

Functional Imaging of Neurotransmission

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Abstract: Functional neurotransmitter imaging (fNTI) is an evolving technique that uses molecular imaging to detect neurotransmitters released during a task performance. This technique provides a tool to study neurochemistry of human cognition and involves dynamic measurement of the concentration of a specific radioligand during the task performance. Since ligands are competitively displaced by endogenously released neurotransmitters, a reduction in ligand concentration during task performance indicates task-induced release of endogenous neurotransmitter. Most of the fNTI experiments have used a specific dopamine receptor ligand ^{11}C -raclopride, which is suitable only for detection of dopamine released in the striatum. Ligands such as ^{18}F -fallypride and ^{11}C -FLB456 are potential candidates for detection of extrastriatal dopamine release. Using this technique, we have studied striatal and extrastriatal dopamine neurotransmission during performance of a variety of cognitive and behavioral tasks. These tasks include, motor planning, conscious and non-conscious motor memory, cued-recall, response inhibition and emotional memory. Since, fNTI is an emerging technique, it has so far been used to study only dopaminergic neurotransmission. Its utility in the study of human brain and cognition depends critically on the development of appropriate ligands for other neurotransmitters.

Keywords: Dopamine, raclopride, fallypride, memory, motor planning, molecular imaging.

INTRODUCTION

Functional neurotransmitter imaging (fNTI) is an evolving technique that provides an opportunity to acquire temporal and spatial information about neurotransmitters released during a task performance. This technique seeks to fill an important gap in understanding of cognitive processes, which are currently being studied using functional MRI (fMRI) techniques. These techniques use markers to detect task-induced changes in the regional cerebral blood flow (rCBF). Over the past decade, investigators have used these techniques to identify a number of brain areas that are involved in the processing of human cognition. The fMRI experiments, do not acquire information concerning neurochemical changes associated with cognitive activation and thus there is a gap in our understanding of brain mechanisms of cognition and the neuropathology of neurocognitive and psychiatric disorders. fNTI techniques could be an important tool to fill this gap.

In recent years, investigators have made several attempts to develop a method to detect task-induced release of neurotransmitters in the human brain. The most promising of these efforts are techniques that have used molecular imaging [1-9,20,31,47]. These techniques exploit the competition between endogenous neurotransmitter and its ligand. Thus, typically, a specific radioligand of the neurotransmitter is injected intravenously and volunteers are asked to perform a task. If the task induces release of endogenous neurotransmitter, it competitively displaces the ligand from receptor sites, reducing its concentration. By comparing the ligand concentration in each voxel or in a region of interest before and during the task performance, it is possible to localize the brain areas where neurotransmitter is released during the task performance.

Since the reliability and accuracy of detection of task-induced release of a neurotransmitter depends on the kinetics of the receptor-ligand interaction, selection of ligand is critical for success [13]. An ideal ligand has high affinity and selectivity for the target receptor, is permeable across the blood brain barrier, and is not metabolized in brain tissue, and has high receptor binding (specific binding) but low nonspecific binding, [34]. The ligand concentration is measured before and during the task performance using a positron emission tomography (PET) camera. The concentration measured by the camera however includes both, specific and nonspecific binding. Since only specific binding provides information concerning neurotransmission, nonspecific binding must be estimated and excluded from the detected ligand concentration. This estimation requires application of kinetic models.

A number of kinetic models have been developed to estimate specific binding. The most commonly used model (three compartment model) assumes that in the brain, a ligand is distributed in three compartments: the plasma compartment, nondisplaceable compartment (free and nonspecifically bound ligand), and the receptor compartment (for review see 21,32). When the ligand has distributed in the three compartments, its concentration in all compartments remains constant [58]. In this state of equilibrium, the concentration of ligand bound to receptors (specific binding; B) can be computed using the Michaelis-Menton equation [36] if the maximum number of receptors available for binding (B'_{\max}), the concentration of free ligand (F), and the dissociation rate constant of the ligand (K_D) are known. The computation involves the equation: $B = B_{\max} \cdot F / K_D + F$. If a very small (tracer) dose of ligand is administered (as is the usual practice), the value of F is very small in relation to K_D . It can therefore be disregarded in the denominator of the equation. Thus, specific binding of a tracer can be expressed as: $B = B_{\max} \cdot F / K_D$. Conventionally, specific binding is expressed in terms of binding potential (BP), which is an index of the capacity of a brain region for specific binding.

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Most investigators define BP as the steady state ratio of the specifically bound (B) to free ligand (F).

In fNTI experiments, changes in the BP are considered the index of change in specifically bound ligand because the other variable, the concentration of free ligand in the brain (F), is assumed to remain constant. Further, because ligands diffuse through the blood brain barrier by passive diffusion, it can be assumed that, at equilibrium the brain concentration equals the plasma concentration [33]. Thus, plasma concentration of ligand is generally used as a substitute for F. Development of the simplified reference region model (SRRM) [24] has even eliminated the use of plasma concentration. This model uses a brain area that is devoid of the target receptor (or has a negligible number of receptors, e.g., cerebellum for dopamine receptor studies), as a reference region. Assuming that the F in the reference and the target areas are similar, a comparison of the ligand concentration in the two regions provides a fairly accurate estimation of the BP. Thus the BP in the target region (TARG) can be calculated if the ligand concentration in the reference region (REF) is known: $(BP = C_{TARG}/C_{REF} - 1)$.

If a task induces release of endogenous neurotransmitter, the number of receptors occupied by the neurotransmitter will increase. Consequently, a smaller number of receptors will be available for the ligand. This effectively reduces ligand BP. Thus a reduction in ligand BP signifies task-induced release of the neurotransmitter. Since, the models that are used to estimate the BP assume that a steady state is maintained through out the experiment, a change in the state would violate its assumption and would not allow accurate measurement of the BP. Therefore, initial fNTI experiments were designed to compare the BP acquired in two separate scan sessions. In one session, resting BP was measured and in the other, the BP during the task performance was computed [31]. This design however was not considered sensitive for detection of neurotransmitter released during performance of a specific cognitive task because, it does not account for changes in the baseline activity of the neurotransmitter during the two scan sessions. Since cognitive experiments are designed to allow comparison of the activations observed during a control condition and a test condition, it is important that the experimental milieu during the two conditions remain constant. This can be ensured only if the control and test conditions are administered in the same scan session. This however, would require that the kinetic models be modified to allow a change in the state during the experiment.

We recently proposed a modification of the SRRM [1]. The modified model takes into account the change in the state by allowing the dissociation rate of ligand to change in response to an altered synaptic level of neurotransmitter. The change was effected by introducing a term $\gamma \cdot \exp(-\tau(t-T)) \cdot v(t-T)$ in the dissociation parameter of the SRRM. In this algorithm, γ represents the amplitude of ligand displacement, τ accounts for initial burst release of dopamine, t denotes the measurement time, T is the time of change in transmitter level, and v is the unit step function. The null hypothesis in this model assumes that the task does not elicit additional dopamine release and there is no change in the rate of ligand displacement (i.e., $\gamma=0$). The solution of

the differential equations describing the model for the instantaneous concentration history of the ligand has the following form:

$$PET(t) = R \cdot C_R(t) + k_2 \int_0^t C_R(u) du - k_{2a} \int_0^t PET(u) du - \gamma \int_0^t v(u-T) e^{-\tau(u-T)} PET(u) du$$

where, C_R is the concentration of radioligand in a region devoid of specific binding (reference), PET is the concentration of radioligand in a voxel with specific binding, R is the ratio of transport rates for the binding and reference regions, k_2 describes the clearance of nonspecifically bound tracer from the voxel, k_{2a} includes the information about dissociation from the receptor, γ represents the amplitude of transient effects, t denotes the measurement time, T is the task initiation time and $v(u-T)$ is the unit step function.

Using this model we conducted a series of experiments to ensure that the task-induced release of the striatal dopamine can be reliably detected.

DETECTION OF STRIATAL DOPAMINE RELEASE

As discussed earlier, all ligands cannot be used to detect task-induced release of a neurotransmitter. For use in fNTI experiments, the ligand must meet the criteria mentioned above, and should be displaceable from receptor sites in detectable amounts, by endogenously released neurotransmitter. A dopamine receptor radioligand ^{11}C -raclopride meets these criteria, but its displacement can be detected only in the striatum where the density of dopamine receptors is high. It cannot be detected in the brain regions outside the striatum. This ligand can therefore be used to detect task-induced release of striatal dopamine. We used this ligand in the following experiments to evaluate sensitivity and reliability of the fNTI technique. In all of these experiments, volunteers received a single intravenous injection of the dopamine receptor ligand ^{11}C -raclopride before task initiation. The ligand concentration was dynamically measured throughout the experiment, and a decrease in concentration indicated displacement of the ligand by the task-induced release of endogenous dopamine.

One of our initial studies involved a motor planning task [7]. In this study we asked six healthy volunteers to perform a finger opposition task in response to a number that was displayed on a computer monitor once every 2 s. The task was preceded by a control condition in which the same numbers were presented at the same frequency, and the subjects were asked to look at the numbers. Immediately after initiation of the task, significant increase in the rate of ligand displacement was observed in the dorsal aspect of the right anterior caudate and in the lateral part of the left putamen (Fig. 1). Detection of dopamine release in the same striatal areas where increased activity has been reported in fMRI studies [10,28,54] suggested that the fNTI experiment is sensitive not only in detecting release of dopamine, but also in localizing areas of activity. In addition, by showing that the release was greater in the initial task blocks, the result confirmed the observations of previous human [10] and animal studies [23,37] and suggest that the fNTI

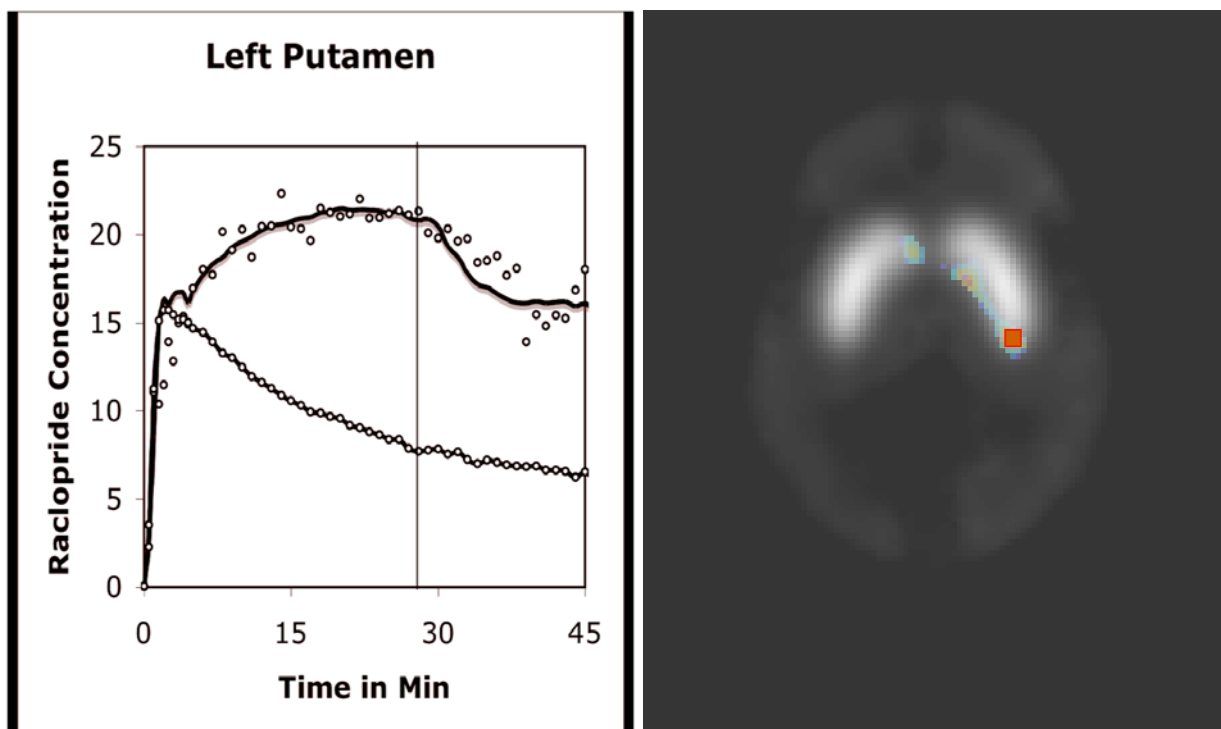


Fig. (1). Changes in the rate of displacement of ^{11}C -raclopride in the left putamen during a motor planning task. The vertical line indicates time of the task initiation and the curve at the bottom depicts displacement in a reference region (cerebellum). The figures also include transverse section of the brain showing the area of putamen (colored) where dopamine was released during the task performance.

techniques can be used to characterize temporal attributes of dopaminergic activity.

To confirm that the technique can detect dopamine released during cognitive processing, we studied motor memory tasks. Both, neuroimaging [17,22,26,27] and lesion [18,25,45,48] studies have suggested involvement of the striatal dopamine in these tasks. We used a serial reaction time (SRT) task [44] to elicit implicit (nonconscious) and explicit (conscious) motor memory. Both of these experiments were associated with striatal dopamine release [4,5,8]. We have also detected striatal dopamine release in non-motor cognitive tasks. These tasks include cued-recall [9], response inhibition and emotional memory [3].

DETECTION OF EXTRASTRIATAL DOPAMINE RELEASE

Since most of human cognitive processing occur in extrastriatal brain areas, it is important to be able to detect task-induced release of dopamine in these areas in order to understand the neurochemistry of human cognition. This however, would require the use of a ligand that can be displaced from receptor sites by endogenously released dopamine in extrastriatal brain regions. Two dopamine ligands, ^{18}F -fallypride and ^{11}C FLB 457 (isoremoxipride) appear to be the most promising candidates for the use in fNTI experiments. The ligand ^{11}C FLB 457 has very high selectivity and affinity for D_2 receptors in the human brain and can be used for imaging extrastriatal dopamine release [15,16,19,29]. The extrastriatal concentration of this ligand has been shown to be 2-8% of the striatal concentration in different brain regions [19,57]. Further, this ligand has

excellent test-retest reproducibility and it is displaced from the receptor sites by amphetamine induced dopamine release in the primate brain [49]. The kinetic property of the other ligand, ^{18}F -fallypride has been studied extensively. Initial receptor kinetic data acquired in laboratory animals [43], and experiments conducted in non-human primates indicate that the receptor binding of this ligand could be detected in a number of extrastriatal areas [40-42,56]. These areas include the thalamus, amygdala, pituitary, temporal cortex and frontal lobe. Recent experiments conducted on human volunteers confirmed that the receptor binding of this ligand is detectable in extrastriatal brain regions both, in healthy volunteers [15,38,46,53] and in psychiatric patients [11,30]. The next critical question, whether ^{18}F -fallypride can be displaced from receptor sites by endogenously released dopamine, and whether the displacement can be detected in extrastriatal brain areas, was examined in monkeys [39,43] and in human volunteers [50,51]. These studies have found that amphetamine-induced release of endogenous dopamine displaces the ligand, and that the displacement can be detected in a number of extrastriatal areas. It has been shown that the BP of ^{18}F -fallypride decreases by as much as 39% in some of these areas following amphetamine administration [39]. Displacement has also been reported following administration of dopamine receptor blockers in psychiatric patients [30], and during a cognitive task performance [14]. Recently, we used this ligand to detect extrastriatal dopamine released during performance of an emotional memory task [2].

In this experiment volunteers were given an intravenous injection of the ligand ^{18}F -fallypride at a high specific

activity (>92500 MBq/micromole). Immediately after the injection, the control condition of the task was administered. In this condition volunteers were shown a series of neutral words that are not likely to elicit emotional memory (e.g., paper, desk). To ensure that the words had no emotional connotation, volunteers were asked to indicate by pressing a key, whether they like, dislike or had no liking/disliking for each word. The control condition was followed by a test condition, which was started 25 min after the ligand injection. In this condition, words that are likely to elicit negative emotions were shown (e.g., murder, fire), and volunteers were asked to indicate their liking/disliking for each word. In both conditions, words were displayed for 5000 ms and there was an inter-stimulus interval of 500 ms. During the interval a fixation cross-mark appeared on the screen. The response time and volunteers' rating for each word were recorded.

Behavioral data indicated that words in the control condition did not elicit emotional memory while those in the test condition elicited strong emotions. These words allowed retrieval of negative emotional memories. Analyses of the PET data indicated that, immediately after the initiation of the test condition, the rate of ligand displacement increased significantly in extrastriatal cortical areas that have previously been implicated in the processing of emotional memory [12,35,52,55]. These areas included ventral prefrontal cortex, medial temporal lobe and amygdala. There was no change in the rate of ligand displacement in the cerebellum, which was used as a reference region.

Since the data indicates that dopamine was released in the same extrastriatal areas that have been implicated in the processing of emotional memory, it appears that the ligand ^{18}F -fallypride can be used in the fNTI techniques to detect and localize task-induced release of extrastriatal dopamine.

The findings of these studies clearly establish that fNTI techniques can be used to reliably detect task-induced release of dopamine. These techniques could be an effective approach for studying dysregulation of neurotransmission in patients with neurocognitive and psychiatric disorders. In the future, application of these techniques to additional neurotransmitter systems will open a new window for viewing the fundamental neurochemistry of neurocognitive function.

POSSIBLE RESEARCH AND CLINICAL USES

Since the fNTI technique provides an opportunity to examine neurotransmission, it could be a valuable research tool for the study of disorders that are associated with dysregulated neurotransmission. The technique could be used not only for the study of pathophysiology of these disorders, but also for their diagnosis. Using cognitive or behavioral 'provocative' techniques, it may be possible to detect dysregulation of neurotransmission at an early stage. In addition, the fNTI technique could play a very important role in the drug development process because it would allow objective evaluation of the ability of a pharmaceutical compound to manipulate human dopaminergic and possibly other neurotransmitter systems.

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