agonists and antagonists. J. Neurochem. **48:** 824–831, 1987.
34. BRAESTRUP, C.: Biochemical differentiation of amphetamine vs. methylphenidate and nomifensine in rats. J. Pharm. Pharmacol. **29:** 463–470, 1977.
35. BROXTERMAN, H. J., NOACH, E. K., VAN VALKENBURG, C. F. M., AND WIJLING, A.: Cross-tolerance of dopamine metabolism to baclofen, gamma-butyrolactone, and HA-966 in the striatum and olfactory tubercle of the rat. Life Sci. **28:** 973–981, 1981.
36. BROXTERMAN, H. J., VANVALKENBURG, C. F. M., AND NOACH, E. L.: HA-966 effects on striatal dopamine metabolism: implications for dopamine compartmentation. J. Pharm. Pharmacol. **32:** 67–69, 1980.
37. BURKI, H. R.: Effects of fluperlapine on dopaminergic systems in rat brain. Psychopharmacologia **89:** 77–84, 1986.
38. BURKI, H. R., RUCH, W., AND ASPER, H.: Effects of clozapine, thioridazine, perlapine, and haloperidol on the metabolism of the biogenic amines in the brain of the rat. Psychopharmacologia **41:** 27–33, 1975.
39. BUU, N. T.: Relationship between catechol-o-methyltransferase and phenolsulfotransferase in the metabolism of dopamine in the rat brain. J. Neurochem. **45:** 1612–1619, 1985.
40. CAMPOCHIARO, P., SCHWARTZ, R., AND COYLE, J. T.: GABA receptor binding in rat striatum: localization and effects of denervation. Brain Res. **136:** 501–511, 1977.
41. CARLSSON, A., AND LINDQVIST, M.: Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol. Toxicol. **20:** 140–144, 1963.
42. CARLSSON, A., LINDQVIST, M., AND KEHR, W.: Postmortal accumulation of 3-methoxytyramine in brain. Naunyn-Schmiedeberg's Arch. Pharmacol. **284:** 365–372, 1974.
43. CARLSSON, A., AND WINBLAD, B.: Influence of age and time interval between death and autopsy on dopamine and 3-methoxytyramine levels in human basal ganglia. J. Neural Trans. **38:** 271–276, 1976.
44. CATTABENI, F., BUGATTI, A., GROPPETTI, A., MAGGI, A., PARENTI, M., AND RACAGNI, G.: GABA and dopamine: their mutual regulation in the nigrostriatal system. *In* GABA-Neurotransmitters, ed. by P. Krogsgaard-Larsen, J. Scheel-Kruger, and H. Kofod, pp. 107–117, Academic Press, New York, 1979.
45. CHANG, R. S. L., TRAN, V. T., AND SNYDER, S. H.: Neurotransmitter receptor localizations: brain lesion induced alterations in benzodiazepine, GABA, beta-adrenergic, and histamine H1-receptor binding. Brain Res. **190:** 95–110, 1980.
46. CHERAMY, A., NIEOULLON, A., AND GLOWINSKI, J.: Blockade of the picrotoxin-induced in vivo release of dopamine in the cat caudate nucleus by diazepam. Life Sci. **20:** 811–816, 1977.
47. CHIUEH, C. C., ZAVADIL, A. P., AND MARKEY, S. P.: Increase of striatal 3-methoxytyramine but not of homovanillic acid following administration of *d*-amphetamine or decapitation. Fed. Proc. **37:** 509 (abst. 1554), 1978.
48. CHURCH, W. H., JUSTICE, J. B., AND BYRD, L. D.: Extracellular dopamine in rat striatum following uptake inhibition by cocaine, nomifensine, and benztropine. Eur. J. Pharmacol. **139:** 345–348, 1987.
49. CIMINO, M., PONZIO, F., ACHILLI, G., VANTINI, G., PEREGO, C., ALGERI, S., AND GARATTINI, S.: Dopaminergic effects of buspirone, a novel anxiolytic agent. Biochem. Pharmacol. **32:** 1069–1074, 1983.

50. COMMISSIONG, J. W.: Monoamine metabolites: their relationship to monoaminergic neuronal activity. Biochem. Pharmacol. **34:** 1127–1131, 1985.
51. COSI, C., ALTAR, C. A., AND WOOD, P. L.: Effects of cholecystokinin on acetylcholine turnover and dopamine release in the rat frontal cortex and striatum. Neuropharmacology (in press), 1988.
52. COSTALL, B., NAYLOR, R. J., AND NOHRIA, V.: Climbing behavior induced by apomorphine in mice: a potential model for the detection of neuroleptic activity. Eur. J. Pharmacol. **50:** 39–50, 1978.
53. DAVIES, C. L., AND HEAL, D. J.: Determination of 3-methoxytyramine in rat brain by HPLC with electrochemical detection and its correlation with dopamine function after administration of a monoamine oxidase inhibitor and L-3,4-dihydroxyphenylalanine. J. Neurochem. **47:** 1919–1923, 1986.
54. DEDEK, J., BAUMES, R., TIEN-DUC, N., GOMENI, R., AND KORF, J.: Turnover of free and conjugated (sulphonyloxy)dihydroxyphenylacetic acid and homovanillic acid in rat striatum. J. Neurochem. **33:** 687–695, 1979.
55. DEMAREST, K. T., SMITH, D. L., AND AZZARO, A. J.: The presence of the type A form of monoamine oxidase within nigrostriatal dopamine-containing neurons. J. Pharmacol. Exp. Ther. **215:** 461–468, 1980.
56. DEUTCH, A. Y., TAM, S.-Y., FREEMAN, A. S., BOWERS, M. B., JR., AND ROTH, R. H.: Mesolimbic and mesocortical dopamine activation induced by phencyclidine: contrasting pattern to striatal response. Eur. J. Pharmacol. **134:** 257–264, 1987.
57. DICHIARA, A. M., PORCEDDU, M. L., FRATTA, W., AND GESSA, G. L.: Postsynaptic receptors are not essential for dopaminergic feedback regulation. Nature (Lond.) **267:** 270–272, 1977.
57a. DICHIARA, G., AND IMPERATO, A.: Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. J. Pharmacol. Exp. Ther. **244:** 1067–1080, 1988.
58. DIGIULIO, A. M., GROPPETTI, A., CATTABENI, F., GALLI, C. L., MAGGI, A., ALGERI, S., AND PONZIO, F.: Significance of dopamine metabolites in the evaluation of drugs acting on dopaminergic neurons. Eur. J. Pharmacol. **52:** 201–278, 1978.
58a. DURING, M. J., ACWORTH, I. N., AND WURTMAN, R. J.: Effects of systemic L-tyrosine on dopamine release from rat corpus striatum and nucleus accumbens. Brain Res. **452:** 378–380, 1988.
59. EWING, A. G., BIGELOW, J. C., AND WIGHTMAN, R. M.: Direct in vivo monitoring of dopamine released from two striatal compartments in the rat. Science (Wash. DC) **221:** 169–171, 1983.
60. EWING, A. G., AND WIGHTMAN, R. M.: Monitoring the stimulated release of dopamine with in vivo voltammetry. II. Clearance of released dopamine from extracellular fluid. J. Neurochem. **43:** 570–577, 1984.
61. FADDA, F., SERRA, G., ARGIOLAS, A., MELIS, M. R., AND GESSA, G. L.: Effect of lithium on 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations in different brain areas of rats. Pharmacol. Res. Commun. **12:** 689–693, 1980.
62. FARNEBO, L., AND HAMBERGER, B.: Drug-induced changes in the release of ^{3}H-monoamines from field stimulated rat brain slices. Acta Physiol. Scand. **371:** 35–44, 1971.
63. FAULL, K. F., AND BECK, O.: Quantification of neurotransmitters and related compounds by electron capture chemical ionization GC-MS. *In* Mass Spectrometry in Biomedical Research, ed. by S. J. Gaskell, pp. 403–441, John Wiley & Sons, New York, 1986.
64. FERRETTI, P., ALGERI, S., BENFENATI, F., CIMINO, M., FERREITI, C., GARRATTITI, M., AND LIPARTITI, M.: Biochemical effects of minaprine on striatal dopaminergic neurons in rats. J. Pharm. Pharmacol. **36:** 48–50, 1984.
65. FINCH, C. E., RANDALL, P. K., AND MARSHALL, J. F.: Aging and basal ganglia functions. Annu. Rev. Gerontol. Geriat. **2:** 49–85, 1981.
66. FRENCH, E. D., PILAPIL, C., AND QUIRION, R.: Phencyclidine binding sites in the nucleus accumbens and phencyclidine-induced hyperactivity are decreased following lesions of the mesolimbic dopamine system. Eur. J. Pharmacol. **116:** 1–9, 1985.
67. FUXE, K., ANDERSON, K., LOCATELLI, V., AGNATI, L. F., HOKFELT, T., SKIRBOLL, L., AND MUTT, V.: Cholecystokinin peptides produce marked reduction of dopamine turnover in discrete areas in the rat brain following intraventricular injection. Eur. J. Pharmacol. **67:** 329–331, 1980.
68. GALLI, C. L., CATTABENI, F., EROS, T., SPANO, P. F., ALGERI, S., DIGIULIO, A., AND GROPPETTI, A.: A mass fragmentographic assay of 3-methoxytyramine in rat brain. J. Neurochem. **27:** 795–798, 1976.
69. GALLOWAY, M. P., WOLF, M. E., AND ROTH, R. H.: Regulation of dopamine synthesis in the medial prefrontal cortex is mediated by release modulating autoreceptors: studies in vivo. J. Pharmacol. Exp. Ther. **236:** 689–698, 1986.
70. GERHARDT, S., GERBER, R., AND LIEBMAN, J. M.: SCH 23390 dissociated from conventional neuroleptics in apomorphine climbing and primate acute dyskinesia models. Life Sci. **37:** 2355–2363, 1985.
71. GIANUTSOS, G., AND MOORE, K. E.: Tolerance to the effects of baclofen and gamma-butyrolactone on locomotor activity and dopaminergic neurons in the mouse. J. Pharmacol. Exp. Ther. **207:** 859–869, 1978.
72. GLAESSER, B. S., BERRY, J. C., BOYAR, W. C., LOVELL, R. A., BRAUNWALDER, A., LOO, P., STONE, G., KALINSKY, H., AND HUTCHISON, A. J.: Dopamine autoreceptor activity of CGS 15855A. Soc. Neurosci. Abstr. **11:** 501, 1985.
73. GOLDSTEIN, J. M., LITWIN, L. C., AND MALICK, J. B.: Electrophysiological effects of repeated administration of apomorphine, EMD 23 448, and (+) 3-PPP on A9 neurons. Soc. Neurosci. Abstr. **11:** 116, 1985.
74. GOLDSTEIN, L. E., BRADBERRY, C. W., ROTH, R. H., AND BUNNEY, B. S.: Simultaneous in vivo measurements of dopamine release and metabolism in the rat medial prefrontal cortex and striatum using intracerebral dialysis. Soc. Neurosci. Abstr. **13:** 457.8, 1987.
75. GOULD, R. J., MURPHY, K. M., REYNOLDS, I. J., AND SNYDER, S. H.: Antischizophrenic drugs of the dipnehylbutylpiperidine type act as calcium channel antagonists. Proc. Natl. Acad. Sci. USA **80:** 5122–5125, 1983.
76. GRACE, A. A., AND BUNNEY, B. S.: Opposing effects of striatonigral feedback pathways on midbrain dopamine cell activity. Brain Res. **333:** 271–284, 1985.
77. GROPPETTI, A., ALGERI, S., CATTABENI, F., DIGIULIO, A. M., GALLI, C. L., PONZIO, F., AND SPANO, P. F.: Changes in specific activity of dopamine metabolites as evidence of a multiple compartmentation of dopamine in striatal neurons. J. Neurochem. **28:** 193–197, 1977.
78. GROPPETTI, A., PARENTI, M., GALLI, C. L., BUGATTI, A., CATTABENI, F., DIGIULIO, A. M., AND RACAGNI, G.: 3-Methoxytyramine and different neuroleptics: dissociation from HVA and DOPAC. Life Sci. **23:** 1763–1768, 1978.
79. GULDBERG, H. C., SHARMAN, D. F., AND TEGERDINE, P. R.: Some observations on the estimation of 3-methoxytyramine in brain tissue. Br. J. Pharmacol. **42:** 505–511, 1971.
80. HEFTI, F., MELAMED, E., AND WURTMAN, R. J.: Partial lesions of the dopaminergic nigrostriatal system in rat brain: biochemical characterization. Brain Res. **195:** 123–137, 1980.
81. HENN, F. A., AND HAMBERGER, A.: Glial cell function: uptake of transmitter substances. Proc. Natl. Acad. Sci. USA **68:** 2686–2690, 1971.
82. HITZEMAN, R. J., LOH, H. H., AND DOMINO, E. F.: Effect of phencyclidine on the accumulation of ^{14}C-catecholamines formed from ^{14}C-tyrosine. Arch. Int. Pharmacodyn. **202:** 252–258, 1973.
83. HJORTH, S., CARLSSON, A., AND CLARK, D.: Central dopamine agonist and antagonist actions of the enantiomers of 3-PPP. Psychopharmacology **81:** 89–99, 1983.
84. HJORTH, S. A., CARLSSON, A., WIKSTROM, H., LUNDBERG, P., SANCHEZ, D., HACKSELL, U., ARVIDSSON, L.-E., SVENSSON, U., AND NILSON, J. L. G.: 3-PPP, a new centrally acting DA-receptor agonist with selectivity for autoreceptors. Life Sci. **28:** 1225–1238, 1981.
85. HOKFELT, T., LJUNGDAHL, A., FUXE, K., AND JOHANSSON, O.: Dopamine nerve terminals in the rat limbic cortex: aspects of the dopamine hypothesis of schizophrenia. Science (Wash. DC) **184:** 177–179, 1974.
86. HOLTZ, R. W., AND COYLE, J. T.: The effects of various salts, temperature, and the alkaloids veratridine and brachotoxin on the uptake of [^{3}H] dopamine into synaptosomes from rat striatum. Mol. Pharmacol. **10:** 746–758, 1974.
87. HORNKIEWICZ, O.: Parkinson's disease: from brain homogenate to treatment. Fed. Proc. **321:** 183–190, 1973.
88. HOWARD-BUTCHER, S., BLAHA, C. D., AND LANE, R. S.: A comparison of CNS stimulants with phencyclidine on dopamine release using in vivo voltammetry. Brain Res. Bull. **13:** 497–501, 1984.
89. HUFF, R. M., AND ADAMS, R. N.: Dopamine release in N. accumbens and striatum by clozapine: simultaneous monitoring by in vivo electrochemistry. Neuropharmacology **19:** 587–590, 1980.
90. IMPERATO, A., AND DICHIARA, G.: Trans-striatal dialysis coupled to reverse phase high performance liquid chromatography with electrochemical detection: a new method for the study of the in vivo release of endogenous dopamine and metabolites. J. Neurosci. **4:** 966–977, 1984.
91. IMPERATO, A., MULAS, A., AND DICHIARA, G.: The D-1 antagonist SCH 23390 stimulates while the D-1 agonist SKF 38393 fails to affect dopamine release in the dorsal caudate of freely moving rats. Eur. J. Pharmacol. **142:** 177–182, 1987.
92. ISHIKAWA, K., SHIBANOKI, S., SAITO, S., AND MCGAUGH, J. L.: Effect of microwave irradiation on monoamine metabolism in dissected rat brain. Brain Res. **240:** 158–161, 1982.
93. IYENGAR, S., HAUSLER, A., KIM, H. S., MARIEN, M., ALTAR, C. A., AND WOOD, P. L.: The dopamine autoreceptor agonist, CGS 15855A, modulates striatal dopamine metabolism and prolactin release. Neuropharmacology (in press), 1988.
94. IYENGAR, S., KIM, H. S., AND WOOD, P. L.: Agonist action of the agonist/antagonist analgesic butorphanol on dopamine metabolism in the nucleus accumbens of the rat. Neurosci. Lett. **77:** 226–230, 1987.
95. JURIO, A. V.: Lesion of selected brain areas as a tool for the demonstration of some trace biogenic amines neural pathways. Gen. Pharmacol. **18:** 1–5, 1987.
96. KAAKKOLA, S., MANNISTO, P. T., AND NISSINEN, E.: Striatal membrane-bound and soluble catechol-*o*-methyl-transferase after selective lesions in the rat. J. Neural. Trans. **69:** 221–228, 1987.
97. KAN, J. P., MOUGET-GONIOT, C., BIZIERE, K., LADINSKY, H., AND GARATTINI, S.: Minaprine, a new atypical stimulant of dopaminergic neurotransmssions. II. Evidence for dual pre- and post-synaptic mechanisms of action. *In* Dopaminergic Systems and Their Regulation, ed. by G. N. Woodruff, pp. 395–396, VCH Publishers, London, 1986.
98. KAPLAN, G. P., HARTMAN, B. K., AND CREVELING, C. R.: Immunohisto-

chemical demonstration of catechol-*o*-methyltransferase in mammalian brain. Brain Res. **167:** 241–250, 1979.

99. KARASAWA, T., FURUKAWA, K., OCHI, V., AND SHIMIZU, M.: Monoamine metabolites as indicators of the effect of centrally acting drugs on monoamine release in rat brain. Arch. Int. Pharmacodyn. **231:** 261–273, 1978.
100. KATO, T., DONG, B., ISHII, K., AND KINEMUCHI, H.: Brain dialysis: in vivo metabolism of dopamine and serotonin by monoamine oxidase A but not B in the striatum of unrestrained rats. J. Neurochem. **46:** 1277–1282, 1986.
101. KEHR, W.: 3-Methoxytyramine as an indicator of impulse-induced dopamine release in rat striatum in vivo. Naunyn-Schmiedeberg's Arch. Pharmacol. **293:** 209–215, 1976.
102. KEHR, W.: 3-Methoxytyramine and normetanephrine as indicators of dopamine and noradrenaline release in mouse brain in vivo. J. Neural Transm. **50:** 165–178, 1981.
103. KEHR, W., CARLSSON, A., LINDQVIST, M., MAGNUSSON, T., AND ATACK, C.: Evidence for a receptor-mediated feedback control of striatal tyrosine hydroxylase activity. J. Pharm. Pharmacol. **24:** 744–747, 1972.
104. KELLY, R. S., AND WRIGHTMAN, R. M.: Detection of dopamine overflow and diffusion with voltammetry in slices of rat striatum. Brain Res. **423:** 79–87, 1987.
105. KILTS, C. D., VRBANAC, J. J., RICKERT, D. E., AND RECH, R. H.: Mass fragmentographic determination of 3,4-dihydroxyphenylethylamine and 4-hydroxy-3-methoxyphenethylamine in the caudate nucleus. J. Neurochem. **28:** 465–467, 1977.
105a. KIM, H. S., IYENGAR, S., AND WOOD, P. L.: Opiate actions on mesocortical dopamine metabolism in the rat. Life Sci. **39:** 2033–2036, 1986.
105b. KIM, H. S., IYENGAR, S., AND WOOD, P. L.: Reversal of the actions of morphine on mesocortical dopamine metabolism in the rat by the kappa agonist MR-2034: tentative mu-2 opioid control of mesocortical dopaminergic projections. Life Sci. **41:** 1711–1715, 1987.
106. KITO, S., SHIMOYAMA, M., AND ARAKAWA, R.: Effects of neurotransmitters or drugs on the in vivo release of dopamine and its metabolites. Jpn. J. Pharmacol. **40:** 57–67, 1986.
107. KONDO, M., FUJIWARA, H., AND TANAKA, C.: Dopamine release and presynaptic dopaminergic regulation in guinea-pig spinal cord. Jpn. J. Pharmacol. **41:** 39–46, 1986.
108. KOPIN, I. J.: Catecholamine metabolism: basic aspects and clinical significance. Pharmacol. Rev. **37:** 333–364, 1985.
109. KUPERSMITH, M. J., AND LIEBERMANN, A. N.: The effect of specific brain lesions on the high affinity binding of GABA in the substantia nigra. Brain Res. **194:** 536–539, 1980.
110. KUSCHINSKY, K., AND HORNYKIEWICZ, O.: Effects of morphine on striatal dopamine metabolism: possible mechanism of its opposite effect on locomotor activity in rats and mice. Eur. J. Pharmacol. **26:** 41–50, 1974.
111. LANE, R. F., AND BLAHA, C. D.: Electrochemistry in vivo: application to CNS pharmacology. Ann. NY Acad. Sci. **473:** 47–63, 1986.
112. LANE, R. F., AND BLAHA, C. D.: Chronic haloperidol decreases dopamine release in striatum and nucleus accumbens in vivo: depolarization block as a possible mechanism of action. Brain Res. Bull. **18:** 135–138, 1987.
113. LANE, R. F., BLAHA, C. D., AND PHILLIPS, A. G.: In vivo electrochemical analysis of cholecystokinin-induced inhibition of dopamine release in the nucleus caudatus. Brain Res. **397:** 200–204, 1986.
114. LEHMANN, J., BRILEY, M., AND LANGER, S. Z.: Characterization of dopamine autoreceptor and tritium labeled spiperone binding sites in vitro with classical and novel dopamine receptor agonists. Eur. J. Pharmacol. **88:** 11–26, 1983.
115. LEHMANN, J., AND LANGER, S. Z.: The pharmacological distinction between central pre- and postsynaptic dopamine receptors: implications for the pathology and therapy of schizophrenia. *In* Advances in Dopamine Research, ed. by M. Kohsaka et al., Pergamon, Oxford, 1982.
116. LILJEQUIST, S., AND CARLSSON, A.: Alteration of central catecholamine metabolism following acute administration of ethanol. J. Pharm. Pharmacol. **30:** 728–730, 1978.
117. LINDVALL, O., AND BJORKLUND, A.: Dopamine- and norepinephrine-containing neuron systems: their anatomy in the rat brain. *In* Chemical Neuroanatomy, ed. by P. C. Emson, pp. 229–255, Raven Press, New York, 1983.
118. LINDVALL, O., BJORKLUND, A., AND FALCK, B.: Fluorescence microscopy of biogenic amines. *In* Methods in Neurobiology, ed. by R. Lahue, vol. 2, pp. 365–431, Plenum Press, New York, 1981.
119. LLOYD, K. G., AND BARTHOLINI, G.: The effects of drugs on the release of endogenous catecholamines into the perfusate of discrete brain areas of the cat in vivo. Experientia (Basel) **31:** 560–562, 1975.
120. LOOPUJUIT, L. D., AND VAN DER KOOY, D.: Simultaneous ultrastructural localization of cholecystokinin- and tyrosine hydroxylase-like immunoreactivity in nerve fibers of the rat nucleus accumbens. Neurosci. Lett. **56:** 329–334, 1985.
121. MAGGI, A., AND ENNA, S. J.: Regional alterations in rat brain neurotransmitter systems following chronic lithium treatment. J. Neurochem. **34:** 888–892, 1980.
122. MARSDEN, C. A.: Measurement of Neurotransmitter Release in Vivo, John Wiley, New York, 1984.
123. MARSDEN, C. A., BROCH, O. J., JR., AND GULDBERG, H. C.: Effect of nigral and raphe lesions on the catechol-o-methyl transferase and monoamine oxidase activities in the rat striatum. Eur. J. Pharmacol. **19:** 35–42, 1972.
124. MARSHALL, J. F.: Somatosensory inattention after dopamine-depleting intracerebral 6-OHDA injections: spontaneous recovery and pharmacological control. Brain Res. **177:** 311–324, 1979.
125. MARSHALL, J. F., AND ALTAR, C. A.: Striatal dopamine uptake and movement disorders of aging. Brain Res. **379:** 112–117, 1986.
126. MCKENZI, G. M., AND SZERB, J. C.: The effect of dihydroxyphenylalanine, pheniprazine, and *d*-amphetamine on the in vivo release of dopamine from the caudate nucleus. J. Pharmacol. Exp. Ther. **162:** 302–308, 1968.
127. MCQUADE, P. S., AND WOOD, P. L.: The effects of beta-phenethylamine on tyramine and dopamine metabolism. Prog. Neuro-Psychopharmacol. Biol. Psychiat. **7:** 755–759, 1983.
128. MCQUADE, P. S., AND WOOD, P. L.: The effects of administration of meta-tyramine and para-tyramine on dopamine and its metabolites in the rat striatum. Prog. Neuro-Psychopharmacol. Biol. Psychiat. **8:** 705–709, 1984.
129. MICHAEL, A. C., IKEDA, M., AND JUSTICE, J. B.: Dynamics of recovery of releasable dopamine following electrical stimulation of the medial forebrain bundle. Neurosci. Lett. **76:** 81–86, 1987.
130. MICHAEL, A. C., JUSTICE, J. B., AND NEILL, D. B.: In vivo voltammetric determination of the kinetics of dopamine metabolism in the rat. Neurosci. Lett. **56:** 365–369, 1985.
130a. MOORE, K. E., DEMAREST, K. T., AND LOOKINGLAND, K. J.: Stress, prolactin, and hypothalamic dopaminergic neurons. Neuropharmacology **26:** 801–807, 1987.
131. MORTIMER, J. A., AND WEBSTER, D. D.: Comparison of extrapyramidal motor function in normal aging and Parkinson's disease. *In* Advances in Neurogerontology: The Aging Nervous System, ed. by J. A. Mortimer, F. J., Pirozzolo, and G. S. Maletta, pp. 217–241, Karger, New York, 1982.
132. MOS, J., BORXTERMAN, H. J., AND VAN BENNEKOM, W. P. V.: In vivo voltammetric investigations into the action of HA-966 on central dopaminergic neurons. Brain Res. **207:** 465–470, 1981.
133. MYERS, S., AND PUGSLEY, T. A.: Decrease in rat striatal dopamine synthesis and metabolism in vivo by metabolically stable adenosine receptor agonists. Brain Res. **375:** 193–197, 1986.
134. NAIR, N. P. V., LAL, S., AND BLOOM, D. M.: Cholecystokinin and schizophrenia. Perspectives on etiology of psychiatric disorders: brain neurotransmission and neuropeptides. Prog. Brain Res. **65:** 237–258, 1986.
135. OZAWA, H., AND MIYAUCHI, T.: Potentiating effect of lithium chloride on methamphetamine-induced stereotypy in mice. Eur. J. Pharmacol. **41:** 213–216, 1977.
136. PALKOVITS, M., AND BROWNSTEIN, M. J.: Microdissection of brain areas by the punch technique. *In* Brain Microdissection Techniques, ed. by A. C. Cuello, pp. 1–36, John Wiley & Sons, New York, 1983.
137. PATRICK, R. L., AND BARCHAS, J. D.: Dopamine synthesis in rat striatal synaptosomes. II. Dibutyryl cyclic adenosine 3′,5′-monophosphoric acid and 6-methylenetetrahydropterine-induced synthesis increases without an increase in endogenous dopamine release. J. Pharmacol. Exp. Ther. **197:** 97–104, 1976.
138. PELTON, E. W., KIMELBERG, H. K., SHIPHERD, S. V., AND BOURKE, R. S.: Dopamine and norepinephrine uptake and metabolism by astroglial cells in culture. Life Sci. **28:** 1655–1663, 1981.
139. PERSSON, S. A.: Effect of morphine on the accumulation of DOPA after decarboxylase inhibition in the rat. Eur. J. Pharmacol. **55:** 121–128, 1979.
140. PETERS, A., PALAY, S. L., AND WEBSTER, H.: The Fine Structure of the Nervous System: The Neurons and Supporting Cells, W. B. Saunders Co., Philadelphia, 1976.
141. PHILLIPS, A. G., LANE, R. F., AND BLAHA, C. D.: Inhibition of dopamine release by cholecystokinin: relevance to schizophrenia. Trends Pharmacol. Sci. **7:** 126–128, 1986.
142. PILEBLAD, E., AND CARLSSON, A.: In vivo effects of the calcium antagonist nimodipine on dopamine metabolism in mouse brain. J. Neural Trans. **66:** 171–187, 1986.
143. PLANTJE, J. F., DIJCKS, F. A., VERHEIJDEN, F. H., AND STOOF, J. C.:Stimulation of D2 dopamine receptors in rat mesocortical areas inhibits the release of [^{3}H]dopamine. Eur. J. Pharmacol. **114:** 401–402, 1985.
144. PLANTJE, J. F., STEINBUSCH, H. W. M., SCHIPPER, J., DIJCKS, F. A., VERHEIJDEN, P., AND STOOF, J. C.: D-2 dopamine receptors regulate the release of [^{3}H]dopamine in rat cortical regions showing dopamine immunoreactive fibers. Neuroscience **20:** 157–168, 1987.
145. PONZIO, F., ACHILLI, G., PEREGO, C., AND ALGERI, S.: Differential effects of certain dopaminergic drugs on the striatal concentration of dopamine metabolites, with special reference to 3-methoxytyramine. Neurosci. Lett. **27:** 61–67, 1981.
146. PORTIG, P. J., AND VOGT, M.: Release into the ventricles of substances with possible transmitter function in the caudate nucleus. J. Physiol. (Lond.) **204:** 687–715, 1969.
147. RACAGNI, C., BRUNO, F., IULIANO, E., AND PAOLETTI, R.: Differential sensitivity to morphine-induced analgesia and motor activity in two inbred strains of mice: behavioral and biochemical correlations. J. Pharmacol. Exp. Ther. **209:** 111–116, 1979.
148. RACKHAM, A., WOOD, P. L., AND HUDGIN, R. L.: Kyotorphan (tyrosine-arginine): further evidence for indirect opiate receptor activation. Life Sci. **30:** 1337–1342, 1982.
149. RADHAKRISHUN, F. S., KORF, J., VENEMA, K., AND WESTERINK, B.: The

release of endogenous dopamine and its metabolites from rat striatum as detected in push-pull perfusates: effects of systemically administered drugs. Pharm. Weekbl. Sci. Ed. **5:** 153–158, 1983.

150. RANDRUP, A., AND MUNKVARD, I.: Biochemical, anatomical, and psychological investigations of stereotyped behavior induced by amphetamines. *In* Amphetamines and Related Compounds, ed. by E. Costa and S. Garattini, pp. 695–713, Raven Press, New York, 1970.
151. RANJE, C., AND UNGERSTEDT, U.: High correlations between number of dopamine cells, dopamine levels, and motor performance. Brain Res. **134:** 83–93, 1977.
152. RAO, T. S., KIM, H. S., LEHMANN, J., MARTIN, L. L., AND WOOD. P. L.: Selective activation of mesocortical dopaminergic pathways by phencyclidine (PCP) receptor agonists: tentative evidence for PCP recognition sites not coupled to N-methyl-D-aspartate (NMDA) receptors, submitted, 1988.
153. RIBAK, C. E., VAUGHN, J. E., SAITO, K., BARBER, R., AND ROBERTS, E.: Immunocytochemical localization of glutamate decarboxylase in rat substantia nigra. Brain Res. **116:** 287–298, 1988.
154. RINNE, U. K., SONNIEN, V., AND HYYPPA, M.: Effect of l-dopa on brain monoamines and their metabolites in Parkinson's disease. Life Sci. **10:** 549–557, 1971.
155. RIVETT, A. J., FRANCIS, A., AND ROTH, J. A.: Distinct cellular localization of membrane-bound and soluble forms of catechol-o-methyltranaferase in brain. J. Neurochem. **40:** 215–219, 1983.
156. ROBINSON, T. E., AND BECKER, J. B.: Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res. **11:** 157–198, 1986.
156a. ROBINSON, T. E., AND WHISHAW, I. Q.: Striatal dopamine release assessed with microdialysis following unilateral nigrostriatal damage. Soc. Neurosci. Abst. **13:** A67.5, 1987.
157. Roffler-Tarlov, S., Sharman, D. F., and Tergerdine, P.: 3,4-Dihydroxyphenylacetic acid in the mouse striatum: a reflection of intra- and extraneuronal metabolism of dopamine? Br. J. Pharmacol. **42:** 343–351, 1971.
158. ROTH, R. H., WALTERS, J. R., AND AGHAJANIAN, G. K.: Effect of impulse flow on the release and synthesis of DA in the rat striatum. *In* Frontiers in Catecholamine Research, ed. by S. H. Snyder and E. Usdin, pp. 567–574, Pergamon Press, New York, 1973.
159. RUGGERI, M., UNGERSTEDT, U., AGNATI, L. F., MUTT, V., HARFSTRAND, A., AND FUXE, K.: Effects of cholecysotokinin peptides and neurotensin on dopamine release and metabolism in the rostral and caudal part of the nucleus accumbens using intracerebral dialysis. Neurochem. Int. **10:** 509–520, 1987.
160. SALLER, C. F., AND SALAMA, A. I.: 3-Methoxytyramine accumulation: effects of typical neuroleptics and various atypical compounds. Naunyn-Schmiedeberg's Arch. Pharmacol. **334:** 125–132, 1986.
161. SCATTON, B., ZIVKOVIC, B., DEDEK, J., LLOYD, K. G., CONSTANTINIDIS, J., TISSOT, R., AND BARTHOLINI, G.: Gamma-aminobutyric acid (GABA) receptor stimulation. III. Effect of progabide (SL 76002) on NE, DA, and 5-HT turnover in rat brain areas. J. Pharmacol. Exp. Ther. **220:** 678–688, 1982.
162. SCHALLERT, T., UPCHURCH, M., LOBAUGH, N., FARRAR, R., SPIRDUSO, W., GULLIAM, P., VAUGHN, D., AND WILCOX, R.: Tactile extinction: distinguishing between sensorimotor and motor asymmetries in rats with unilateral nigrostriatal damage. Pharmacol. Biochem. Behav. **16:** 455–462, 1982.
163. SCHOEPP, D. D., AND AZZARO, A. J.: Effects of intrastriatal kainic acid injection on [^{3}H]dopamine metabolism in rat striatal slices: evidence for postsynaptic glial cell metabolism by both the type A and B forms of monoamine oxidase. J. Neurochem. **40:** 1340–1348, 1983.
164. SCHOEPP, D. D., AND AZZARO, A. J.: Further studies on the nature of postsynaptic dopamine uptake and metabolism in rat striatum: sodium dependency and investigation of a possible role for carrier-mediated uptake into serotonin neurons. J. Neurochem. **44:** 1747–1752, 1985.
165. SHEEL-KRUGER, J.: Studies on the accumulation of o-methylated dopamine and noradrenaline in the rat brain following various neuroleptics, thymoleptics, and aceperone. Arch. Int. Pharmacodyn. **195:** 372–378, 1972.
166. SKAPER, S. D., ADELSON, G. L., AND SEEGMILLER, J. E.: Metabolism of biogenic amines in neuroblastoma and glioma cells in culture. J. Neurochem. **27:** 1065–1072, 1976.
167. SMITH, T. L.: Increased synthesis of striatal dopamine by N,N-dimethyltryptamine. Life Sci. **21:** 1597–1602, 1977.
168. SPARKS, D. L., SLEVIN, J. T., AND HANSAKER, J. C.: 3-Methoxytyramine on the putamen as a gauge of the postmortem interval. J. Forensic Sci. **31:** 962–971, 1986.
169. STACHOWIAK, M. K., KELLER, R. W., STRICKER, E. M., AND ZIGMOND, M. J.: Increased dopamine efflux from striatal slices during development and after nigrostriatal bundle damage. Neuroscience **7:** 1648–1654, 1987.
170. STAMFORD, J. A., KRUK, Z. L., AND MILLAR, J.: Measurement of stimulated dopamine release by in vivo voltammetry: the influence of stimulus duration on drug responses. Neurosci. Lett. **69:** 70–73, 1986.
171. STAMFORD, J. A., KRUK, Z. L., AND MILLAR, J.: Accommodation of rat nigrostriatal dopamine neurones to high frequency electrical stimulation of the medial forebrain bundle: in vivo voltammetric data. Neurosci. Lett. **82:** 172–176, 1987.
172. STANLEY, M., AND WILK, S.: Striatal DOPAC elevation predicts antipsychotic efficacy of metoclopramide. Life Sci. **24:** 1907–1912, 1979.
173. STARKE, K., REIMANNM, W., ZUMSTEIN, A., AND HERTTING, G.: Effect of dopamine receptor agonists and antagonists on release of dopamine in the rabbit caudate nucleus in vitro. Naunyn-Schmiedeberg's Arch. Pharmacol. **305:** 27–36, 1978.
174. STARKE, K., SPAETH, L., LANG, J. D., AND ADELUNG, C.: Further functional in vitro comparison of presynaptic and postsynaptic dopamine receptors in the rabbit caudate nucleus. Naunyn-Schmiedeberg's Arch. Pharmacol. **323:** 298–306, 1983.
175. STENSTROM, A., HARDY, J., AND ORELAND, L.: Intra- and extra-dopaminesynaptosomal localization of monoamine oxidase in striatal homogenates from four species. Biochem. Pharmacol. **36:** 2931–2935, 1987.
176. STOOF, J. C., DE BOER, T., SMINIA, P., AND MULDER, A. H.: Stimulation of D2 dopamine receptors in rat neostriatum inhibits the release of acetyl choline and dopamine but does not affect the release of GABA, glutamate or serotonin. Eur. J. Pharmacol. **84:** 211–214, 1982.
176a. STOOF, J. C., DEN BREEJEN, E. J. S., AND MULDER, A. H.: GABA modulates the release of dopamine and acetylcholine from rat caudate nucleus slices. Eur. J. Pharmacol. **57:** 35–42, 1979.
177. STROMBOM, U.: Catecholamine receptor agonists effects on motor activity and route of tyrosine hydroxylation in mouse brain. Naunyn-Schiedeberg's Arch. Pharmacol. **292:** 167–173, 1976.
178. SWANSON, L. W.: The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res. Bull. **9:** 321–353, 1982.
179. TALMACIU, R. K., HOFFMAN, I. S., AND CUBEDDUX, L. X.: Dopamine autoreceptors modulate dopamine release from the prefrontal cortex. J. Neurochem. **47:** 805–817, 1986.
180. TAM, S.-Y., AND ROTH, R. H.: Selective increase in dopamine metabolism in the prefrontal cortex by the anxiogenic beta-carboline FG 7142. Biochem. Pharmacol. **34:** 1595–1598, 1985.
181. TAMMINGA, C. A., DEFRAITES, E. G., GOTTS, M. D., AND CHASE, T. N.: Apomorphine and N-n-propylnorapomorphine in the treatment of schizophrenia. *In* Apomorphine and Other Dopaminometics, ed. by G. U. Corsis and F. L. Gessa, pp. 49–56, Raven Press, New York, 1981.
182. TAMMINGA, C. A., GOTTS, M. D., GUNVANT, K. T., ALPHS, L. D., AND FOSTER, N. L.: Dopamine agonist treatment of schizophrenia with N-propylnorapomorphine. Arch. Gen. Psychiatry, **43:** 398– 402, 1986.
183. URETSKY, N. J., AND IVERSON, L. L.: Effects of 6-hydroxydopamine on catecholamine containing neurones in the rat brain. J. Neurochem. **17:** 269–278, 1970.
184. VAN VALKENBURG, C. F. M., NOACH, E. L., AND WIJLING, A.: Involvement of the nerve impulse flow in the release of extragranular dopamine. Eur. J. Pharmacol. **57:** 191–199, 1979.
185. VAN VALKENBURG, C., VAN DER KROGT, J., MOLEMAN, P., VAN BERKUM, H., TJADEN, U., AND DE JONG, J.: A procedure to measure the specific activities of dopamine and its metabolites in rat striatum, based on HPLC, electrochemical detection, and liquid scintillation counting. J. Neurosci. Methods **11:** 29–38, 1984.
186. VOIGT, M. M., AND WANG, R. Y.: In vivo release of dopamine in the nucleus accumbens of the rat: modulation by cholecystokinin. Brain Res. **296:** 189–193, 1984.
187. VOIGT, M. M., WANG, R. Y., AND WESTFALL, T. C.: The effects of cholecystokinin on the in vivo release of newly synthesized [^{3}H]dopamine from the nucleus accumbens of the rat. J. Neurosci. **5:** 2744–2749, 1985.
188. VON VOIGTLANDER, P. F., AND MOORE, K. E.: The release of ^{3}H-dopamine from cat brain following electrical stimulation of the substantia nigra and caudate nucleus. Neuropharmacology **10:** 733–741, 1971.
189. VULTO, A. G., SHARP, T., AND UNGERSTEDT, U.: Rapid postmortal increase in extracellular concentration of dopamine in the rat brain as assessed by intra-cranial dialysis. Soc. Neurosci. Abst. **11:** A353.7, 1985.
190. VULTO, A. G., WESTENBERG, H. G. M., MEIJER, L. B. A., AND VERSTEEG, D. H. G.: The dopamine metabolite 3-methoxytyramine is not a suitable indicator of dopamine release in the rat brain. J. Neurochem. **47:** 1387–1393, 1986.
191. WALDMEIER, P. C., LAUBER, J., BLUM, W., AND RICHTER, W. J.: 3-Methoxytyramine: its suitability as an indicator of synaptic dopamine release. Naunyn-Schiedeberg's Arch. Pharmacol. **315:** 219–225, 1981.
192. WALDMEIER, P. C., AND MAITRE, L.: On the relevance of preferential increases of mesolimbic versus striatal dopamine turnover for the prediction of antipsychotic activity of psychotropic drugs. J. Neurochem. **27:** 589–597, 1976.
193. WALDMEIER, P. C., AND MAITRE, L.: Neurochemical investigations of the interaction of N,N-dimethyltryptamine with the dopaminergic system in rat brain. Psychopharmacology **52:** 137–144, 1977.
194. WALTERS, J. R., ROTH, R. H., AND AGHAJANIAN, G. K.: Dopaminergic neurons: similar biochemical and histochemical effects of gamma-hydroxybutyrate and acute lesions of the nigrostriatal pathway. J. Pharmacol. Exp. Ther. **186:** 630–639, 1973.
195. WANG, R. Y., WHITE, F. J., AND VOIGT, M. M.: Cholecystokinin, dopamine, and schizophrenia. Trends Pharmacol. Sci. **5:** 436–438, 1984.
196. WELLER, M. E., ROSE, S., JENNER, P., AND MASDEN, C. D.: In vitro characterization of dopamine receptors in the superior colliculus of the rat. Neuropharmacology **26:** 347–354, 1987.

197. WESTERINK, B. H. C.: Effects of drugs on the formation of 3-methoxytyramine, a dopamine metabolite, in the substantia nigra, striatum, nucleus accumbens, and tuberculum olfactorium of the rat. J. Pharm. Pharmacol. **31:** 94–99, 1979.
198. WESTERINK, B. H. C.: Further studies on the sequence of dopamine metabolism in the rat brain. Eur. J. Pharmacol. **56:** 313–322, 1979.
199. WESTERINK, B. H. C.: Sequence and significance of dopamine metabolism in the rat brain. Neurochem. Int. **7:** 221–227, 1985.
200. WESTERINK, B. H. C., DAMSMA, G., ROLLEMA, H., DE VRIES, J. B., AND HORN, A. S.: Scope and limitations of in vivo brain dialysis: a comparison of its application to various neurotransmitter systems. Life Sci. **41:** 1763–1776, 1987.
201. WESTERINK, B. H. C., AND SPAAN, S. J.: Estimation of the turnover of 3-methoxytyramine in the rat striatum by HPLC with electrochemical detection: implications for the sequence in the cerebral metabolism of dopamine. J. Neurochem. **38:** 342–347, 1982.
202. WESTERINK, B. H. C., AND SPAAN, S. J.: On the significance of endogenous 3-methoxytyramine for the effects of centrally acting drugs on dopamine release in the rat brain. J. Neurochem. **38:** 680–686, 1982.
203. WESTERINK, B. H. C., AND SPAAN, S. J.: Simultaneous determination of the formation rate of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in various rat brain areas. Brain Res. **252:** 239–245, 1982.
204. WOLF, M. E., AND ROTH, R. H.: Dopamine neurons projecting to the medial prefrontal cortex possess release-modulating autoreceptors. Neuropharmacology **26:** 1053–1059, 1987.
205. WOOD, P. L.: Actions of GABAergic agents on dopamine metabolism in the nigrostriatal pathway of the rat. J. Pharmacol. Exp. Ther. **222:** 674–679, 1982.
206. WOOD, P. L.: A selected ion monitoring assay for dopamine and its metabolites using negative chemical ionization. Biomed. Mass Spec. **9:** 302–306, 1982.
207. WOOD, P. L.: Opioid regulation of CNS dopaminergic pathways: a review of methodology, receptor types, regional variations, and species differences. Peptides **4:** 595–601, 1983.
208. WOOD, P. L.: The significance of multiple CNS opioid receptor types: a review of critical considerations relating to technical details and anatomy in the study of central opioid actions. Peptides **9:** 49–55, 1988.
209. WOOD, P. L., ALTAR, C. A., AND KIM, H. S.: Presynaptic inhibition of nigrostriatal dopamine release in the mouse: lack of cross-tolerance between apomorphine, GBL, and CGS 10746B. Life Sci. **42:** 1503–1506, 1988.
210. WOOD, P. L., AND CHENEY, D. L.: Gas chromatography-mass fragmentography of amino acids. Neuromethods **3:** 51–80, 1985.
211. WOOD, P. L., ETIENNE, P., LAL, S., AND NAIR, N. P. V.: GABAergic regulation of nigrostriatal neurons: coupling of benzodiazepine and GABA receptors. Prog. Neuro-Psychopharmacol. Biol. Psychiat. **6:** 471–474, 1982.
212. WOOD, P. L., ETIENNE, P., LAL, S., AND NAIR, N. P. V.: Benzodiazepines and GABAergic regulation of nigrostriatal neurons: lack of tolerance. Prog. Neuro-Psychopharmacol. Biol. Psychiat. **8:** 779–783, 1984.
213. WOOD, P. L., KIM, H. S., AND ALTAR, C. A.: In vivo assessment of dopamine and norepinephrine release in rat neocortex: gas chromatography-mass spectrometry measurement of 3-methoxytyramine and normetanephrine. J. Neurochem. **48:** 574–579, 1987.
214. WOOD, P. L., KIM, H. S., BOYAR, W. C., AND HUTCHISON, A.: Inhibition of rat nigrostriatal dopamine release by adenosine receptor agonists: A1 receptor mediation. Neuropharmacology (in press), 1988.
215. WOOD, P. L., KIM, H. S., AND MARIEN, M. R.: Intracerebral dialysis: direct evidence for the utility of 3-MT measurements as an index of dopamine release. Life Sci. **41:** 1–5, 1987.
216. WOOD, P. L., KIM, H. S., STOCKLIN, K., AND RAO, T. S.: Dynamics of the striatal 3-MT pool in rat and mouse striatum: species differences as assessed by steady-state measurements and intracerebral dialysis. Life Sci. **42:** 2275–2281, 1988.
217. WOOD, P. L., AND MCQUADE, P. S.: Ciclindole and flucindole: novel tetrahydrocarbazolamine neuroleptics. Prog. Neuro-Psychopharmacol. Biol. Psychiat. **8:** 773–777, 1984.
218. WOOD, P. L., MCQUADE, P. S., ETIENNE, P., LAL, S., AND NAIR, N. P. V.: Differential actions of classical and atypical neuroleptics on mouse nigrostriatal neurons. Prog. Neuro-Psychopharmacol. Biol. Psychiat. **7:** 765–768, 1983.
219. WOOD, P. L., NAIR, N. P. V., AND BOZARTH, M.: Striatal 3-methoxytyramine as an index of dopamine release: effects of electrical stimulation. Neurosci. Lett. **32:** 291–294, 1982.
220. WOOD, P. L., NAIR, N. P. V., LAL, S., AND ETIENNE, P.: Buspirone, a potential atypical neuroleptic. Life Sci. **33:** 269– 273, 1983.
221. WOOD, P. L., AND PELOQUIN, A.: Increases in choline levels in rat brain elicited by meclofenoxate. Neuropharmacology **21:** 215–219, 1982.
222. WOOD, P. L., AND RAO, T. S.: Morphine stimulation of mesolimbic and mesocortical but not nigrostriatal dopamine release in the rat. Life Sci. (in press), 1988.
223. WOOD, P. L., AND RAO, T. S.: Differential actions on dopamine synthesis and release in the rat striatum after cessation of impulse flow with GBL or TTX (submitted), 1988.
224. WOOD, P. L., AND RICHARD, J. W.: Morphine and nigrostriatal function in the rat and mouse: the role of nigral and striatal opiate receptors. Neuropharmacology **21:** 1305–1310, 1982.
225. WOOD, P. L., SOTLAND, M., RICHARD, J. W., AND RACKHAM, A.: Actions of mu, kappa, sigma, delta, and agonist/antagonist opiates on striatal dopaminergic function. J. Pharmacol. Exp. Ther. **215:** 697–703, 1980.
226. YONEHARA, N., AND CLOUET, D. H.: Effects of delta and mu opiopeptides on the turnover and release of dopamine in rat striatum. J. Pharmacol. Exp. Ther. **231:** 38–42, 1984.
227. ZETTERSTROM, T., SHARP, T., AND UNGERSTEDT, U.: Effect of neuroleptic drugs on striatal dopamine release and metabolism in the awake rat studied by intracerebral dialysis. Eur. J. Pharmacol. **106:** 27–37, 1985.
228. ZETTERSTROM, T., SHARP, T., AND UNGERSTEDT, U.: Effect of dopamine D1 and D2 receptor selective drugs on dopamine release and metabolism in rat striatum in vivo. Naunyn-Schmiedeberg's Arch. Pharmacol. **334:** 117–124, 1986.
229. ZETTERSTROM, T., AND UNGERSTEDT, U.: Effects of apomorphine on the in vivo release of dopamine and its metabolites, studied by brain dialysis. Eur. J. Pharmacol. **97:** 29–36, 1984.
230. ZIGMOND, M. J., AND STRICKER, E. M.: Adaptive properties of monoaminergic neurons. *In* Handbook of Neurochemistry, ed. by A. Lajtha, vol. 9, pp. 87–107, Plenum Press, New York, 1985.
231. ZUMSTEIN, A., KARDUCK, W., AND STARKE, K.: Pathways of dopamine metabolism in the rabbit caudate nucleus in vitro. Naunyn-Schmiedeberg's Arch. Pharmacol. **316:** 205–216, 1981.