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STUDIES OF THE BRAIN CANNABINOID SYSTEM USING POSITRON EMISSION TOMOGRAPHY

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Introduction. Studies using radiolabeled cocaine in conjunction with positron emission tomography (PET) have permitted the imaging of cocaine binding sites in the human brain *(1)*, and the demonstration that the concentration of these sites is decreased in chronic cocaine abusers *(2)*. Similar studies of marijuana have been hampered by the unsuitability of radiolabeled THC for PET studies *(3)*, and the current unavailability of other in vivo imaging agents for cannabinoid receptors. Recent developments in medicinal chemistry suggest that a PET radiotracer for cannabinoid receptors will soon become available. This chapter briefly reviews these developments, together with the results of PET studies of the effects of marijuana and other abused drugs on brain metabolism. It also reviews PET studies of cocaine binding sites, to demonstrate the kind of investigations that will be possible when a cannabinoid receptor PET radioligand becomes available.

Drugs of Abuse. Figure 1 is a greatly simplified cartoon of a synapse in which synthetic enzymes, neurotransmitter storage vesicles, receptors, transporters are shown, together with enzymes which destroy neurotransmitters and so terminate their action. The adult human brain contains about one hundred million million synapses.

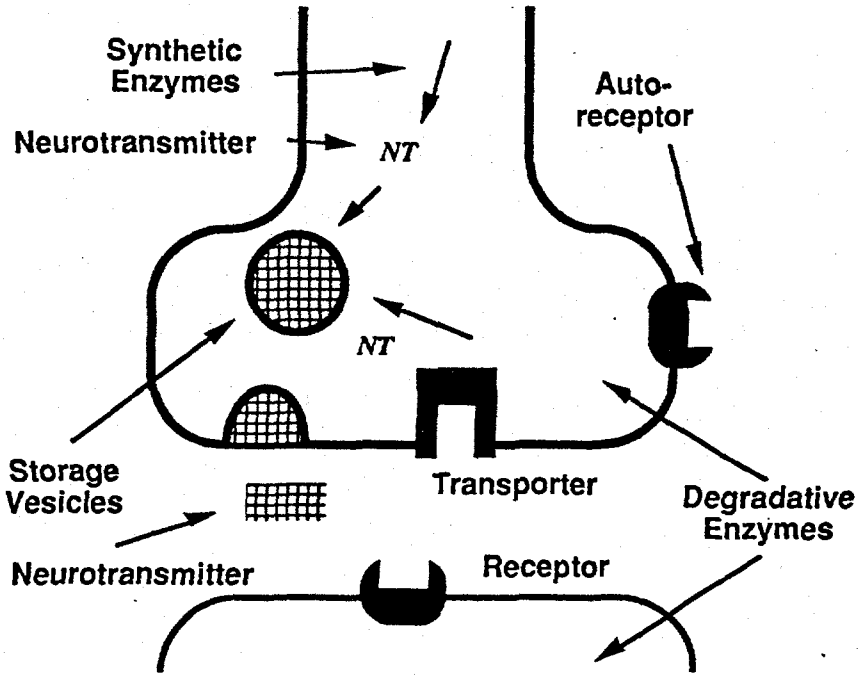

Figure 1. Cartoon of a generalized synapse, showing the location of neurotransmitter receptors and transporters, storage vesicles and synthetic and degradative enzymes.

The mechanisms of action of most abused drugs involves their binding to various classes of neurotransmitter receptors which are involved in the operation of the brain. The drugs may be

either receptor agonists or antagonists. Receptor stimulation may be either direct, as for cannabinoid and opioid receptors, or indirect, as exemplified by cocaine which blocks clearance of the neurotransmitter dopamine from the synaptic cleft between nerve cells and so increases signalling at neurons containing dopamine receptors *(4)*. The abuse of the compounds shown in Table 1 involves self administration of small amounts of the drugs, ranging from about 50 μg of LSD to about 50 mg of cocaine. Drugs such as alcohol or nitrous oxide which are abused in larger quantities are exceptions to this picture, and probably alter subjective states via effects on neuronal membranes.

Table 1. Some Abused Drugs and their Neurotransmitter Binding Sites.

Drug of Abuse	Target	Endogenous Ligand
Agonists		
Cocaine	Dopamine transporter	Dopamine
Nicotine	Nicotinic acetylcholine receptor	Acetylcholine
Marijuana	Cannabinoid receptor	Anandamide?
Morphine	Opioid receptors	Endorphins
Valium	Benzodiazepine receptor	Unknown, steroid(s)?
LSD	Serotonin receptor	Serotonin
Antagonists		
Caffeine	Adenosine receptor	Adenosine
Phencyclidine	Glutamate receptor	Existence uncertain

Abused and Therapeutic Drugs. Substances which interact with neurotransmitter systems can have medically beneficial effects as well as permit abuse. Often, as for example with valium or morphine, the same drug can be viewed as belonging to either category depending on the circumstances of their administration. Often, too, the same binding site may be the target of both abused and therapeutic drugs. For example cocaine which appears to have uniquely addictive properties acts by blocking the dopamine transporters. However, methylphenidate which blocks the dopamine transporter with a similar affinity to cocaine is prescribed to millions of American children to treat attention deficit disorder *(5)*. The divergent characteristics of these two drugs may result from different routes of administration, or from different affinities for other binding sites, or conceivably because they interact with the dopamine transporter in distinct ways. As another example, hallucinogens such as LSD act via stimulation of serotonin receptors *(6)*. However, elevation of brain serotonin by administration of drugs such as tricyclic antidepressants or selective serotonin reuptake inhibitors does not produce hallucinations.

In addition to helping understand mechanisms of addiction, PET studies of drugs of abuse may help our understanding of mental diseases, and produce useful leads for the development of drug therapies for these illnesses. A better understanding of the cannabinoid receptor system might produce useful drugs with, for example, antiemetic or analgesic properties, but with minimal abuse potential *(7)*.

Radioligand Studies. Compounds which have been radiolabeled to high specific radioactivities, and which bind with high affinity to neurotransmitters receptors and transporters, ("radioligands") are important tools in neuroscience. The development of tritium and iodine-125 labeled radioligands, and of autoradiographic techniques, have enabled the mapping of receptor and transporter distributions in the brains of experimental animals, and in post mortem human

brains *(8)*. These labeled compounds have also greatly facilitated molecular pharmacological studies of receptor/neurotransmitter interactions, as well as the screening of compounds with potential pharmacological properties.

Positron Emission Tomography (PET). PET is a nuclear medicine imaging technique which is able to measure in a quantitative fashion the regional and temporal concentrations of positron emitting nuclides in small volumes of the human body *(9)*. PET therefore allows the extension of radioligand binding studies to the living human and non-human primate brain, provided that suitable labeled compounds are available *(10)*. Carbon, nitrogen and oxygen all have positron emitting isotopes which in principle can be used to label organic compounds, including drugs of abuse, without altering their pharmacological behavior. In practice, nitrogen-13 (half-life = 10 minutes) and oxygen-15 (half-life = 2 minutes) are rarely used except in the form of simple inorganic radiotracers. In contrast, organic compounds which incorporate atoms of carbon-11 (half-life = 20 minutes) and also fluorine-18 (half-life = 110 minutes) are frequently employed as PET radiotracers. Over the last 20 years the chemistry of these positron emitters has evolved to the point where many radioligands are available in major PET centers. The relatively short half-lives of the "physiological" positron emitters is associated with reasonably benign radiation dosimetry which often allows repeated studies on the same subject. Thus longitudinal studies as well as "test-retest" studies involving administration of pharmacologically active drugs are possible. The 20-minute half-life of carbon-11 allows repeated studies in a single scanning session.

PET can be used to study drugs of abuse in several ways. Firstly, abused substances can be labeled with carbon-11. This permits the distribution of drugs in the brain to be directly measured following intravenous administration. Furthermore, if a very low mass of the labeled drug is administered (so that only a small fraction of the binding sites is occupied) and the drug has suitable properties (see below) then PET images will reflect local concentrations of drug binding sites. Secondly, the ability of an abused drug to compete with or to displace a different radioligand for the same binding sites can be measured. It may then be possible to measure the degree of receptor occupancy achieved by the abused drug and to compare the occupancy with behavioral and subjective changes caused by the drug. Thirdly, by using radioligands which bind to different sites it may be possible to examine effects of abused drugs on other neurotransmitter systems. For example, the in vivo binding of the dopamine D2 receptor radioligand C-11 raclopride, and the muscarinic cholinergic radioligand C-11 benztropine, have both been shown to be sensitive to alterations in levels of endogenous neurotransmitters (dopamine and acetylcholine, respectively) *(11-13)*. Fourthly, in addition to receptor and transporter radioligand studies, PET may be used to measure local values of cerebral blood flow (lCBF) *(14)* and of cerebral metabolic rate for glucose (lCMRglu) *(15)*. The most common radiotracers are oxygen-15 labeled water (for lCBF) and fluorine-18 labeled 2-deoxy-2-fluoro-D-glucose (FDG; for lCMRglu). These radiotracers allow effects of abused drugs on overall brain physiology to be evaluated, since flow and metabolism depend on nerve terminal activity. Both acute and chronic effects of abused drugs may be examined using these four general approaches.

Cannabinoid receptors. Autoradiographic studies with tritiated CP 55,940 *(16, 17)* have demonstrated high concentrations of cannabinoid receptors in substantia nigra and its output regions including the basal ganglia. High concentrations are also found in hippocampus and cerebellum. The cerebral cortex also contains appreciable concentrations of cannabinoid receptors, the highest being the cingulate gyrus. Some other regions including most of the brainstem and the thalamus contain low or negligible concentrations. The pattern of distribution of cannabinoid receptors in many brain regions is similar to that of dopamine D1 receptors, which has led to the suggestion that a function of the cannabinoid system may be to indirectly modulate brain dopaminergic activity *(17)*.

Compounds which bind to cannabinoid receptors. The major psychoactive ingredient of marijuana, delta-9 THC, binds to cannabinoid receptors with moderate affinity, as do several related compounds. In recent years, synthetic molecules with higher affinities have been developed. These include the cannabinoid receptor agonists CP 55,244 *(18)* and the structurally dissimilar WIN 55,212-2 *(19)* (Figure 2). Very recently, a high affinity cannabinoid receptor antagonist, SR141716A has been synthesized *(20)*. It can be expected that these new ligands will allow the design of experiments which will greatly expand our knowledge of the role that the cannabinoid receptor plays in normal brain functions. Additionally, they will facilitate studies of the effects of chronic stimulation of the cannabinoid receptor, and of withdrawal from marijuana intoxication.

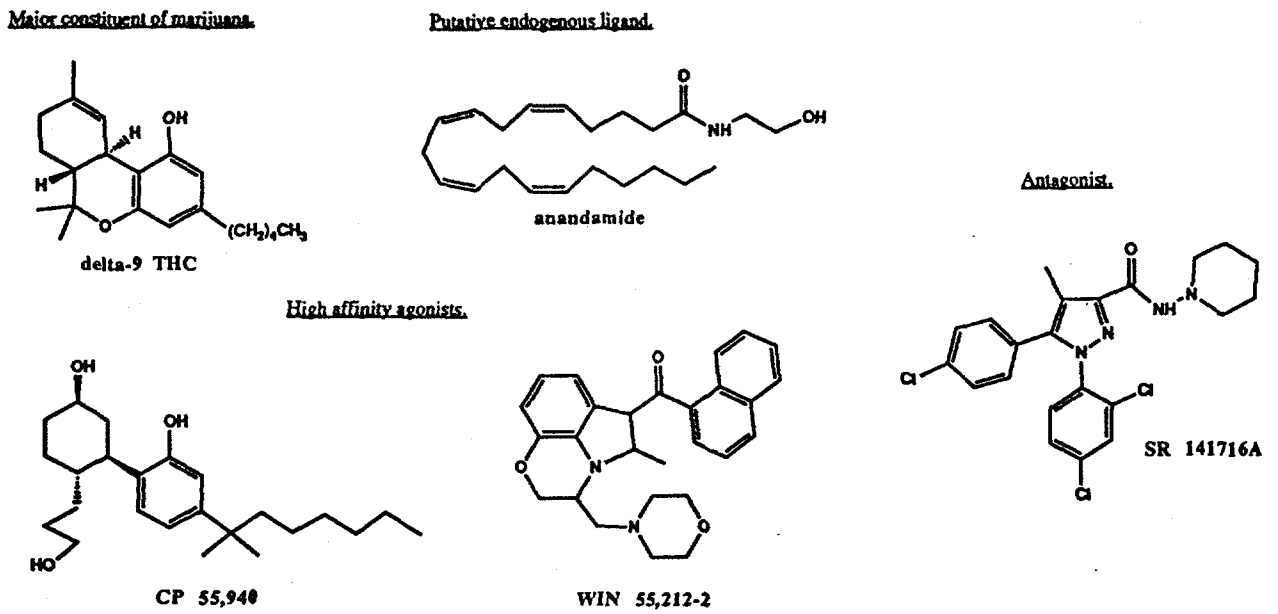

Figure 2. Chemical structures of some natural and synthetic compounds which bind to cannabinoid receptors.

Progress towards a PET radioligand for cannabinoid receptors. A PET radioligand for the cannabinoid receptor does not yet exist. A logical starting point for development of such a ligand is to incorporate an atom of carbon-11 or fluorine-18 into the structure of one of the molecules shown in Figure 2. In fact, THC has been modified by labeling with fluorine-18 in the hydrocarbon sidechain. Unfortunately, this compound did not produce PET images which showed any particular regional pattern of brain localization when injected into a baboon. *(3)* It was widely distributed in the brain with a relatively higher concentration in the cerebellum. Furthermore, preadministration of a pharmacological dose of THC failed to alter the regional distribution of the F-18 compound. This suggests that the PET images represented only non-specific uptake of the tracer with a negligible component due to specific binding to cannabinoid receptors. Further developments in this area will probably necessitate the synthesis of compounds with a combination of higher affinity for the cannabinoid receptor to increase specific binding, and of lower lipophilicity, to decrease non-specific binding. The structures of CP 55,940, WIN 55,212-2 and SR141716A (Figure 2) may in principle serve as lead compounds for these efforts. CP 55,940 may be the best option, since it possesses the highest affinity *(20)*. However, the structure is such that labeling with C-11 would probably be quite difficult. Incorporation of an F-18 atom into the alkyl side-chain might be a more fruitful approach, since the labeling chemistry would be easier, and in addition the longer half-life of F-18 would allow more time for non-specific binding to clear. Naturally, there is no guarantee that the fluorine-containing analog of CP 55,940 would retain the same affinity for the cannabinoid receptor. The

structure of SR141716A contains three chlorine atoms. One of these might be replaced with an atom of the longer lived positron emitter bromine-76 (half-life = 16 hours) which is occasionally used in PET experiments (*21*). Alternatively, a chlorine atom could be replaced with iodine-123 (half-life = 13 hours) which is not a positron emitter, but which can be imaged using single-photon emission computed tomography (SPECT) (*22*). These possible approaches are outlined in Figure 3.

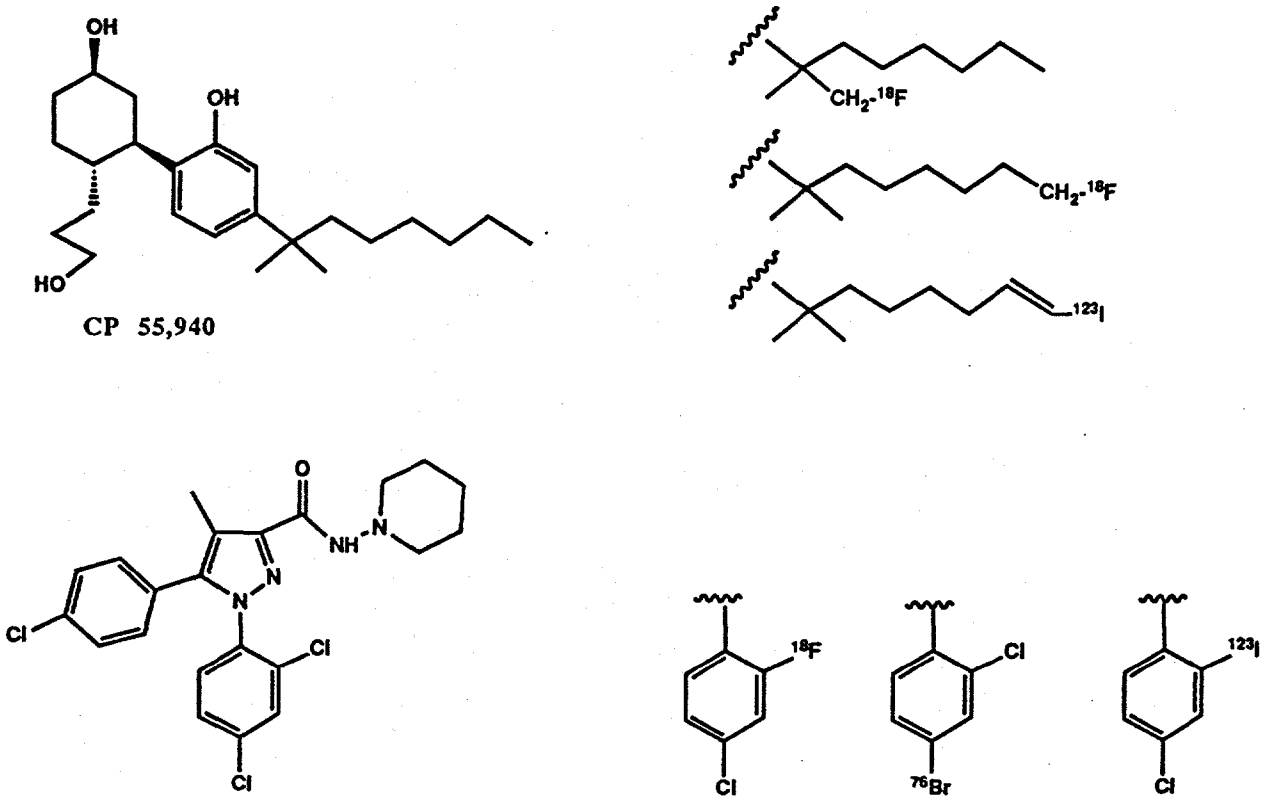

Figure 3. Possible approaches to labeling synthetic, high affinity cannabinoid receptor ligands with suitable radionuclides for PET or SPECT imaging.

Dealing with the issue of excessive lipid solubility will probably be key to the successful in vivo direct imaging of THC binding sites. The importance of lipophilicity may be illustrated by the experience of the last few years in imaging cocaine binding sites. Table 3 shows several compounds, including C-11 cocaine, which successfully visualize the dopamine transporter. These four tracers give essentially identical results, when their different affinities for the transporter are accounted for. However, two other positron emitting compounds with very high affinity for the dopamine transporter, F-18 labeled GBR 13119 and GBR 12909 (*23, 24*), with lipophilicities similar to that of THC (log P >> 3), were much less satisfactory PET radioligands. This is probably because of widespread brain uptake, followed by extremely slow clearance from tissue lipid pools in all brain areas. When these problems have been overcome for cannabinoid receptor radioligands, PET studies similar to those now underway with C-11 cocaine and its congeners, and with C-11 methylphenidate will become possible. Studies in cocaine abusers have shown that while there are increases in measures of dopamine transporter density shortly after withdrawal begins, there are decreases or no changes with protracted withdrawal *(25)*. Other drugs of abuse also alter transporter availability. For example, a preliminary study done in violent alcoholics showed significant elevations of DAT when compared with non alcoholic subjects *(26)*. In contrast non violent alcoholics had lower DAT levels than controls.

Table 2. Dopamine transporter radioligands based on cocaine and methylphenidate.

Radioligand	Ki in vitro (nM)	log P	Peak ST (nCi/cc/mCi)	ST/CB	Tmax (min)	Bmax/Kd' (mL/g)
RTI-55	1.5	1.6	130	12+	>1200	6.7
WIN 35,428	12	0.8	150	4+	>120	5
dtMP	40	1.4	100	2.3	30-40	1.6
Cocaine	100	1.3	85	1.7	5-7	0.6

Legend. RTI-55 and WIN 35,428 are, respectively, the I-123 labeled 3β-iodophenyl and the C-11 3β-fluorophenyl analog of cocaine *(27, 28)*. dtMP is C-11 *d-threo*methylphenidate, the active isomer of methylphenidate *(27, 28)*. log P is lipophilicity measured using an HPLC method *(30)*. Peak ST is the fraction of administered radioactivity localizing in the striatum. ST/CB is the ratio of concentration of radioactivity in striatum to that in the cerebellum. "Tmax" is the time after injection of radioligand at which striatal accumulation of radioactivity is at a maximum. Bmax/Kd' is the ratio of binding parameters in vivo estimated from the radioligand distribution volume *(32)*.

Effects of THC on brain glucose metabolism. Effects of acute intravenous injection of THC on lCMRglu have been investigated in both chronic marijuana abusers and in normal control subjects *(33, 34)*. The experimental design involved a baseline scan on day 1 and a second scan on day 2, 30-40 minutes after administration of 2 mg of THC. The most consistent observation in both in normal controls and habitual marijuana users was an increase in relative metabolic rate in the cerebellum. This increase was positively correlated both with concentrations of THC in the plasma, and with the intensity of the subjective sense of intoxication. However, the average increase in cerebellar metabolism after THC administration was less in marijuana users than in controls. Additionally, the marijuana users had lower cerebellar metabolism than the controls during the baseline scans. Thus it appears that the brain area showing the greatest metabolic increase in response to acute THC, the cerebellum, responds to chronic marijuana exposure with a decrease in baseline metabolic rate. The FDG PET studies also demonstrated that marijuana users responded to THC administration with increased metabolic activity in the prefrontal cortex, orbitofrontal cortex and basal ganglia. These increases were not seen in controls. In contrast to the robust effects of THC on relative metabolic rates, absolute global changes in CMRglu in response to THC were quite variable. In approximately one third of the subjects metabolism increased by more than 10%, and in another third metabolism decreased by more than 10%. There was also variability in subjective response to marijuana; most subjects reported the experience as pleasant, but some reported only minimal effects, and a few became anxious or paranoid.

The involvement of the cerebellum in the psychoactive effects of marijuana and in changes in cerebral metabolic rate is consistent with the view that THC interacts with the high concentration of cannabinoid receptors in this brain area. Decreases cerebellar metabolic rates in habitual marijuana users may reflect the effects of chronic exposure to the drug. Functions known to be associated with the cerebellum, such as motor coordination, proprioception and learning, have been documented to be adversely affected both during acute marijuana intoxication and in habitual users of the drug *(35)*. The PET scanner used in these investigations lacked sufficient resolution to examine metabolic rates in other brain areas, such as hippocampus, substantia nigra and caudate nucleus, which contain high concentrations of cannabinoid receptors. PET cameras with improved performance will allow further studies of the changes in brain metabolic rates induced by acute and chronic THC. Development of a PET radioligand for cannabinoid receptors will allow changes in metabolism to be related to local receptor concentrations.

Effects of other abused drugs on brain glucose metabolism. The effects of acute admistration of cocaine, alcohol, morphine, amphetamine and benzodiazepines have also been evaluated in PET experiments in normal subjects *(36-41)*. In each case a decrease in global brain metabolism has been found, in spite of the different mechanisms of action of these drugs. Thus THC behaves dissimilarly to these other abused drugs, in that single acute administration did not reduce overall metabolic rates. Findings that have been reported in terms of local drug induced changes in glucose metabolism are summarized in Table 3. For alcohol, activity in the thalamus is decreased relatively less than in other brain areas *(42)*, while occipital cortex and cerebellum show larger decreases; for diazepam, low doses do not change while high doses markedly decrease thalamic metabolic rates. Activity in occipital cortex is also markedly decreased by benzodiazepines.

Chronic cocaine abusers and alcoholics, like marijuana abusers, show metabolic abnormalities in the orbito frontal cortex and basal ganglia. Cocaine abusers have increased metabolic activity in the OFC and basal ganglia, relative to normal controls, during early detoxification. Later (>6 days) in detoxification activity in the OFC is decreased relative to controls *(40)*. Alcoholics on the other hand show decreased activity in the OFC and basal ganglia during detoxification *(43)*. By 30 days after withdrawal the decrements in activity in these areas have been partially restored, relative to controls. Thus patterns of metabolic rates after cessation of drug self administration are time dependent.

Table 3. PET studies of glucose metabolism involving drugs of abuse.

Drug	MR	Relative Metabolic Rates					Resp.	Recovery
		OFC	OC	BG	TH	CB		
Acute								
Heroin	↓							
Cocaine	↓						—	—
Amphetamin	↓						—	—
Alcohol	↓		↓		↑	↓	—	—
Marijuana	NC.	↑[2]				↑	—	—
Diazepam	↓		↓		↑low ↓high		—	—
Chronic								
Cocaine	↑	↑early		↑			ND	≈ 6 d (early)
Alcohol	↓	↓early		↓			↑	≈ 30 d (partial)
Alcohol[1]							↓	
Marijuana	NC.	↑		↑			↓	

Legend. MR, global metabolic rate; OFC, orbital frontal cortex; OC, occipital cortex; BG, basal ganglia; TH, thalamus; CB, cerebellum; Resp., response to administration of drug; NC, no change; ND, not determined. [1]alcoholics responded to a diazepam challenge with lower responses in BG, OFC and TH *(42)*. [2]Marijuana abusers only, not normal controls.

Conclusions. Compared with PET investigations of cocaine binding sites, imaging researches into the brain cannabinoid system of the living human brain are still in their infancy due to the high lipophilicity of available cannabinoid receptor ligands. Studies of the effects of THC on cerebral glucose metabolic rates have been conducted, and have shown that in contrast to other

drugs of abuse THC increases metabolism in the cerebellum, in both habitual marijuana users and normal controls. The relationship between this increase and the high concentration of cannabinoid receptors in the cerebellum seen in post mortem and animal brains may become clear when radioligands are developed which allow PET studies of these receptors in human subjects. The recent development of high affinity agonists and antagonists for THC receptors encourage us to anticipate that suitable imaging agents will soon become available. When that happens, several avenues of investigations will be opened up. These may include: measurement of long-term alterations of binding site densities in conditions such as disease states, withdrawal from habitual marijuana use, and treatment with therapeutic drugs; measurement of spatial and temporal patterns of cannabinoid receptor occupancies in the brain, and of relationships between receptor occupancies and the subjective and physiological effects of marijuana and related compounds; and assessment of short-term alterations in concentrations of endogenous cannabinoid receptor ligand (s) caused by administration of drugs which modulate other neurotransmitter systems via the cannabinoid system.

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