In Vivo Imaging of Neuromodulatory Synaptic Transmission Using PET: A Review of Relevant Neurophysiology

Ronald E. Fisher, Evan D. Morris, Nathaniel M. Alpert, and Alan J. Fischman

Division of Nuclear Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

Abstract: Recent data from positron emission tomography (PET) imaging studies suggest the possibility of studying synaptic transmission in vivo in humans. The approach will require a synthesis of two established techniques: brain activation studies (conventionally performed by measuring regional cerebral blood flow or metabolism) and neurotransmitter receptor imaging (using radiolabelled ligands that bind to specific neuroreceptors). By comparing neuroreceptor binding in subjects at rest and while performing an activation task, it may be possible to determine whether a particular neurotransmitter is involved in performance of the task. The underlying principle is that endogenous neurotransmitter competes with the injected radioligand for the same receptors, thereby inhibiting ligand binding. This effect will be even more pronounced during activation, as the synaptic concentration of transmitter rises. Thus, activation of a specific neurotransmitter will be detected as a *decrease* in specific binding of the radioligand. In this paper we review neurophysiological and biochemical literature to estimate the endogenous neurotransmitter concentration changes that will be expected to occur during an activation task, using the dopamine system as an example. We calculate that the average synaptic dopamine concentration is ≈ 100 nM and that it approximately doubles during activation. This, along with consideration of the concentration of radioligand and affinities of the ligand and dopamine for dopamine receptors, suggests that physiological activation of a specific neurotransmitter system is likely to be detectable with PET.

Key words: receptor, positron emission tomography, dopamine, endogenous, brain, activation

INTRODUCTION

Information is transmitted through the brain electrically along neurons and electrochemically between neurons (across synapses). These processes can occur extremely fast: passive electrical decay, axonal conduction, and fast excitatory synaptic transmission all occur on a time scale of a few milliseconds. Neural transmission at these speeds is essential in order for animals to perceive, interpret, and respond to stimuli in their environment.

This constant process of high-speed information processing is under the influence of much slower processes that reflect an organism's environment and mental state. These slower neural processes are provided by groups of neurons that originate in the subcortical nuclei or brainstem and project diffusely throughout the brain. The main neurotransmitters of these pathways are norepinephrine, acetylcholine,

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Address reprint requests to Dr. Alan Fischman, Division of Nuclear Medicine, MZ-TIL-201, Massachusetts General Hospital, 32 Fruit Street, Boston, MA 02114.

dopamine, and serotonin. (There are dozens of other, less well-studied transmitters that may eventually be shown to be equally important.) These so-called "neuromodulatory pathways" in the brain appear to play an important role in a variety of mental functions, including attention and arousal, and learning and memory. Abnormalities of these pathways have been implicated in many psychiatric and neurological disorders.

Although there is ample evidence that such widely projecting neuromodulatory pathways play important roles in cerebral function, there is only indirect (and clearly insufficient) evidence suggesting which transmitters are involved in which functions. In vivo observation of neurochemical processes during behavioral tasks (with, for example, intracellular electrical recording, microdialysis, or voltammetry) is technically difficult and limited to animal studies. Thus, the precise roles of modulatory neurons in human thought and behavior have remained undefined.

PET scanning has offered a means of studying brain activity noninvasively. However, neuromodulatory activity has been difficult to measure. This is because regional cerebral blood flow and metabolic imaging are nonspecific with regard to transmitter type; these studies measure signals from large numbers of spatially clustered neurons. Unfortunately, neuromodulatory synapses are distributed widely and sparsely in the brain—generally accounting for only a small percentage of synapses in any region [Cooper et al., 1991; Squire, 1987]. An alternative approach, positron emission tomography (PET) imaging of specific neurotransmitter receptors, has been successful in demonstrating the number and distribution of modulatory receptors, but not their function.

More recently, PET imaging of dopamine receptors has been shown to be sensitive to fluctuations in the level of endogenously released dopamine [Dewey et al., 1990; Ross and Jackson, 1989; Seeman et al., 1989]. In fact, drugs believed to increase or decrease dopamine levels have been shown to cause detectable effects on PET receptor images [Dewey et al., 1990, 1992, 1993b]. The mechanism for the effect is suspected to be inhibition of PET radioligand binding by competition from endogenous dopamine. Relying on a similar physiological mechanism, we will describe an approach by which PET neuroreceptor imaging may be used to assess the role of neuromodulatory neurons in a variety of human brain activities. The primary goal of this paper is to determine what synaptic parameters will influence the outcome of this type of PET study, and to estimate the values of these parameters by examining a variety of published experimental data. The basic design of a neuromodulatory activation study will also be addressed. The results of computer simulations of such an experiment are given in the accompanying paper [Morris et al., 1995]; the computer model takes into account endogenous neurotransmitter levels, using the synaptic parameter values estimated in this paper.

COMPETITION BETWEEN ENDOGENOUS TRANSMITTER AND THE PET RADIOLIGAND

The proposed paradigm relies on the competition between endogenous neurotransmitter and radioligand for the same postsynaptic receptors. Briefly, for any modulatory neurotransmitter system for which a PET neuroreceptor ligand is available, the following conditions are expected to hold:

1. Endogenous neurotransmitter is released into synapses when action potentials arrive at the presynaptic terminal.
2. Endogenous neurotransmitter will compete with the PET neuroreceptor radioligand for the same receptors.
3. The amount of endogenous neurotransmitter released during an appropriate activation task will be greater than that at rest.
4. This increased competition for receptors will be sufficient to cause a measurable decrease in radioligand binding in the activated regions.
5. By varying the activation tasks and PET receptor ligands, one can begin to determine the roles of different neurotransmitter systems in many psychological and physical activities.

This type of PET experiment will therefore involve imaging subjects both at rest and while performing an activation task. Neuromodulatory activation will be observed as a difference in radiotracer binding between the two studies. More specifically, increased neuromodulatory neural activity will appear as a *decrease* in binding during the activation study.

CAN SYNAPTIC TRANSMISSION BE IMAGED IN VIVO? ESTIMATION OF RELEVANT SYNAPTIC PARAMETERS

The success of such an approach depends on a number of parameters (Table I). We will evaluate the approach using the DA system as an example, since much is already known about this system, particularly with regard to PET imaging. A second advantage of DA imaging is that dopaminergic synapses are rela-

TABLE I. Summary of estimates of important synaptic parameters

Neurotransmitter[a]	Method	Best estimate	Probable range	Reference
I. Peak neurotransmitter concentration in synapse				
Ach (v, p)	Single channel recording	1 mM	1–3 mM	Franke, 1991; Dudel et al., 1992
Glu (i, p)	Single channel recording	1 mM	1–5 mM	Dudel et al., 1992
Glu (v, c)	Reduction in synaptic currents by Glu antagonists	1.1 mM	0.5–2 mM	Clements et al., 1992
DA (v, c)	See text	*10 μM*	10–100 μM	
II. Rate of clearance of neurotransmitter from synapse				
Ach (i, p)	Noise analysis of miniature end plate potentials	$\tau \leq 1$ ms	0.1–1 ms	Magleby and Stevens, 1972
Glu (i, p)	Single channel recording	$\tau \leq 2$ ms	1–5 ms	Dudel et al., 1992
Glu (v, c)	Displacement of Glu ligands by endogenous Glu	$\tau \approx 1$ ms	0.5–2 ms	Clements et al., 1992
5-HT (i, p)	Time course of synaptic currents and 5-HT reuptake (electrical/optical measurements)	$\tau \approx 50$ ms	30–80 ms	Bruns et al., 1993
DA (v, c)	See text	$\theta = 2$ *ms*	1–20 ms	
III. Firing rate of DA neurons (substantia nigra, pars compacta)				
Rest (awake)	Rats, cats, monkeys	*4–5 Hz*	2–6 Hz	Schultz et al., 1983; Freeman et al., 1985; Diana et al., 1989
Activation	Walk in circles (rats); rewarded motor task (monkeys)	*8–10 Hz*	4–14 Hz	Diana et al., 1989; Schultz et al., 1983
IV. Average concentration of DA in synapse				
Rest				
	Change in D2 antagonist binding induced by pharmacologic depletion of endogenous DA	40 nM	40–100 nM	Ross and Jackson, 1989
	In vivo voltammetry	30 nM[b]	20–50 nM	Gonon and Buda, 1985; Gonon, 1988
	In vivo microdialysis	40 nM[b]	20–50 nM	Church et al., 1987; Zetterstrom et al., 1983
	Calculated from above (see text)	*100 nM*	10–100 nM	
Activation				
	In vivo voltammetry	100 nM[b]	80–200 nM	Gonon and Buda, 1985; Gonon, 1988
	Calculated from above	*200 nM*	20–300 nM	
V. Other relevant parameters				
K_D (*dopamine* for D2 receptors)		100 nM	10–1,000 nM	Gingrich and Caron, 1993
K_D (*raclopride* for D2 receptors)		7 nM		Farde et al., 1988
Raclopride CSF concentration		0.2–1 nM (varies during imaging)		Farde et al., 1988; see also Morris, in press

[a] Ach, acetylcholine; Glu, glutamate; 5-HT, serotonin; v, vertebrate; i, invertebrate; p, peripheral nervous system; c, central nervous system.
[b] These are concentrations of *extra*synaptic DA (near, but not in, the synapse; synaptic DA levels would be expected be somewhat higher).

tively clustered in the striatum, which will predictably and reliably demonstrate high radioligand binding. Furthermore, motor tasks performed on one side of the body have been shown to activate only the *contralateral* striatum [Playford et al., 1992], thus providing the possibility of an internal control (the ipsilateral striatum).

It is reasonable to assume that some endogenously released dopamine (DA) will bind to DA receptors and thereby inhibit binding of the radioligand, but the question remains: Is this competition sufficient to cause a *measurable* decrease in binding? Convincing evidence has already been obtained that such competition causes detectable changes in conventional PET DA neuroreceptor imaging [Ross and Jackson, 1989; Seeman et al., 1989; Dewey et al., 1993b]. For the purposes of this paper, the critical question is: Will the *difference* in competition between the resting and activated states cause a measurable change in binding? The answer depends on the values of a number of parameters, including: the concentrations of DA and radioligand in the synaptic cleft, the change in DA concentration with activation, and the affinities (K_D) of both substances for the DA receptor. The next few sections will be devoted to estimation of these parameters.

The first parameter to consider is the concentration of radiotracer in the synaptic cleft during PET imaging. It will be assumed that it is roughly equal to the concentration in the cerebrospinal fluid (CSF). For raclopride, a D2 antagonist often used in PET DA receptor imaging, the CSF concentration reaches a peak of ≈1 nM shortly after injection; it then declines quickly and remains at about *0.2 nM* during most of the PET imaging time [Farde et al., 1988]. The radioligand concentration, therefore, is always lower than its K_D for D2 receptors, which is *5–10 nM* [Farde et al., 1989].

The value of K_D of DA for the DA receptor depends on the receptor type [Gingrich and Caron, 1993]:

D1 receptors: ≈1,000 nM.
D2 receptors: ≈100–1,000 nM (low affinity state)
D2 receptors: ≈10 nM (high affinity state).

These values are derived from measurements in a variety of in vitro preparations and a variety of animal species, including humans. They reflect the fact that the D2 receptor can exist in at least two states having different affinities for dopamine [Rubinstein et al., 1990; Seeman et al., 1994]. For our calculations, since we will be using a D2 antagonist as PET radiotracer (raclopride), we will assume a K_D (*DA binding to D2 receptors*) of *100 nM*. This represents a rough average of the K_D values for the high and low affinity states. This same value (100 nM) was obtained in vitro by Ross and Jackson [1989], who measured the K_I of DA in inhibiting binding of a D2 antagonist (NPA) to D2 receptors in mouse striata.

This leads us to the next synaptic parameter that must be estimated: the concentration of dopamine in the cleft. Unfortunately, this has not been measured directly, and an estimation requires synthesis of data from a wide variety of neurophysiological and biochemical experiments.

CONCENTRATION OF DOPAMINE IN THE SYNAPTIC CLEFT

The concentration of dopamine in the cleft is not constant; in fact, it is continuously fluctuating. Following the arrival of an action potential at the presynaptic terminal, DA is released into the synaptic cleft. This causes the DA concentration in the cleft to rise nearly instantaneously. (Diffusion time to reach the postsynaptic receptors is ≈*2 microseconds;* to diffuse evenly throughout the cleft is *10–20 microseconds;* see Eccles and Jeager, 1958.) Subsequently, the DA concentration declines rapidly as the result of two processes: diffusion out of the cleft (into the extracellular space) and active reuptake by the presynaptic neuron. The arrival of the next action potential again causes a dramatic increase in the DA concentration in the cleft, which quickly disappears, and so on. Thus, it is necessary to estimate several parameters: the peak concentration of DA in the cleft following a single action potential, the rate of decay of DA between action potentials, and the firing rate of DA neurons in vivo (both at rest and during activation of dopaminergic neurons).

The *peak concentration of DA* in the cleft is not known, but accurate estimates are available for other neurotransmitter systems, and indirect estimates can be made for DA.

Fast excitatory synapses

At the vertebrate neuromuscular junction, comparison of the rise-times of the synaptic current with kinetics of acetylcholine (Ach) receptor/channels (from single channel recordings) in the presence of known concentrations of Ach yields an estimate of *1 mM* in the synaptic cleft [Franke et al., 1991; Dudel et al., 1992]. Using a similar approach in invertebrate peripheral neurons, Dudel et al. [1992] estimated the peak glutamate (Glu) concentration in the synaptic cleft to be ≈*1 mM.* In cultured hippocampal neurons, Clements et al. [1992] measured the reduction in synaptic currents by rapidly dissociating Glu antagonists. By varying the concentration of antagonist, they arrived at a fairly precise estimate of peak Glu concentration in the synaptic cleft: *1.1 mM.*

The above measurements provide good estimates of peak neurotransmitter concentration for the two main *fast* excitatory transmitters—Ach (peripheral neurons) and Glu (peripheral neurons of invertebrates and central neurons of vertebrates): about *1 mM,* or perhaps a few mM. Neuromodulatory transmitters, which are designed for less rapid information transfer, might be different. Slower information transfer, for example, might be achieved using lower, but more prolonged, synaptic transmitter concentrations. Unfortunately, estimates for the peak concentration of neuromodulatory transmitters are less precise, but one is available for DA.

Neuromodulatory synapses—Dopamine

Extracellular DA was measured directly in olfactory tubercle and striatal neurons in vivo in anesthetized rats using electrochemically treated carbon fiber electrodes combined with voltammetry [Gonon, 1988; Gonzalez-Mora et al., 1988]. These measurements, however, reflect DA concentrations in the interneuronal, extracellular space, that is, *outside* the synapse. Extracellular DA has also been measured in vivo in rat striatum using microdialysis techniques [Church et al., 1987; Zetterstrom et al., 1983]. Both types of studies have found a basal (non-stimulated) concentration of 20–50 nM (although other studies have found lower levels, about 5 nM; see Kawagoe et al. [1992]). Elevations ranging from 60 to over 500 nM were seen following electrical stimulation (10–15 Hz) of the dopaminergic neurons [Gonon, 1988; Gonon and Buda, 1985]. This, however, is the average concentration in the extracellular space, *not* the peak concentration in the synaptic cleft. Gonon [1988] suggests that the synaptic concentration of DA probably reaches 1–100 μM. This estimate, though certainly rough, seems reasonable: First of all, such extrasynaptic measurements represent an average of brief moments of transmitter release combined with much longer periods of quiescence. Secondly, DA concentration decreases with distance from the synapse because of both diffusion and reuptake. Our estimate of peak synaptic concentration of DA from these data is *10–100 μM.* This fits reasonably well with estimates of the other neurotransmitters discussed above. Such a concentration, if sustained, would easily be sufficient to prevent most of the radiotracer from binding to DA receptors. The question is, how long does it stay at that concentration?

TIME COURSE OF DISAPPEARANCE OF DA FROM THE SYNAPTIC CLEFT

Relatively accurate measurements of transmitter clearance are available only for Ach and Glu at "fast" excitatory synapses. An indirect measure of clearance is available for the neuromodulatory transmitter serotonin.

Fast synapses

At the vertebrate neuromuscular junction, Magleby and Stevens [1972] showed that very brief (<1 ms) exposures of Ach will induce Ach-gated channel openings of sufficient duration to mimic a synaptic current. This finding suggests that, during normal synaptic transmission, Ach stays in the cleft just a very short time: τ (time constant of disappearance of transmitter) ≤ 1 *ms.* At the invertebrate neuromuscular junction, Glu-activated channel openings are too brief to mimic a synaptic current unless Glu stays in the mM range (or just under) for 1–2 ms [Dudel et al., 1992]. Slightly longer durations of Glu exposure can also mimic synaptic currents, since desensitization partly compensates for increased channel activation. A reasonable estimate of decay, then, is $\tau = 1\text{–}5$ *ms.* Clements et al. [1992], in cultured hippocampal neurons, made perhaps the most direct measurement of clearance. They measured the displacement of rapidly dissociating Glu antagonists by endogenous Glu during synaptic transmission. Their kinetic analysis of the data yielded a convincing estimate of $\tau \approx 1$ *ms.*

Neuromodulatory synapse

In invertebrate serotonergic neurons, Bruns et al. [1993] measured both postsynaptic currents and presynaptic serotonin reuptake currents (serotonin reuptake is electrogenic: Na^+ ions flow inward during reuptake). They also measured serotonin reuptake directly by following reuptake of a fluorescent serotonin analog. They found that all three processes occur over a similar time course and concluded that serotonin disappears from the synapse with a time constant $\tau \approx 50$ *ms.*

Thus, τ at fast synapses is likely to be *1 ms.* One might intuitively expect this value to be larger at neuromodulatory synapses, since they are involved in mental processes occurring on a slower time scale. This is, as we have seen, the case for the neuromodulatory transmitter serotonin at an invertebrate synapse.

On the other hand, dopaminergic neurons fire up to 20 Hz on occasion [Schultz et al., 1983] and, in fact, fire several times per second even at rest (see below). This requires prompt removal of transmitter (to prevent build-up) and suggests that DA clearance may be more comparable to that at fast synapses than at the leech serotonergic synapse. It is not immediately clear, then, which value of τ is most likely correct for DA. The range of possible values for τ is approximately 1–50 ms. Fortunately, careful consideration of the firing rates narrows this range considerably. Therefore, this will be discussed before making our final estimate of the time course of synaptic DA concentration.

FIRING RATES OF DOPAMINERGIC NEURONS IN THE RESTING AND ACTIVATED STATES

The average DA concentration in the synaptic cleft is affected by the firing rate of the dopaminergic neuron. In vivo dopaminergic firing rates in humans, however, have never been measured. In rats, guinea pigs, and monkeys they have been measured directly using in vivo microelectrode recording. Recordings in awake animals from DA neurons of the substantia nigra (pars compacta) revealed considerable variability in resting firing rates, but were generally about 4–5 Hz [Schultz et al., 1983; Freeman et al., 1985; Diana et al., 1989]. During physiological activation, by either forced walking in a circle (rats) or a visual cue—motor response—food reward task (monkeys), firing increased to roughly *8–10 Hz.* Interestingly, a small minority of DA neurons in the rat *decreased* to ~2 Hz upon certain types of sensory stimulation [Chiodo et al., 1980].

Since the precise function of DA neurons is unknown, it is difficult to design an experiment in which the animal maximally uses its DA pathways. Furthermore, animals do not follow commands easily. Thus, it is possible that activation tasks may be found in humans that cause even greater DA activation. However, given the lack of data, we will assume that in humans, DA neuronal firing is *5 Hz at rest* and increases to at least *10 Hz with activation.* Possible strategies for activating DA neurons in humans will be discussed later.

BEST ESTIMATE OF PEAK AND DECAY OF DOPAMINE CONCENTRATION

The peak concentration of DA in the synaptic cleft, as discussed, is probably ~10–100 μM. The time course of disappearance from the cleft is more difficult to estimate. We will next discuss how consideration of the resting firing rate helps narrow the possible range of rates of clearance.

Let us first assume a peak dopamine concentration of *10 μM.* The possible range of time constants of transmitter disappearance (given above) is 1–50 ms—a very broad range. If the time constant of disappearance is >20 ms, however, the *average* DA concentration will be > 1 μM at rest.[1] This concentration is enough to bind >90% of the postsynaptic D2 receptors at rest,[2] hardly an efficient information transfer system. Therefore, a time constant on the order of a few milliseconds seems more reasonable. $\tau = 2$ ms yields an average DA concentration of ~100 nM, resulting in ~50% of receptors bound at rest. This affords the dopaminergic synapses the advantageous possibility of modulation in either direction (increasing or decreasing the number of bound receptors), according to behavioral or psychological conditions. That is, information can be conveyed equally well by either an increase or a decrease in firing rate. (This is supported by the finding, as mentioned previously, that some DA neurons respond to certain sensory stimulation by *decreasing* their firing frequency [Chiodo et al., 1980].)

One must also consider the possibility of a peak DA concentration of 100 μM. Even if the time constant of decay is short—2 ms—the average concentration of DA at rest will be ~1 μM. This will result in ~90%

[1]Average DA concentration in the cleft was calculated assuming that the concentration instantly increases following each action potential and decays exponentially until the arrival of the next action potential. That is,

$$[DA] = [DA]_p \cdot e^{-t/\tau}$$

where $[DA]_p$ = peak concentration of DA (i.e., immediately following arrival of an action potential), [DA] = concentration of DA at time t, where t = time since the action potential, and τ = the time constant of clearance of transmitter from the cleft.

When the next action potential arrives at the presynaptic terminal, the identical cycle is repeated. At rest, each cycle takes about 200 ms (the firing rate is ~5/sec). Thus, the average concentration of DA from the moment of transmitter release until the arrival of the next action potential is:

$$[DA]_{avg} = 1/200 \int_0^{200} [DA]_p \cdot e^{-t/\tau}\, dt$$

$$= (t/200)\,[DA]_p\,(1 - e^{-200/\tau}).$$

[2]% receptors bound (at equilibrium) is calculated as:

$$\%\ \text{bound} = \frac{[DA]}{K_D + [DA]} \times 100$$

[see Cooper, et al., 1991].

receptor binding at rest. If the time constant is 1 ms, as it is for Glu, the average DA concentration will be ~0.5 μM: ~83% of the receptors bound at rest. To achieve 50% receptor binding at rest, the time constant would have to be ~0.2 ms. Although this is theoretically possible (unrestricted diffusion allows disappearance to occur with a time constant of ~0.15 ms [Eccles and Jeager, 1958]), it seems unlikely. A faster transmitter clearance for a neuromodulatory synapse than a fast synapse does not seem commensurate with its function. Thus, we favor *10 μM peak concentration and a time constant of disappearance of 2 ms. This yields, at rest, an average synaptic DA concentration of 100 nM.*[3] During activation, this concentration will roughly double.

The *average* concentration of DA in the synaptic cleft was also estimated by Ross and Jackson [1989]. They measured the apparent K_D of the in vivo binding of [^{3}H]NPA (a D2 antagonist) in the mouse striatum under pharmacologic conditions that decreased endogenous DA release. This resulted in increased binding of the labelled D2 antagonist (due to less inhibition from endogenous DA). By comparing the apparent K_D in pretreated to untreated rats, they estimated the average DA concentration in the synapse at rest to be ~40 nM. This is in fairly close agreement with our estimate. Furthermore, their measurement assumes that the pharmacologic manipulations caused a total depletion of synaptic DA; if these drugs caused only a partial decrease in DA release, which is likely, the true value of resting DA must be higher.

Kawagoe et al. [1992] measured extrasynaptic DA levels in rat striatum and constructed a model based on a reuptake system (described by Michaelis-Menten kinetics) and passive diffusion. Their model predicted the average synaptic DA concentration (concentration at the synaptic-extrasynaptic interface) to be 97 nM, in close agreement with our estimate. Their model, however, assumed much lower peak concentrations (200–300 nM) and much slower disappearance from the cleft ($\tau \approx 30$ ms).

EXTRASYNAPTIC D2 RECEPTORS

It is likely that some D2 receptors exist outside the synaptic cleft, although the fraction of such receptors is unknown. A significant percentage of *D1* receptors appear to be extrasynaptic, possibly the majority of them [Smiley et al., 1994]. It is presumed that DA reaches these receptors by diffusion through the extrasynaptic space. Extrasynaptic receptors have also been demonstrated for the glycinergic [Smiley and Yazulla, 1990], glutamatergic [Baude et al., 1993], and muscarinic [Mrzljak et al., 1993] systems.

Extrasynaptic D2 receptors will be exposed to raclopride (or any other injected PET radioligand) and will also be exposed to endogenous DA, although the DA concentrations will be lower. What will be their contribution to the PET signal during rest and activation? It will be minimal if these receptors represent a small fraction of the total D2 receptors exposed to raclopride. If these receptors are comparable or greater in number than the synaptic receptors, then their effect may be substantial and extrasynaptic DA levels at rest and activation must be considered. These concentrations have been measured directly using microdialysis and in vivo voltammetry, as discussed above. Most studies suggest that extrasynaptic DA levels are approximately 30 nM at rest, and rise to about 90 nM at 10–15 Hz stimulation [Gonon and Buda, 1985; Gonon, 1988; Church et al., 1987; Zetterstrom et al., 1983]. Thus, raclopride binding will be less during activation compared with rest, very much as it is with synaptic receptors. The hypothetical situation of D2 receptors being predominantly extrasynaptic was simulated in our computer model [Morris et al., this issue]; the effect on the PET signal is similar to that found with synaptic D2 receptors [Morris et al., in press].

CHOICE OF ACTIVATION TASKS

As mentioned above, the PET imaging must be performed both at rest and while the subject performs an activation task designed to increase the firing of DA neurons. The choice of an appropriate activation task can be based on at least two sets of data:

1. *From animal studies:* rewarded motor tasks appear to activate DA neurons [Schultz et al., 1983]. Light flashes and tactile sensations also can stimulate these neurons [Chiodo et al., 1980], although it is not clear how long the response can be maintained.
2. *From motor deficits in patients with Parkinson's disease:* one can deduce activities that are likely to require dopaminergic neuronal firing. Possibilities include: tasks involving repeated initiation of movements; fine motor tasks such as handwriting; performance of two motor tasks simultaneously; or "internally cued" movements. Internally cued movements of a joystick were used

[3]It may be noted that, at this DA concentration, approximately 10% of D1 receptors will be bound (assuming K_D = 1,000 nM).

successfully to activate several brain regions (including the contralateral caudate) in normals, with decreased activation in Parkinson's patients, as measured by PET regional cerebral blood flow (rCBF) studies [Playford et al., 1992].

FACTORS THAT MAY IMPROVE THE LIKELIHOOD OF DETECTING AN ACTIVATION EFFECT

Burst firing

Nigrostriatal neurons have been shown, in vivo, to fire not only at regularly spaced intervals, but also in short bursts. Each burst usually consists of three to eight action potentials at a frequency of about 15 Hz [Diana et al., 1989; Freeman et al., 1985; Sun et al., 1993]. It has also been shown in freely moving rats that certain movements (turning) are associated with significant increases not only in overall firing frequency, but in the percentage of burst firing (compared with non-burst firing) and in the number of spikes per burst [Diana et al., 1989]. This finding becomes particularly significant in light of the evidence, in dopaminergic neurons and in other neuronal types, that burst firing results in increased release of transmitter [Gonon, 1988; Gillary and Kennedy, 1969]. Thus, during a PET study, this may cause more DA to be released during activation, leading to a greater inhibition of radioligand binding. Presynaptic facilitation (discussed next) may be partly responsible for the increased transmitter release during bursts.

Facilitation

When two action potentials arrive at a presynaptic terminal separated in time by less than about 200 ms, a larger postsynaptic potential will be induced by the second impulse compared with the first. This phenomenon is known as facilitation, and is believed to be due to a presynaptic process that results in increased transmitter release by the second impulse [Katz and Miledi, 1968]. The magnitude of the facilitation effect diminishes with increasing time between impulses, with a time constant of decay of about 100 ms [Creager et al., 1980]. Thus, there is increased transmitter release per impulse during activation (10 Hz firing, or 100 ms between action potentials) compared with rest (5 Hz firing, or 200 ms between impulses). Our calculation of the additional transmitter release resulting from facilitation during 10 Hz activation is ~40%.[4]

High affinity/low affinity states of the D2 receptor

The D2 receptor is capable of existing in two states: the high affinity state ($K_D \approx 10^{-6}$–10^{-7} M for *DA*) and low affinity state ($K_D \approx 10^{-8}$ M) [Gingrich and Caron, 1993]. These two states do not appear to affect the affinity of the receptor for *raclopride* [Seeman et al., 1994]. There is evidence that increases in dopamine concentration induce a shift of the low affinity to the high affinity state [Seeman et al., 1994; Agnati et al., 1993]. Thus, during activation of the DA pathways, there may be a concomitant shift of D2 receptors toward a high affinity (for DA, but not for raclopride) state. This would noncompetitively inhibit raclopride binding, thereby increasing the difference in PET signal between the resting and activated states.

FACTORS THAT MAY DECREASE THE LIKELIHOOD OF DETECTING AN ACTIVATION EFFECT

Autoregulation

For DA, there exist autoreceptors on the presynaptic terminals that appear to inhibit release of transmitter [Cooper et al., 1991]. That is, they form a negative feedback system that inhibits DA release when the synaptic DA level rises. Thus, even though a dopaminergic neuron may double its firing rate for an extended period of time, the amount of DA released during this time may not actually double.

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[4]Ignoring facilitation, each impulse at rest or activation will release the same amount of transmitter (e.g., 1 unit, or 1 U). At rest (5 Hz firing rate), total transmitter release, R, during time, t, is

$$R_{rest} = 5 \cdot U \cdot t$$

and during activation (10 Hz)

$$R_{activ} = 10 \cdot U \cdot t$$

$$R_{activ}/R_{rest} = 2.$$

Accounting for facilitation during both rest and activation,

$$R_{rest} = 5 \cdot U \cdot (1 + e^{-200\,ms/100\,ms}) \cdot t = 5.7 \cdot U$$

$$R_{activ} = 10 \cdot U\,(1 + e^{-100\,ms/100\,ms}) \cdot t = 13.7 \cdot U$$

$$R_{activ}/R_{rest} \approx 2.4.$$

Adaptation

Some neurons are unable to sustain an increased (or decreased) firing rate despite a prolonged stimulus. A common example is adaptation of sensory neurons during a constant sensory stimulus [Kuffler et al., 1984]. It is not known if human DA neurons will continue to fire at increased frequency throughout an activation task. In vivo recordings from animals, however, suggest that DA neurons can maintain increased firing rates for at least several minutes [Steinfels et al., 1983; Diana et al., 1989].

Blood flow effects

The activation task, by activating specific neuromodulatory neurons, will presumably also increase CBF to these neurons [Sergent, 1994]. This will result in increased delivery of radiotracer to these neurons and therefore increased radiotracer binding, thus possibly offsetting the inhibitory effect of endogenous neurotransmitter competition. That is, blood flow effects, if significant, will have an opposite effect on radioligand binding compared with the competitive effects described in this paper. This is advantageous in the sense that, if decreased ligand binding is observed during activation, it cannot easily be ascribed to blood flow effects. Since most neuromodulatory neurons are sparsely distributed in the brain (see Introduction), the local blood flow changes may be minimal, although they might be significant for dopaminergic neurons in the striatum [Playford et al., 1992].

OTHER DATA SUGGESTING THAT ENDOGENOUS DOPAMINE CAN INHIBIT RACLOPRIDE BINDING

Several studies have demonstrated that endogenous DA can inhibit binding of D2 ligands, including raclopride, in the striatum of rats [Ross and Jackson, 1989; Seeman et al., 1990; Young et al., 1991]. Rats depleted of endogenous DA by pretreatment with reserpine show a 40–60% increase in D2 ligand binding; treatment with DA reuptake blockers or amphetamine, which increases DA release, caused decreases in D2 ligand binding of a similar magnitude. Unpublished data by Farde and Halldin [Farde et al., 1992] indicate a mild decrease in raclopride binding, measured by PET, following amphetamine administration in humans ($\sim 10\%$). Similar effects of amphetamine administration in humans have been reported using ^{123}I-IBF, a γ-emitting D2 ligand, and single photon emission computed tomography (SPECT) [Laruelle et al., 1994]. PET scanning of baboons has demonstrated increased binding of raclopride in the striatum following administration of GABA-ergic agonists and decreased raclopride binding following administration of cholinergic antagonists [Dewey et al., 1992; Dewey et al., 1993a]. Both results are consistent with the known inhibitory actions of GABA and acetylcholine on DA release in the striatum. Finally, preliminary results have been published using SPECT imaging of a highly specific D2 ligand ([^{123}I]IBZM) before and after total sleep deprivation in depressed patients [Ebert et al., 1994]. Patients that improved following sleep deprivation demonstrated decreased radioligand binding in the striatum, using semiquantitative techniques. The data were interpreted as suggestive that endogenous DA, liberated during sleep deprivation, inhibited radioligand binding.

These data demonstrate that endogenous DA can inhibit radioligand binding of such a magnitude that it can be measured by PET. It is still not known if mental or physical task activation can also stimulate enough DA release to do the same thing, but the estimates discussed above are encouraging. In the accompanying paper [Morris et al., in press], we describe a compartmental model of radioligand receptor binding that takes into account the concentration of endogenous neurotransmitter. Using the model, we ran computer simulations that predict that neurotransmitter activation is likely to be detectable.

ALTERNATIVE PET TRACERS

Other postsynaptic receptor ligands

Radiolabelled neuroreceptor ligands for PET have been used to study cholinergic (muscarinic), adrenergic, serotonergic, opiate, and GABA-A receptors [Fischman et al., in press]. The approach described in this paper may be equally effective for studying these neuromodulatory transmitter systems. We have not yet attempted to estimate the values of the relevant synaptic parameters for these systems.

Reuptake ligands

PET tracers that bind to reuptake sites are available for several modulatory neurotransmitter systems, including DA and serotonin [Fischman et al., in press]. These may be useful for studying synaptic transmission, as endogenous transmitter will complete for these binding sites also. It must be noted, however, that all reuptake sites are not necessarily located in the synaptic cleft. A significant fraction appear to be located outside the cleft, either elsewhere on the neuron or on glial cells [Cerruti et al., 1991; Iversen and Kelly, 1975]. This means that they may experience a lower peak concentration of transmitter compared with synaptic receptor ligands. However, the change in average transmitter concentration and the relative K_D values of transmitter and radioligand for the reuptake site may prove favorable. As mentioned earlier, direct measurements of *extrasynaptic* DA indicate a basal concentration of ~20–50 nM, which rises to anywhere from ~60 to 500 nM when the nigrostriatal pathway is electrically stimulated at 10–14 Hz [Gonon and Buda, 1985, Gonon, 1988]. Thus, with activation the extrasynaptic space may experience a larger change in DA than does the synapse. This means that a greater change in PET signal (activation vs. rest) might be obtained by using a reuptake radioligand rather than a postsynaptic receptor radioligand. This type of experiment is addressed further in the accompanying paper.

CONCLUSIONS

The above estimations suggest that activation of specific neuromodulatory pathways in the brain may be detectable with PET. A quantitative prediction of the results of such an activation study can be made with the use of a compartmental model; the model, in fact, does predict that neuromodulatory activation is likely to be detectable [Morris et al., 1995]. The example used in this discussion is the DA system, using a D2 receptor antagonist as the radioligand. Other neurotransmitter systems and other types of radioligands (e.g., reuptake site ligands) may also be amenable to study in this manner. This approach offers the possibility of in-depth study of neuromodulatory processes in the human brain in vivo. Since such processes are likely to be abnormal in many psychiatric and neurological diseases, these studies could provide insight into psychopathological mechanisms and allow objective diagnostic criteria to be applied to these diseases.

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