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Neurobiological Approaches to Brain-Behavior Interaction

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Preface

Neurobiological Approaches to Brain-Behavior Interaction is the result of a NIDA-sponsored technical review meeting held September 5-6, 1989, in Bethesda, MD. (For an overview of the presentations see "Neurobiological Approaches to Brain-Behavior Interaction: Summary and Overview.") Neuroscientists and behavioral scientists were brought together to share ideas and techniques for studying brain mechanisms that underlie or modify behavior. Technically, we have come a long way from the early 1960s when large inferential leaps were necessary to describe a behavior or function with respect to neurophysiological, neuropharmacological, and neurochemical events. With this approach it was necessary, for example, to run drug dose-response or time-response evaluations on a behaving group of animals in parallel with a different set of animals that was used for neurochemical determinations; less precise and specific methods were used to determine whole-brain monoamine levels and metabolite data to explain synaptic events as well as to provide a basis for drawing correlations with behavioral effects of a drug.

Recently, however, advances in technology and methodological developments have been made that allow for direct correlations between brain changes and behavioral events. Direct measurement of synaptic events occurring in discrete brain regions now can be assessed during ongoing behavior. This capability has the potential for immensely magnifying our understanding of relationships between brain structure and function tremendously enhances our capacity for dealing with malfunctions at the cellular level, especially with respect to understanding the direct effects of drugs of abuse on the central nervous system (CNS), and further, to reveal how these drug-CNS interactions alter behavior.

The purpose of this technical review was twofold. First, an important aim was to examine the various techniques for monitoring neural activity, synaptic events, and biochemical interactions during ongoing behavior by discussing the strengths and weaknesses of the current methodologies. Toward this end, discussions are provided in this monograph on the 2-deoxyglucose technique ("Importance of Behavioral Controls in the Analysis of Ongoing Events); *in vivo*

voltammetry and microdialysis ("Microdialysis in the Study of Psychostimulants and the Neural Substrate for Reinforcement: Focus on Dopamine and Serotonin"); computer imaging techniques such as positron emission tomography, single-photon emission computed tomography, and magnetic resonance tomography ("Studying Psychoactive Drugs With Positron Emission Tomography: Relationships Between Mood and Metabolic Rate" and "New Neuroimaging Techniques for Investigating Brain-Behavior Relationships"); and neurophysiological recording methods ("Neurophysiological Approaches to Receptor Pharmacodynamics" and "Psychomotor Stimulant Effects on Single Neurons in Awake, Behaving Rats").

As we enter the Decade of the Brain, information is becoming available concerning the complex response of the brain to drugs of abuse as well as the underlying events that unfold during the development of addiction. Drug addiction begins with a relatively simple neuropharmacological action of a drug acting at a specific site. A resultant cascade of neural and chemical events takes place to produce an experience, which is usually positive. This positive experience is highly reinforcing and often leads to the compulsive behavior and abuse of the drug despite its damaging consequences. With repeated drug exposure, brain mechanisms most likely change or adapt to the presence of the drug, often resulting in an alteration in the ability to process information. Because drug addiction is a behavioral phenomenon resulting from a modification of neural function, the role of the behavioral sciences in understanding drug abuse is absolutely essential. The Neuroscience Research Branch of the National Institute on Drug Abuse strongly encourages interaction between the neurobiological sciences and the behavioral sciences. Consequently, the second purpose of this technical review was to emphasize the equally important perspective that behavioral events influence the brain and, thus, the drug-brain-behavior interaction is an important consideration in evaluating neuropharmacological data.

Neurobiologists should be aware that a behavioral action of a psychotropic drug depends on many factors, of which the interaction between a drug and its corresponding receptor or receptors is only one. The modification of behavior is the final common pathway of a drug's action at a synapse whose influence might lie on a continuum from being very critical to being far removed from other circuits that determine behavior. Consequently, the ultimate effect of a drug on behavior might be modified by changes in activity within the brain's complex circuitry. These changes might come from environmental or situational factors that affect neural events, such as previous history, environmental

stimuli, and situation (“Importance of Behavioral Controls in the Analysis of Ongoing Events”). Locomotor activity is sometimes thought to be a simple drug screen, yet even this seemingly simple behavior is made up of complex components (“Multivariate and Nonlinear Approaches to Charcterizing Drug Effects on the Locomotor and Investigatory Behavior of Rats”). The use of behavior to study the neural basis of drug action is a powerful tool (“Brain-Stimulation Reward: A Model for the Study of the Rewarding Effects of Abused Drugs” and “Cocaine Self-Administration: Pharmacology and Behavior”).

Assuredly, during the Decade of the Brain and thereafter, multidisciplinary approaches to behavioral neuroscience will have major influences in dealing with behavioral problems and diseases of the brain. The overall strength of this meeting on brain-behavior interactions was that it reviewed some state-of-the-art neuroscience and neurobehavioral techniques currently in use and also allowed for direct interaction between neuroscientists studying specific aspects of brain function with those interested in the precise nature of behavioral events related to drug abuse.

Microdialysis in the Study of Psychostimulants and the Neural Substrate for Reinforcement: Focus on Dopamine and Serotonin

Bartley G. Hoebel, Luis Hernandez, Gregory P. Mark, and Emmanuel Pothos

INTRODUCTION

Microdialysis is a useful technique in the study of addiction. It provides a means of measuring neurochemical changes in localized regions of the brain (Delgado et al. 1972; Kissinger 1990; Ungerstedt 1984). Neurotransmitter release can be determined periodically while animals receive drugs or engage in drug-seeking behavior (Becker et al. 1988; Di Chiara and Imperato 1988; Hernandez et al. 1987; Hurd and Ungerstedt 1989; Hurd et al. 1989; Kuczenski and Segal 1989; Pettit et al. 1989; Sharp et al. 1987; Ungerstedt 1984; Westerink et al. 1987; Zetterström et al. 1983). Basal release of a neurotransmitter can also reveal individual differences between animals and differences due to changes in an animal's physiology or environment. This chapter illustrates the use of microdialysis in the study of changes in extracellular dopamine (DA) and serotonin (5-HT) due to (1) rewarding brain stimulation, (2) natural ingestive behaviors, (3) classical conditioning, (4) locally infused addictive drugs, (5) peptides that influence DA release, (6) anorexia in relation to drugs of abuse, (7) body weight loss, and (8) chronic treatment with haloperidol (HAL) and clozapine (CLOZ). The goal is to understand (1) the neurochemical actions of abused psychostimulants, (2) potentiation of these actions by conditioned stimuli, peptides, and low body weight, (3) development of addiction and psychosis, and (4) treatment.

THE MICRODIALYSIS TECHNIQUE

Microdialysis has an enormous advantage over earlier neurochemical techniques that required analyses of brain slices, synaptosomes, or tissue homogenates (Robinson and Justice 1991; Rollema et al. 1991). For most experiments it is no longer necessary to extract the brain. In vivo microdialysis, however, has a major disadvantage relative to in vivo voltammetry; researchers typically must wait 5 to 30 minutes to obtain a microdialysis sample that contains enough of the neurochemical to be detected by present analytical techniques. This sampling rate is frustratingly slow when one is dealing with rapidly changing physiology or behavior. On the other hand, in vivo voltammetry lacks the wealth of criteria for identifying a variety of neurochemicals. Compared with open-tip, push-pull cannulas, microdialysis probes (figure 1) are easier to use because they do not pressurize the brain or become plugged up for several days. For molecules such as insulin that are too large to pass through a microdialysis fiber, however, the push-pull approach is advantageous. We use semipermeable membranes with a molecular weight cutoff of 6,000 that excludes all enzymes that would degrade the sample. Membranes with a molecular weight cutoff of 20,000 are also useful, and a variety of materials are available.

Microdialysis samples are typically analyzed by high-pressure liquid chromatography (HPLC) or radioimmunoassay. Most of the known neurotransmitters have been measured in this way, including catecholamines, 5-HT, excitatory and inhibitory amino acids, acetylcholine, and several peptides. In addition, the second messenger cyclic adenosine monophosphate can be measured in the extracellular space and could be a valuable tool in a new approach to the study of addiction (Egawa et al. 1988; Hoebel et al. 1990).

The microdialysis technique involves passing Ringer's solution through a probe constructed of input and output tubes with a section of semipermeable dialysis membrane between them. Neurochemicals diffuse in through the dialysis membrane and are carried out to a collection vial or directly to the analyzer. The input and output tubes can go through the brain horizontally, carrying the Ringer's solution in one side of the head and out the other (Hamberger et al. 1983; Imperato and Di Chiara 1984; Ungerstedt 1984), vertically side by side in the form of a U tube (Hernandez et al. 1983; Kalen 1988; Marsden et al. 1986; Quan and Blatteis 1989; Ungerstedt 1984) or with one tube inside the other (Benveniste 1989; Hernandez et al. 1986; Johnson and Justice 1983; Kapoor and Chalmers 1987; Nakahara et al. 1989; Ungerstedt 1986; Westerink et al.

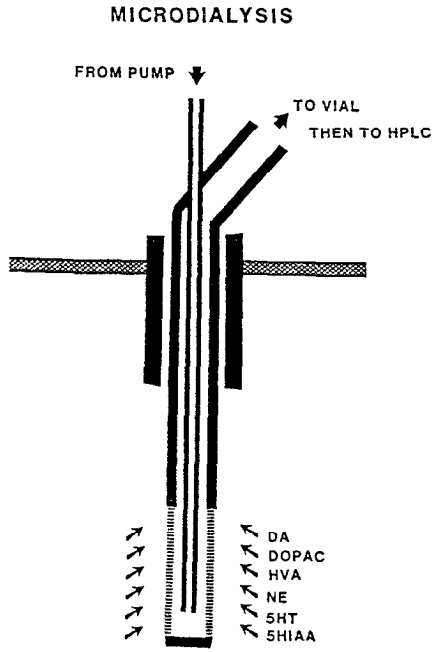


FIGURE 1. *A removable microdialysis probe in a guide shaft cemented to the skull. Ringer's solution from a remote pump enters through the small inner tube and exits the larger tube to a vial attached above the rat's head. Drugs added to the Ringer's solution diffuse outward from the semipermeable tip (0.2 mm outer diameter) to bathe neural tissue. (From Hernandez et* al. *1986.)*

1987). The horizontal arrangement causes the least local damage because the microdialysis tube floats in the brain without any steel tubing inside. The U tube has the most local surface area and therefore collects the most neurotransmitter in a given region, but it does the most local damage. Any probe will cause some brain damage but usually not enough to disrupt operant behavior such as bar pressing for food, self-stimulation, or drugs. If large probes were placed bilaterally in a small structure, one would have to worry about bilateral lesions. The concentric arrangement we chose is the smallest and allows the probe to be removed and reinserted later via an implanted guide shaft. Small probes, however, require very sensitive analytical chemistry. We are developing capillary electrophoresis to improve sensitivity for future experiments (Guzman et al. 1990).

MICRODIALYSIS IN BEHAVING ANIMALS

This laboratory has specialized in the measurement of neurotransmitter release in freely moving animals (Hoebel 1988; Hoebel et al. 1989). This method avoids any abnormalities caused by anesthesia, and the animals are able to engage in operant or classical conditioning. We simply adapted well-known microinjection techniques to microdialysis by implanting a guide shaft with a concentric probe design (Hernandez et al. 1986). Permanent guide shafts made from hypodermic needle tubing are implanted in animals while they are anesthetized. The animals recover for a week before the microdialysis probe is lowered into the brain. The probe is constructed from two pieces of concentric tubing, with the dialysis membrane forming the tip in the shape of a 0.2-mm by 3-mm cylinder (figure 1). There is a tradeoff between choosing a small size and obtaining enough neurochemical to be detectable. A cellulose dialysis tip only 150 µm by 2 mm can be used if the neurochemical is abundant, for example, DA in the striatum (STR). For DA in the nucleus accumbens (NAC), we usually use a probe 200 µm by 3 mm, and for the prefrontal cortex (PFC), a probe 200 µm by 4 mm; in the lateral hypothalamus, DA is usually undetectable unless a drug such as amphetamine (AMPH) is administered (Hernandez et al. 1986; Parada et al. 1988*a,* 1988*b).* In all these experiments, we typically collect 20-µl samples over 20 min and then perform a 20-min assay for DA and its metabolites, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA). In this way, sample collection and assay keep pace. It is also possible to measure DA as often as every 5 min by using smaller HPLC columns and skipping the measurement of metabolites (Church and Justice 1987; Phebus et al. 1986). Frequent sampling is a great advantage in behavioral experiments. For measurements second by second one must turn to in vivo voltammetry, by which monoamines are oxidized on the tip of an electrode in the brain; in this case, however, accurate neurochemical identification can be difficult (Gerhardt et al. 1984; Marsden 1984; Wightman et al. 1988). Microdialysis is more reliable and more versatile.

The most difficult aspect of behavioral microdialysis is the neurochemical assay. It is not difficult to place a microdialysis probe with its tip in the hypothalamus or NAC in a rat. The animal moves freely in a test cage such as a Skinner box with flexible tubing leading to and from its head via a watertight swivel (figure 1). Probes are available commercially from Carnegie Medicin (Sweden), Biological Analytical Systems (United States), and Europhor (France), although many laboratories make their own. Probe construction is difficult but can be mastered by a patient person with good eyesight and manual

dexterity. We measure 5-HT with an amperometric electrochemical detector from EG&G: Princeton Applied Research Corp. and DA with a coulometric detector from ESA Company, although many other brands are also suitable. If transmitter is abundant in the sample, the Coulochem Electrode Array System (ESA Co.) with 16 electrodes can be used to detect all the monoamines at once, along with their metabolites and precursor amino acids. The aim with HPLC is to achieve very high sensitivity in the low picogram or femtogram range by having very little dead space and a very smooth baseline free of electronic noise or pump pulsations. The neurochemical of interest must come off the column after the massive front of oxidizable compounds has gone by but not so late that it becomes a broad, smeared-out peak.

A 4:1 signal-to-noise ratio is desirable but not always obtainable. Reuptake blockers or enzyme inhibitors can be introduced locally by way of the probe to boost the neurotransmitter signal, but this method has the disadvantage of departing from natural conditions. The Ringer's solution itself can be disruptive (Westerink et al. 1988). Many early studies used high-calcium Ringer's solution that boosted DA recovery, but now most laboratories match the Ringer's solution calcium level to extracellular levels (about 1.2 mM). Fortunately, high-calcium Ringer's solution did not seem to alter the dopaminergic effects of drugs of abuse in the early studies, but Westerink reports that certain DA-acetylcholine interactions are blocked by high-calcium Ringer's solution (Electrochemical Detection Conference, Nottingham, 1989). Most researchers still use Ringer's solution, not a true artificial cerebrospinal fluid. The absence of magnesium or glucose may be a concern in some experiments. Microdialysis also has an inherent problem in that it depletes neurotransmitter in the extracellular space (Benveniste 1989), but this problem is partly solved by the use of low perfusion rates (0.5 to 1 µl per minute). Nonetheless, microdialysis has shown logical and consistent effects with drugs of abuse in several laboratories.

Last, but not least, it is essential to prove beyond doubt that the analytical procedure actually measures what one claims. This has been done for DA and 5-HT (Brazell et al. 1985; Carboni and Di Chiara 1989; Imperato et al. 1988; Westerink et al. 1987; Zetterström et al. 1988). For 5-HT we find it is necessary to oxidize the samples and standards at more than one potential for positive identification. We also test with 8-OH-DPAT, which is a 5-HT autoreceptor agonist that stops 5-HT neurons from firing. When treatment with this compound causes the putative "serotonin peak" to shrink, then (1) the lost 5-HT probably had come from 5-HT neurons, not glia or platelets and (2) it probably

was real 5-HT, not a different compound with the same oxidation characteristics (Auerbach et al. 1989; Schwartz et al. 1989*c)*.

THE MESOLIMBIC SYSTEM

The mesolimbic DA system has attracted the most attention in the study of the neural circuitry underlying drug abuse (figure 2). Its importance is undeniable. If the mesolimbic system is blocked or almost completely depleted of DA, animals refuse to work for food, self-stimulation, or self-injection (Fibiger and Phillips 1986; Hoebel and Novin 1982; Koob and Bloom 1988; Roberts and Koob 1983; Wise and Bozarth 1987), whereas depleting norepinephrine has the opposite effect by increasing feeding and self-stimulation (Hernandez and Hoebel 1989*a)*. Not only is the DA system necessary, its functions are sufficient to motivate and reward behavior. Rats will work to stimulate certain DA cell regions electrically or chemically. For example, they will electrically self-stimulate the mesolimbic DA cell body region in the ventral tegmental area (VTA) (Phillips et al. 1989). They will also self-inject morphine (Bozarth and Wise 1981) or neurotensin directly into the VTA (Glimcher et al. 1987); they self-inject opioids (Goeders et al. 1984), AMPH (Hoebel et al. 1983), or DA into the NAC (Dworkin et al. 1985), and cocaine into the PFC (Goeders and Smith 1983). Apparently the mesolimbic system is a substrate for both natural rewards such as feeding and the drug rewards that lead to addiction (Fibiger and Phillips 1986; Hoebell 1988; Mogenson 1987; Wise 1982). The findings of many researchers are consistent with this suggestion; however, distinguishing among DA's roles in stress, arousal, locomotion, priming, and reinforcement is often troublesome, and much remains to be learned (Salamone et al. 1989). Numerous conditioning experiments now show that mesolimbic DA is not just part of a locomotor system; it also mediates reward processes involved in positive reinforcement (Ettenberg 1989; Fouriezos and Wise 1976; Gallistel et al. 1982; Spyraki et al. 1983; White and Franklin 1989). It is clear from in vivo neurochemistry research that the mesolimbic DA system could play similar roles in natural appetitive behavior and drug appetite.

Microdialysis has been used to study the release of DA by reinforcing stimuli. Dopaminergic drugs of abuse given systemically caused large increases, e.g., ninefold, in extracellular DA in freely moving rats (Imperato and Di Chiara 1984). We have focused on measurement of NAC DA while presenting the rat with hypothalamic stimulation, food, or intra-accumbens drugs of abuse. Currently we are testing conditioned stimuli associated with food or drugs.

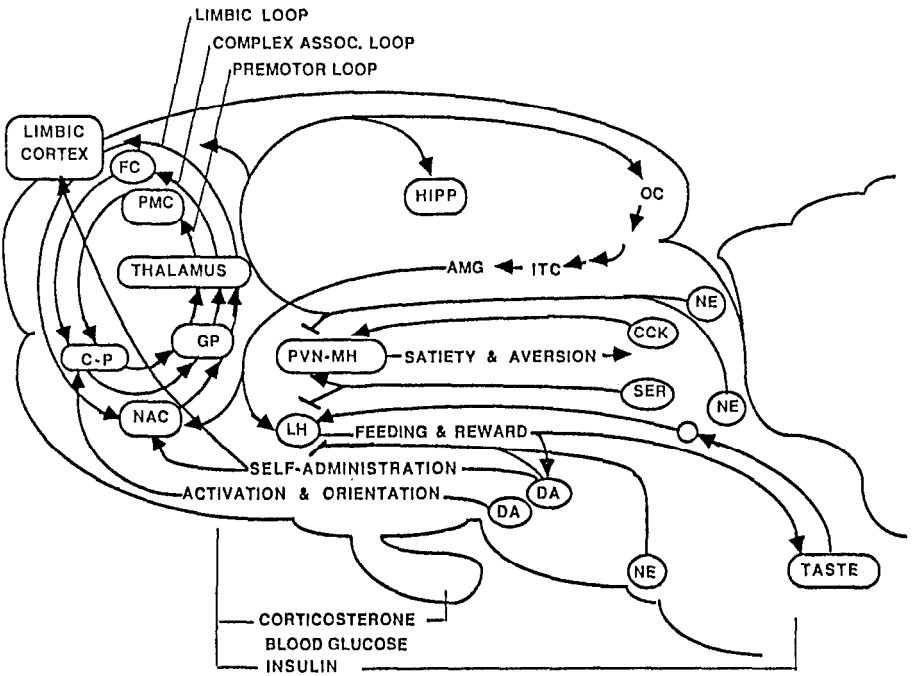


FIGURE 2. *Side view of the rat brain showing the brain regions discussed in the text with some of their interconnections, Sharp arrows indicate facilitation of the labeled behavioral output function. Blunt arrows indicate suppression of the function. For example, palatable "taste" inputs from the nucleus tractus solitarius (NTS) facilitate the lateral hypothalamic "feeding and reward" system, which in turn facilitates the ascending "self-administration" DA paths to the NAC and limbic PFC. Serotonin probably inhibits this process in combination with postingestive factors by promoting satiety in the PVN-MH and suppressing feeding-reward in the LH. Not shown are 5-HT projections to the NAC, HIPP, and related regions where 5-HT might facilitate behavioral arousal. See text for description of AMPH effects in each of these brain sites corresponding to monoamine arrows in the diagram. (From Hoebel 1988.)*

Dopamine Released by Brain Stimulation

Our first step in finding the natural substrate for drug abuse was to stimulate the perifornical lateral hypothalamus (PFH) using electrodes that induced feeding and self-stimulation. With electrodes of this type, self-stimulation rate varied with the animal's appetite for food (Hernandez and Hoebel 1978; Hoebel 1985). The PFH is only one of many medial forebrain bundle (MFB) self-stimulation sites. Electrodes that elicited copulation in males yielded self-stimulation at a rate that varied with androgen level (Caggiula and Hoebel 1966). Thus, MFB self-stimulation can reflect natural appetitive behaviors.

The reinforcing properties of MFB self-stimulation can be enhanced by drugs of abuse. Self-stimulation is often considered an accelerated model of addictive behavior (Carden and Coons 1990; Hubner et al. 1988; Trujillo et al. 1989). (See also "Brain-Stimulation Reward, A Model for the Study of the Rewarding Effects of Abused Drugs.") MFB self-stimulation sends action potentials along axons that descend to the midbrain VTA region (Mogenson 1987; Shizgal et al. 1982) and on to taste-sensory cells in the hindbrain (Murzi et al. 1986) as part of a circuit that is thought to excite the ascending mesolimbic DA system (Wise 1982, 1983). If so, PFH stimulation should release NAC DA. Depending on electrode configuration and placement, MFB stimulation can also activate the ascending DA neurons directly as they pass through the hypothalamus (Nicolaysen et al. 1988; Shizgal 1989; Yeomans 1989). We used placements and parameters designed to activate descending systems that excite DA neurons indirectly by way of their normal inputs to the cell bodies and dendrites (figure 2).

We find that PFH stimulation that induces feeding does release DA in the NAC. This finding was true whether the rat ate food or not (Hernandez and Hoebel 1989*a)* (figure 3). As a control, rats with microdialysis probes in the STR showed no detectable increase in extracellular DA, suggesting that PFH electrodes for stimulation-induced feeding release much more DA in the NAC than in the nearby STR. It also showed that the DA that overflowed out of synapses in the NAC diffused to the local microdialysis probe in the NAC but did not diffuse as far as the probe in the STR. This demonstration by electrical stimulation of anatomical specificity between DA subsystems is crucial in the study of drug abuse. DA-releasing drugs such as AMPH do not distinguish between DA terminal regions the way PFH-stimulation-induced feeding can. AMPH would be expected to release DA in all terminal regions whether involved in abuse or not. Our result suggests that PFH stimulation could have

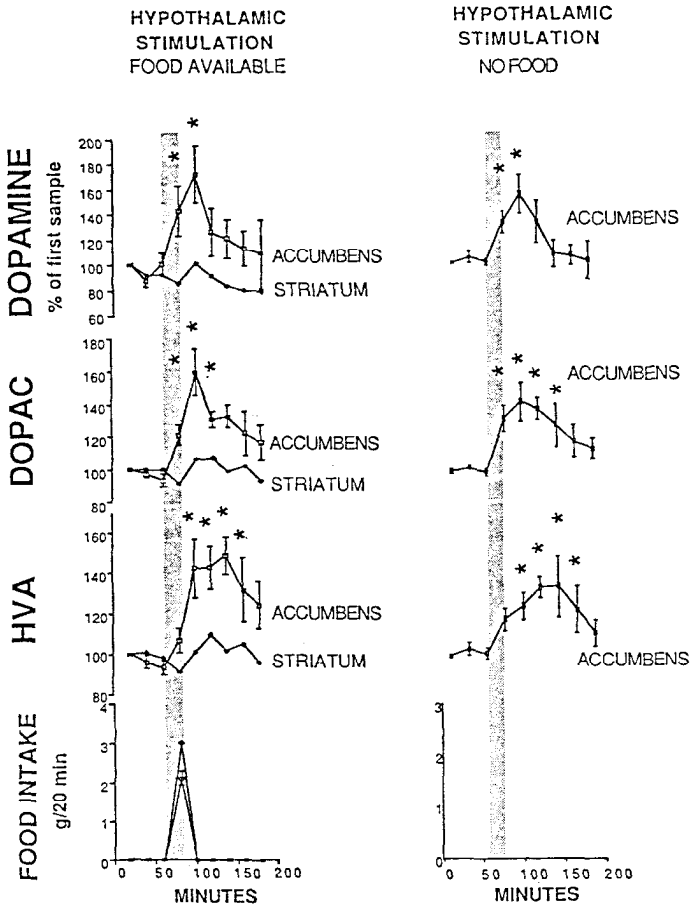


FIGURE 3. *Perifornical lateral hypothalamic (PLH) stimulation releases dopamine (DA) in the nucleus accumbens with food available (stimulation-induced feeding, left side). The same* electrical *stimulation releases DA even without feeding (right side). Self-stimulation or deprivation-induced feeding will do the same thing. Feeding also releases DA in the PFC. Psychostimulants may generate reward by their action in these DA systems. (From Hernandez and Hoebel* 1988a.)

acted by way of the VTA (figure 2). It is unlikely that we stimulated mesolimbic DA axons directly during stimulation-induced feeding, because release was localized to the NAC rather than to the STR. We are finding, however, that self-stimulation of the MFB for an hour (approximately 2,500 bar presses) gives evidence of DA release at both sites (Hunter and Hoebel, unpublished manuscript). DA in the STR is undoubtedly involved in arousal, stereotypy, and other motor activities, some of which are involved in feeding (Church et al. 1987), but present evidence suggests DA in the NAC bears some special relevance to appetitive behavior and reinforcement.

Dopamine Released by Eating

DA release by natural behavior was a crucial test. Eating released DA in the NAC in rats tested at 80 percent of normal body weight and in rats that were deprived of food for just a day (Hernandez and Hoebel 1988*a;* Radhakishun et al. 1988). The same was true whether rats ate Purina chow or bar-pressed for food pellets. DA was also released in the PFC during operant feeding, but there was no comparable release in the part of the STR we sampled. This finding suggests that DA in the PFC as well as the NAC is involved in the elicitation or reinforcement of feeding. Therefore, drugs that release DA in the NAC and PFC mimic natural processes that accompany operant feeding.

Feeding is not the only behavior that releases DA. We found DA release in the NAC during angiotensin-induced drinking and diuretic-induced salt intake (Hoebel et al. 1989). Sexual-approach behavior also releases DA (Mas et al. 1990).

Dopamine Release Can Be Conditioned

How does one know whether DA is related only to arousal or whether it carries the positive valence implied by terms such as elicitation, reward, incentive, positive reinforcement, preference, or pleasure? That this is a difficult problem is attested to by long debate. The clearest answers come from conditioning studies that test animals' remembrance of past events by having them make choices long after the training is completed and experimental drugs are gone (Wise and Bozarth 1987). Therefore, we measured DA release in the NAC before and after a conditioned taste aversion. Initially, saccharin squirted into the mouth through an oral catheter released DA. Saccharin taste was then paired with lithium chloride malaise. As a result, the same concentration of saccharin had the opposite effect: it caused a significant decrease in DA below

baseline levels. This finding suggests that a good-tasting substance can release DA, but when it is associated with nausea in the animal's memory, the effect on DA release is reversed. Thus two opposite effects on DA were obtained even though the animals appeared to be aroused in both cases (Hoebel et al. 1989; Mark et al. 1991 **a).** To save the arousal hypothesis, one would need to postulate two kinds of arousal: one that increases DA within the NAC and one that decreases it. We offer instead the view that DA release in the NAC facilitates appetitive behavior, providing positive reinforcement; conversely, a decrease in DA release facilitates avoidance, enhancing escape reflexes and providing negative reinforcement. Because DA in the NAC is released in response not only to food intake but also to water intake, salt intake (Hoebel et al. 1989), and sexual appetite (Mas et al. 1990), we can hypothesize that DA release is naturally involved in many appetitive behaviors. Similarly, it is possible that DA decreases are involved in numerous avoidance behaviors, but so far this effect has only been shown to be related to conditioned taste aversion.

DA can also be conditioned to increase. A novel taste became the conditioned stimulus for DA release after it had been paired with nutritious food delivered via a gastric fistula (Mark et al. 1991*b).*

Dopamine and Addiction

On the basis of the studies that have been described, there are at least two ways an animal or a person could rectify a conditioned DA decrease: avoid the stimulus situation that caused it, or take some other ingestant or a drug that increases extracellular DA. To achieve conditioned DA release, one merely needs to mimic the stimulus situation and environment.

Many drugs of abuse given systemically increase extracellular DA (Di Chiara and Imperato 1988; Kuczenski 1986). Similarly, when AMPH, cocaine, phencyclidine (PCP), or nicotine was injected locally into the NAC or infused through the microdialysis probe itself, this local treatment increased levels of extracellular DA in a manner similar to an intraperitoneal injection (Hernandez and Hoebel 1988*b;* Hernandez et al. 1987, Hernandez et al. 1988*a,* 1991; Kuczenski and Segal 1989; Mifsud et al. 1989; Robinson et al. 1988; Sharp et al. 1987). The extracellular concentration of the DA metabolite DOPAC decreased, indicating depletion of intracellular DA or interference with DA metabolism. This interference adds to the length of time DA would be expected to persist in the synapses, helping to explain why drugs of abuse are more

potent than normal appetitive stimuli such as food. When rats ate a meal, DA metabolism increased, but after drugs of abuse were administered, DA metabolism decreased (figure 4, AMPH; figure 5, PCP).

When cocaine is injected locally there is a danger in that it acts as a local anesthetic. As a control, equimolar procaine and lidocaine were tested. Procaine is both a mood elevator and a local anesthetic; lidocaine is just a local anesthetic. Cocaine released the most DA; procaine released less (Hernandez

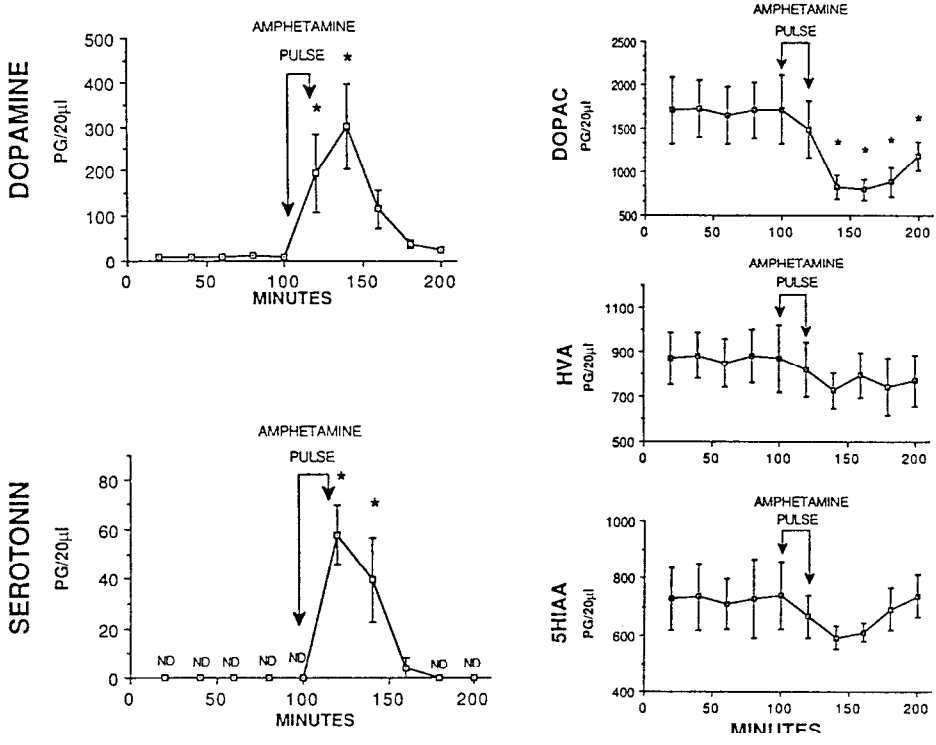
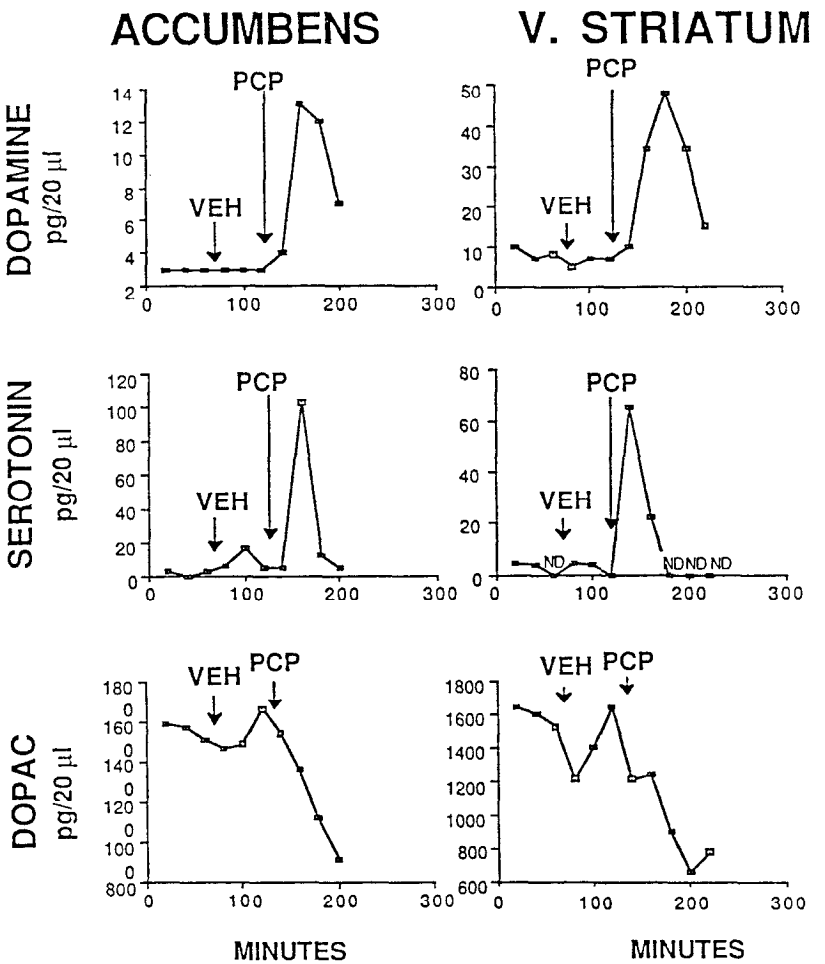


FIGURE 4. *Amphetamine (AMPH) infused through the nucleus accumbens (NAC) microdialysis probe for 20 min, caused both extracellular dopamine and serotonin to increase, and dihydroxyphenylacetic acid to decrease 1 µL per minute perfusate flowrate; 3.37mM $CaCl_2$ Ringer; 2 µg/µL AMPH in 20 µL of Ringer perfusate. Assuming 10 percent outward diffusion comparable to 10 percent monoamine relative recovery, then 4 µg AMPH reached the NAC during 20 min. (From Herncndez et al. 1987.)*



In rats with two microdialysis probes, vehicle (VEH) and phencyclidine (PCP) were injected into the nucleus accumbens and striatum by temporarily removing the probes. Dopamine and serotonin recovery increased; dihydroxyphenylacetic acid decreased (3.37 *mM* $CaCl_2$ *Ringer, 1 µL per minute perfusate flow rate, PCP 20 µg in 0.5 µL local injection). Postmortem tissue assays failed to show the increased turnover that is clearly revealed by microdialysis. (From Hernandez et* al. 1988*a.)*

et al. 1990). Lidocaine released no detectable DA at all, thereby controlling for local anesthesia (figure 6).

Microdialysis can be used to measure extracellular DA during ongoing self-administration (Hurd et al. 1989; Pettit et al. 1989). Animals self-injecting cocaine intravenously maintained a level of extracellular DA that varied with dose, prior experience, and the microdialysis site within the NAC (data presented at Society for Neuroscience satellite, *In vivo* Neurochemistry and Addiction, 1989: Hurd, Ungerstedt, Weiss and Koob; Pettit, Neill and Justice).

Why are drugs of abuse addicting? Solomon (1977) hypothesized an opponent process in which euphoric effects engender longer lasting dysphoric effects, thereby creating a need for more of the euphorigenic drug. Koob and Bloom (1988) have suggested that opiate euphoria and dysphoria are both dependent on the NAC. Wise and Bozarth (1987) have proposed that the mesolimbic system mediates euphoria and the periaqueductal gray region is important for the physical symptoms of opiate withdrawal. We are exploring the possibility that during withdrawal, release of DA decreases and ACh increases in the NAC (Rada et al. 1991 a and 1991*b;* Pothos et al. 1991). In such ways, microdialysis can be used to search for opponent processes.

THE UNKNOWN IMPORTANCE OF SEROTONIN

We found that local AMPH can increase extracellular serotonin in the NAC (Hernandez et al. 1987). The same was true of cocaine (Hernandez and Hoebel 1988*b)* and PCP (Hernandez et al. 1988*a,* 1988*b),* showing that these are all serotonergic drugs at high, local concentrations. AMPH given systemically also increases extracellular serotonin (Kuczenski and Segal 1989). As a general rule, the firing rate of serotonin cells is proportional to arousal level (Jacobs et al. 1984), although local presynaptic control of release and local interactions with other neurotransmitters must be crucial to local serotonergic function. On the basis of serotonin-depletion experiments, Dworkin et al. (1988) suggested that serotonergic innervation of the NAC is involved in the reinforcing effects of morphine. Perhaps serotonin also plays a role in psychostimulant reinforcement, but the nature of the serotonin-DA interaction is not clear.

PRESYNAPTIC CONTROL OF DA RELEASE

Nicotine is not in the same class with the other psychostimulants. It is a cholinergic drug, and yet it too is a drug of abuse. The reason is clear from

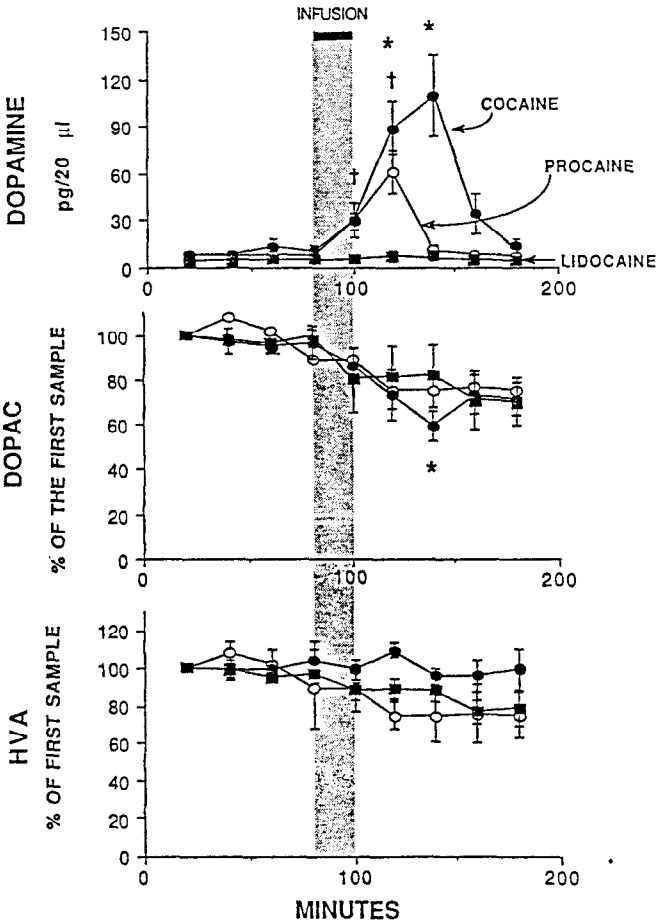


FIGURE 6. *Dopaminergic effect of equimolar (7.3 mM) cocaine, procaine, and lidocaine infused locally in the nucleus accumbens (NAC) for 20 min. In vitro measurement of drug efflux by capillary electrophoresis assay showed approximately 28 percent relative diffusion (outside the probe relative to inside), which suggests about 40 nmol of drug reached NAC tissue. Local cocaine increased extracellular dopamine; procaine was about half as effective; lidocaine had no effect. (From Hernandez et al. 1991.)*

microdialysis. Nicotine given systemically increased extracellular DA in the NAC and STR (Di Chiara and Imperato 1988). To determine whether it acts in the NAC, we infused nicotine locally. It released DA at the microdialysis site, and the effect could be blocked by systemic administration of a nicotinic-receptor antagonist, mecamylamine (Mifsud et al. 1989) (figure 7). This finding suggests that nicotine can act directly in the NAC to release DA by way of nicotinic receptors on the DA terminals or somewhere in the immediate region.

What is the role of peptides in local control of DA release? In our pilot tests, local cholecystokinin injected in the posterior NAC gave negative results (Schwartz et al. 1988). Ruggeri and colleagues (1987), however, observed an increase in DA when cholcystokinin was given in the anterior NAC or with neurotensin in the posterior NAC of anesthetized rats. Route of administration, doses, anesthesia, and microdialysis site within the NAC are all crucial

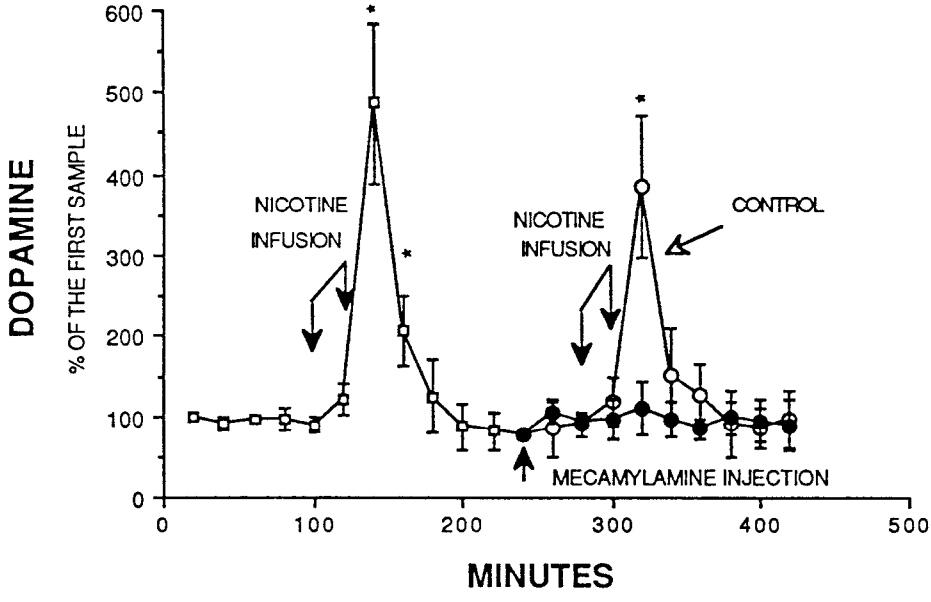


FIGURE 7. *Nicotine infused into the nucleus accumbens via the microdialysis Ringer, caused release of dopamine from nearby terminals (first peak and second peak). Mecamylamine given intraperitoneally to block nicotinic receptors prevented the DA release. (From Mifsud et al. 1989.)*

variables in such experiments (De Witte et al. 1987; Wang and Schoenfeld 1988). Bombesin given locally gave positive results; it increased extracellular DA (Merali 1989). The result of administration of bombesin is exciting because the administration was local, the dose was low, and the rats were awake. This suggests that bombesin may exert presynaptic control of DA release along with acetylcholine.

We need to understand both presynaptic control of release and postsynaptic synergy of multiple neurotransmitters. DA cell bodies fire at a fairly constant rate (Steinfels et al. 1982), and DA depletion or blockade causes rather nonspecific impairments in locomotor and reinforcement functions. Presynaptic control of DA release, however, by acetylcholine or bombesin might endow the local system with circuit specificity and, thereby, behavioral specificity. Postsynaptic synergy between DA and other transmitters could also help create a behavior-specific code.

MICRODIALYSIS AND DRUG DISCRIMINATION

In a drug discrimination study that involved training rats at 80 percent body weight, we noted that high doses of phenylpropanolamine (PPA), i.e., *d,l-norephedrine,* generalized to AMPH, which was the training drug (Lee et al. 1989). This finding, in turn, suggested that PPA might have some effect like AMPH in the NAC. This was also suggested by the finding that rats trained on systemic AMPH generalized to the cue properties of AMPH injected locally in the NAC (Nielson and Scheel-Kruger 1986). Rats apparently sense the effects of AMPH in the NAC and interpret these sensations as they do those of systemic treatment. PPA and AMPH shared some stimulus property after the animals received extensive AMPH training.

When we used microdialysis to compare the dopaminergic effects of PPA and AMPH, the two drugs bore a resemblance in that both increased extracellular DA, although PPA was about 20 times weaker. Therefore, the effects of DA in the NAC could be part of what the rats feel. In another way, however, the two drugs were different. With PPA, the metabolites DOPAC and HVA increased, as happens after feeding, whereas AMPH decreased these metabolites. Therefore, PPA might be nonaddicting in part because the released DA is metabolized relatively rapidly (Hoebel and Hernandez, 1990). Both drugs had insignificant effects in a group of naive rats at 80 percent body weight; however, the extensive AMPH training used in drug discrimination has never been duplicated in a microdialysis experiment.

DISTINGUISHING A DRUG'S ANORECTIC ACTION FROM ABUSE POTENTIAL

Both PPA and AMPH presumably increase monoamines in various parts of the hypothalamus as well as the NAC. In the medial and paraventricular hypothalamus, norepinephrine induces feeding at night and serotonin inhibits it. Thus fenfluramine and fluoxetine have a serotonergic anorectic action in the medial regions (Weiss et al. 1986). In the lateral hypothalamic area, norepinephrine and DA can inhibit feeding (Leibowitz 1980; Leibowitz and Stanley 1986); AMPH has been shown to cause anorexia when injected there (Leibowitz 1982; Wellman and Cockroft 1990). We find that local AMPH can release DA, norepinephrine, and serotonin in the PFH region (Parada et al. 19886). Therefore, we can hypothesize that systemic AMPH acts, in part, in the PFH to inhibit feeding. At the same time, these drugs mimic food by increasing extracellular DA in the NAC. Certain doses of AMPH given locally in the NAC can decrease food and water intake, perhaps by increasing secondary or distracting reinforcers in the environment (Carr and White 1986). We have come to suspect that all drugs that increase synaptic DA and decrease its metabolites are prime candidates for both NAC reward and PFH anorexia. This view is supported by observations that DA-receptor blockers at moderate doses can blunt reward (Fibiger and Phillips 1986; Wise and Bozarth 1987) and cause overeating (Parada et al. 1988*a).*

Some drugs cause anorexia without addiction. Besides PPA, the classic examples are serotonergic drugs. Microdialysis shows that d-fenfluramine increases extracellular serotonin in both the medial and lateral hypothalamus when given systemically (Schwartz et al. 1989*a,* 1989*c).*

Microdialysis also showed us that a meal or the anticipation of one releases serotonin naturally in these regions (Schwartz et al. 1989*b,* 1989*c).* In the medial nuclei serotonin clearly inhibits feeding (Weiss et al. 1986), but we have only a hint of what it does in the PFH. It may inhibit reward because intraperitoneal d-fenfluramine increases synaptic serotonin (Schwartz et al. 1989*a)* and decreases the rate of self-stimulation (McClelland et al. 1989). Apparently, serotonin acts somewhere to inhibit aspects of a reward system for feeding. This does not necessarily mean that serotonin will inhibit rewards of drug self-administration, because the addictive drugs may act "downstream," for example, by releasing DA in the NAC.

In summary, according to present evidence, if a drug increases extracellular DA in the NAC, it probably will be a drug of abuse. If it does so in the PFH, it will also be an anorectic (for example, AMPH), but if it acts primarily to increase extracellular 5-HT, it is likely to be an anorectic without abuse potential. AMPH releases all three—DA, norepinephrine, and serotonin-and interferes with their metabolism. This helps to explain why (1) AMPH in the PVN/medial hypothalamus induces noradrenergic feeding (Leibowitz and Stanley 1986); (2) AMPH in the PFH induces dopaminergic and adrenergic satiety (Leibowitz 1982); (3) AMPH given intraperitoneally after norepinephrine depletion loses its catecholamine satiety effects, but fenfluramine gains serotonergic satiety (Ahlskog et al. 1984; Hoebel et al. 1989); (4) AMPH in the NAC supports local self-injection (Hoebel et al. 1983) and enhances self-stimulation (Broekkamp et al. 1975), and (5) systemic AMPH can both facilitate and inhibit stimulation-induced feeding depending on the dose (Colle and Wise 1988). Extra-cellular DA can be conditioned to increase or decrease (Mark et al. 1991), and it decreases during opiate withdrawal (Pothos et al. 1991). Also in the NAC, acetylcholine release apparently increases during withdrawal (Rada et al. 1991 *b),* and its relation to DA and reinforcement is under study as a potential new approach to addiction.

EFFECTS OF BODY WEIGHT ON DA AND DRUG SELF-ADMINISTRATION

Body weight loss augments self-administration (Carroll 1985; Carroll et al. 1979; Carroll and Meisch 1980; Meisch 1987; Papasava and Singer 1985). Having found that PPA can generalize to AMPH in underweight rats and that both drugs have dopaminergic effects in the NAC, we next injected PPA (20 mg/kg) and AMPH (1 mg/kg) during a dialysis session in underweight rats. The result was puzzling. First, basal DA levels in the underweight rats seemed to be lower than in the control group. Second, systemic PPA and AMPH injections had no significant effect in these underweight animals (Hoebel and Hernandez, 1990). To determine whether the low levels of NAC DA were real, the experiment was repeated using rats as their own controls. Extracellular DA again decreased when male rats were diet restricted for a week to 80 percent of their starting weight. Subsequent ad libitum refeeding for a week failed to reverse the effect (figure 8).

Why do starving animals want more of the addictive drugs? Carroll et al. (1979) suggest that weight loss has a cueing effect that enhances drug intake. Perhaps animals find dopaminergic drugs more rewarding under these circumstances. A number of neurochemical possibilities exist; for example,

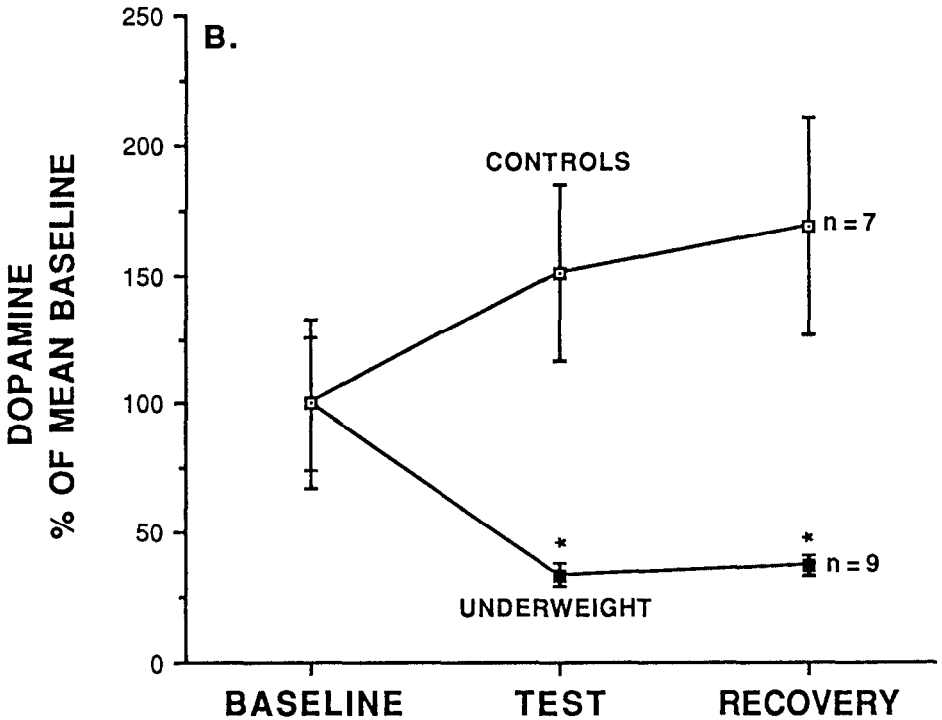


FIGURE 8. *Decrease of 40 to 50 percent in extracellular dopamine in rats deprived of food to 80 percent of normal body weight. This treatment is known to increase drug intake (see text). (From Pothos et al. 1989.)*

(1) self-administration might increase in underweight rats to compensate for decreased extracellular DA release in the NAC; (2) self-administration might increase because of receptor supersensitivity when extracellular DA levels are low; or (3) there may be an augmented reward effect in some other area such as the PFC. More research is needed before we can understand the interaction of body weight and drug taking. All we can add now is that low body weight seems to lower extracellular DA in the NAC.

MICRODIALYSIS AND ANTIPSYCHOTIC DRUGS

Abusers of AMPH, cocaine, or PCP can suffer from drug-induced psychosis. Therefore, a clue to the neural basis of drug abuse and psychosis can be obtained from drugs used to treat schizophrenia.

Antipsychotic drugs (neuroleptics) are DA-receptor blockers (Carlsson 1988). HAL is the classic example. It has some antipsychotic effect immediately, especially at high doses. Lower doses are used in nonemergencies and then continued for weeks to gain improvement in symptoms. Microdialysis confirms earlier work showing that an immediate effect of DA receptor block by HAL or clozapine (CLOZ) is a compensatory release of DA (Hernandez et al. 1990; Hernandez and Hoebel 1989*b;* O'Connor et al. 1989; Zhang et al. 1989). This extra DA would mitigate the receptor blocking effect. When HAL is used to treat the psychosis produced by psychostimulants, the situation is further complicated by drug-induced DA release, which necessitates high doses of HAL to successfully compete for the DA receptors.

It is believed that HAL given chronically to humans gives therapeutic results by altering DA circuits in some way. The problem is to determine what changes take place after chronic treatment. Therefore, rats were prepared with microdialysis probes in three DA terminal regions: PFC, NAC, and STR. Rats were given HAL for a month and DA was measured at the beginning and end of the period. The result at the end of the treatment was a selective decrease in basal DA in the PFC (figure 9). This finding may help explain the improvement in cognitive functioning that is gained with long-term treatment in psychotic patients, because it suggests that endogenous psychosis involves an excess of PFC DA. It follows that stimulant-induced psychosis with AMPH, cocaine, or PCP probably also involves DA excess in the PFC. As a chronic treatment, HAL can have disastrous motor side effects in some patients, presumably due to actions in the nigrostriatal system and its associated pathways (Ichikawa and Meltzer 1990). Atypical neuroleptics such as CLOZ, however, offer some hope. New triple microdialysis studies suggest that CLOZ has effects on DA level and turnover in the PFC and not in the STR (Hernandez et al. 1990; Hernandez and Hoebel 1989*b).*

CONCLUSION

In summary, our studies illustrate the use of microdialysis in freely moving rats and show that psychostimulants can increase extracellular DA by a local action

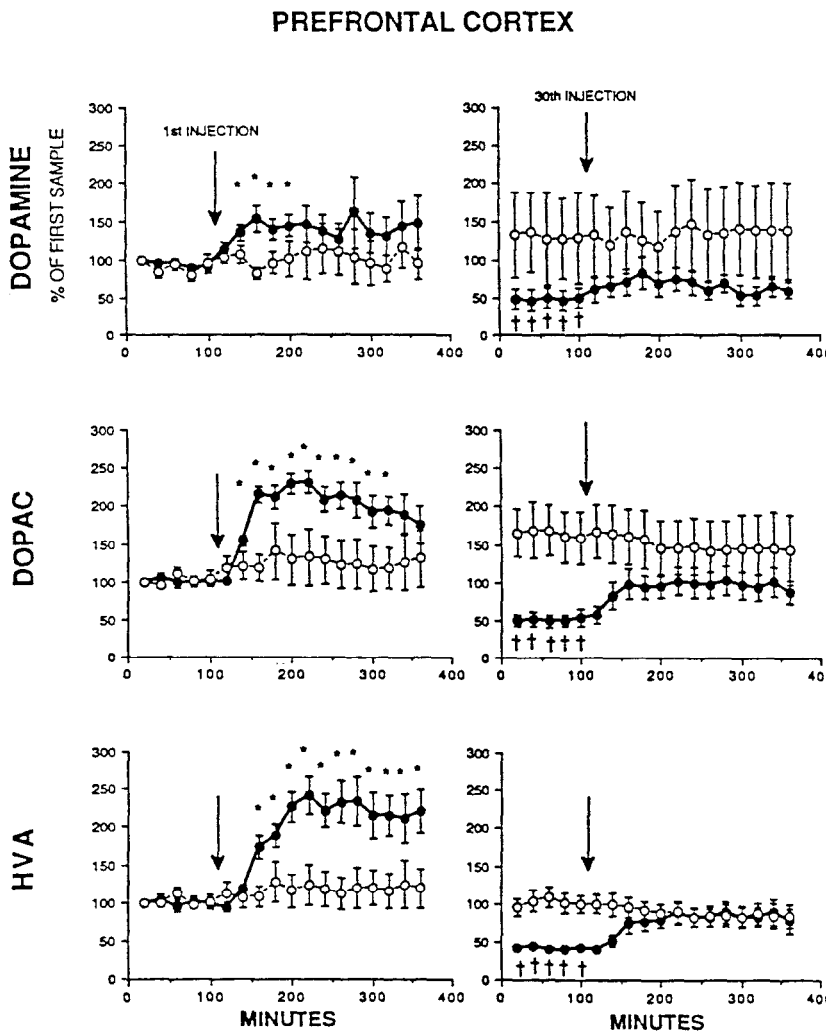


FIGURE 9. *Acute haloperidol (HAL) (1st injection) increasing extracellular dopamine (DA) in the prefrontal cortex (upper left asterisks). After a month of daily HAL injections, the basal DA level is significant/y decreased (upper right graph, daggers), and the 39th injection* was *relatively ineffective, showing decreased DA turnover. HAL had less effect in the nucleus accumbens and striatum of the same rats, (From Hernandez and Hoebel 1989*b, 1990.)

in the NAC. Also, electrical stimulation of the PFH feeding-reward system increases extracellular DA in the NAC. Natural appetitive behavior patterns such as feeding and drinking do the same thing. The release of DA by a sweet taste can be counterconditioned by pairing the taste with malaise. When the taste becomes a conditioned signal for toxicity, it causes DA release to decrease instead of increase. Drugs of abuse that release DA apparently mimic natural rewarding stimuli. Psychostimulants such as AMPH, cocaine, and PCP increase extracellular serotonin as well as DA, but serotonin's role in the NAC is not clear. In feeding-control centers such as the hypothalamus, serotonin probably suppresses appetite and self-stimulation. This serotonergic effect, along with a dopaminergic satiety effect partially explains the anorectic effects of psychostimulants. Drugs that selectively increase synaptic serotonin are anorectic and not abused. PPA, which is an over-the-counter anorectic, was interesting in that it increased extracellular DA in the NAC and also DA metabolites, showing a marked departure from the dopaminergic drugs of abuse that decreased DOPAC and HVA. Nicotine was also a special case, because it acted via nicotinic cholinergic receptors to release DA presynaptically without measurably altering DA metabolism. The drugs like cocaine that interfere with the formation of DOPAC and HVA seem to be those with a high potential for causing psychosis. Microdialysis after chronic antipsychotic drug treatment showed that basal DA was selectively lowered in the PFC. Therefore, we suspect that psychosis is partly a PFC abnormality, and DA antagonists such as CLOZ that act with some selectivity in the PFC might be effective in cases of drug-induced psychosis. It is known from a variety of self-injection studies that dopaminergic drugs are positive reinforcers in both the NAC and the PFC, so it is hard to say which DA projection system will prove to be more important in addiction therapy. The observation that DA release can be controlled presynaptically by neurotransmitters such as acetylcholine and bombesin offers new avenues to addiction treatment. Clearly, microdialysis will be a valuable tool in the evaluation of these treatments in the experimental stages.

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Neurophysiological Approaches to Receptor Pharmacodynamics

Francis J. White

INTRODUCTION

A variety of specific neurophysiological techniques have been used with in vivo and in vitro preparations to further our understanding of how drugs affect neurotransmission at both the molecular (ion channel gating, conductance changes) and cellular (single-cell firing) levels; how such actions are related to changes at a systems level (alterations in activity of identified neuronal populations); and how these changes are ultimately translated into behavioral output. Although it is clear that the most direct means of correlating changes in neuronal activity with specific behaviors would involve the use of single-cell recordings in freely moving animals (see “Psychomotor Stimulant Effects on Single Neurons in Awake, Behaving Rats”), such techniques greatly limit the extent to which pharmacologic manipulation is possible. From the perspective of receptor pharmacodynamics, the use of in vivo anesthetized-animal preparations and in vitro slice or cell preparations allows considerably better definition of relevant drug effects and underlying mechanisms at the single-cell level.

As an example of the tremendous potential of single-cell recording procedures in neurobehavioral research, one need look no further than the pioneering studies of Aghajanian and colleagues (Aghajanian 1978; Andrade and Aghajanian 1985). The work of this group typifies the use of single-cell recording techniques as a means of studying receptor pharmacodynamics and the extension of such findings to clinical investigation. For example, with the ability to identify defined populations of monoamine-containing neurons, Aghajanian and colleagues identified similar inhibitory effects of opioids and alpha-2 selective agonists on norepinephrine-containing neurons within the rat locus coeruleus (Aghajanian 1978). In addition, they were able to demonstrate that these drugs worked via separate receptor populations to alter a common

transduction mechanism, i.e., inhibition of adenylate cyclase (Andrade and Aghajanian 1985). This knowledge and the finding that cyclic adenosine monophosphate (cAMP) formation is greatly exaggerated during opiate withdrawal provided clinical investigators with a theoretical framework on which to base the use of clonidine for opiate withdrawal (Gold et al. 1978).

This chapter reviews recent electrophysiological findings from my laboratory that have identified behaviorally relevant interactions between D_1 and D_2 dopamine (DA) receptors within the mesoaccumbens and nigrostriatal DA systems. I hope to demonstrate that although the physiological and behavioral results were obtained from separate groups of rats, the close parallels between the findings make it possible to reach certain important conclusions regarding the cellular sites and mechanisms of action of dopaminergic drugs as they relate to specific components of behavior and as they may affect clinical aspects of dopaminergic transmission.

MULTIPLE DA RECEPTORS: FUNCTIONAL INTERACTIONS

Although D_1 and D_2 DA receptors have historically been defined on the basis of their (often opposing) effects on cAMP production (Kebabian and Calne 1979; Stoof and Kebabian 1981) and have been shown to exert apparently opposing effects on a variety of other biochemical measures, several behavioral and electrophysiological studies have indicated that these receptor subtypes can also interact synergistically to control DA-mediated functions (Clark and White 1987; Waddington and O'Boyle 1987). The first evidence of an apparent synergistic interaction between D_1 and D_2 receptors was provided by Gershanik and colleagues (1983), who demonstrated that the presence of both the D_1 selective agonist SKF 38393 and the D_2 selective agonist LY 141865 was required to reverse the akinesia produced by administering reserpine to mice.

We provided the initial electrophysiological evidence for synergistic interactions between D_1 and D_2 DA receptors. In these studies (White 1986; White and Wang 1986), we tested the effects of iontophoretic administration of D_1 and D_2 agonists on neurons within the rat nucleus accumbens (NAC), the terminal area for the majority of DA neurons within the ventral tegmental area (A10 DA cells). Both spontaneously active and quiescent NAC neurons, which were made to fire by the iontophoretic administration of glutamate, were inhibited during iontophoretic administration of either SKF 38393 or LY 141865. In addition, receptor selectivity was apparent, because the D_2 antagonist sulpiride blocked only the effects of LY 141865, whereas the D_1 antagonist SCH 23390

completely blocked the effects of SKF 38393 while partially attenuating the effects of LY 141865.

Not only were we able to identify both D_1 and D_2 receptive NAC neurons, but we also determined that a subpopulation of neurons was inhibited by both agonists. More important, on those cells that were responsive to both D_1 and D_2 receptor agonists, coadministration of SKF 38393 and LY 141865 produced a supra-additive or synergistic inhibition. These findings indicated that certain NAC neurons may possess both D_1 and D_2 receptors and that these receptor subtypes can interact in a synergistic manner to regulate NAC neuronal activity (White and Wang 1986).

Accounts of several behavioral results were subsequently published, furthering the notion of synergistic D_1-D_2 receptor interactions and providing hints as to how such synergism might occur. For example, Braun and Chase (1986) reported that in rats acutely depleted of DA with the tyrosine hydroxylase inhibitor alphamethylparatyrosine (AMPT), the production of various stereotyped behaviors (licking, gnawing) required the combined administration of D_1 and D_2 selective agonists. Subsequently, this group demonstrated that SKF 38393 potentiated circling behavior induced by the D_2 agonist quinpirole (QUIN; the active enantiomer of LY 141865) in rats with excitotoxin-induced lesions of the striatum, a rotational model in which DA receptors remain normosensitive. Moreover, when AMPT was used to deplete DA in these rats, the QUIN response was markedly reduced unless the D_1 agonist was also injected (Barone et al. 1986). At nearly the same time, Jackson and Hashizume (1986) reported that the locomotor-stimulant effect of the D_2 agonist bromocriptine was abolished by acute DA depletion, but reinstated by SKF 38393.

Taken together, these various behavioral studies suggested that D_1 receptor stimulation may be required for D_2 agonist-induced responses. Thus, when DA was acutely depleted, the effects of D_2 agonists were abolished unless a D_1 agonist was also administered, suggesting that the critical event missing in the depleted animal was D_1 receptor stimulation by endogenous DA. Several previous studies had reported the apparently paradoxical finding that the D_1 antagonist SCH 23390 blocked the ability of D_2 agonists to induce locomotion and stereotypy without directly blocking the D_2 receptor (Clark and White 1987). In hindsight, this earlier finding could also be interpreted as indicating a necessary role for D_1 receptor stimulation in these particular D_2 agonist-induced behavioral responses.

THE ENABLING ROLE OF D_1 RECEPTORS: ELECTROPHYSIOLOGICAL STUDIES

In view of these various behavioral findings indicating that D_1 receptor stimulation was necessary for (or enabled) certain behavioral effects of D_2 selective agonists, we continued our electrophysiological studies by determining whether the synergistic interactions we had previously observed between D_1 and D_2 receptors within the NAC might also represent an enabling effect of D_1 receptors. We first observed that SKF 38393 potentiated the inhibitory effects of quinpirole even on neurons not inhibited by the D_1 agonist alone (White 1986). We then studied the interaction of the two receptors in the absence of receptor occupation by endogenous DA. When we acutely depleted DA levels (by 80 percent) within the NAC by pretreating with AMPT (White et al. 1988), the normal inhibitory effects of QUIN on NAC cells were significantly attenuated (White 1986, 1987). Importantly, SKF 38393 administration (at low ejection currents, which alone produced little inhibition) “reinstated” the inhibitory response to QUIN (figure 1). Thus, activation of D_1 receptors by SKF 38393 in rats acutely depleted of DA enabled D_2 receptor occupation by QUIN to produce an inhibition of neuronal activity. This finding clearly suggested that endogenous DA may act at D_1 receptors to enable D_s-mediated events in the intact rat (White 1986, 1987).

We have now confirmed and extended these original observations (Wachtel et al. 1989). First, we demonstrated that D_1 receptor activation is required for the inhibitory effects of other selective D_2 DA receptor agonists, such as RU 24213 (Wachtel et al. 1989) and B-HT 920 (Johansen et al. 1991), on NAC neurons. We also found that iontophoretic administration of other D_1 agonists, SKF 75670 and SKF 81297 (Andersen et al. 1987; Arnt et al. 1988), also enabled the inhibitory effects of QUIN on NAC neurons in AMPT-pretreated rats (Johansen et al. 1991). Thus, the requirement of D_1 receptor activation for D_2 receptor-mediated inhibition of NAC neurons is not specific to the particular agonists employed but is a general phenomenon of D_1-D_2 receptor interaction.

Second, we reported that the inhibitory effects of the mixed D_1-D_2 receptor-agonist apomorphine (APO), which possesses nearly equivalent affinity for the two receptors (Arnt and Hyttel 1985), were still evident in AMPT-pretreated rats (figure 2). Thus, acute DA depletion failed to attenuate the effect of a DA agonist that activates both D_1 and D_2 receptors (Wachtel et al. 1989).

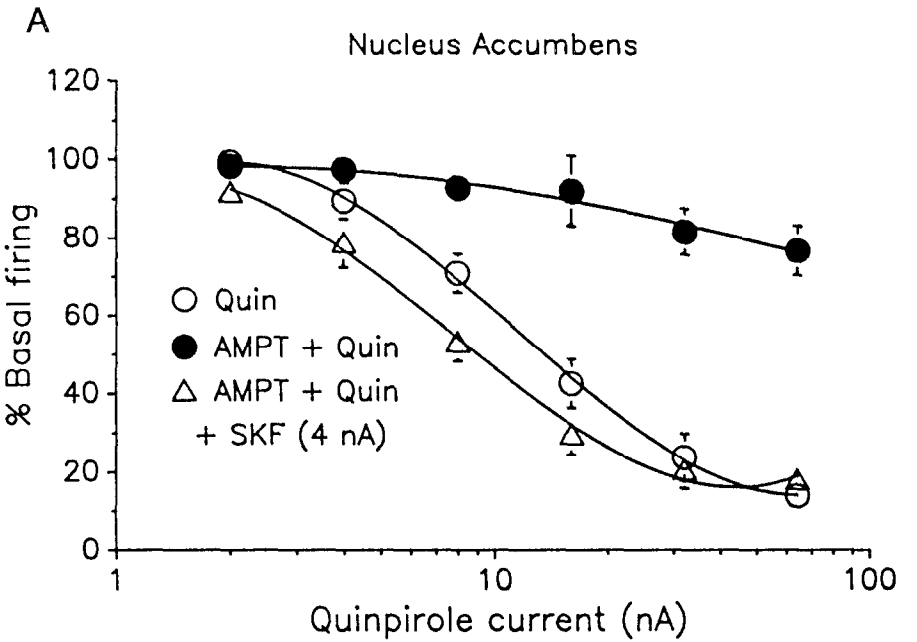

FIGURE 1. *Inhibition of the firing of nucleus accumbens neurons by iontophoretic administration of the D_2 agonist quinpirole (QUIN). Current-response curves illustrate the attenuation of the inhibitory effect of QUIN by pretreatment with alphamethylparatyrosine (AMPT and the reinstatement of the QUIN-induced inhibition on the same neurons with coiontophoresis of SKF 38393 (4 nA). Each point represents the mean±SE_M (QUIN control,* n = 11; *AMPT,* n = *12, AMPT + SKF 38393,* n = *11). (From Wachtel et* al. *1989.)*

Next, we extended our findings by showing that D_1 receptor stimulation also enables the inhibitory effects of D_2 receptors within the lateral caudate-putamen. AMPT pretreatment nearly abolished the inhibitory effects of QUIN and RU 24213 (figure 3), but not of APO, on caudate-putamen neurons, effects that were readily reversed by coiontophoretic administration of SKF 38393 (Wachtel et al. 1989). Therefore, the enabling role of D_1 receptor stimulation for D_2 receptor-mediated inhibition of neuronal activity appears to exist throughout both the dorsal and ventral striata, although apparently not within the rat medial prefrontal cortex (Sesack and Bunney 1989).

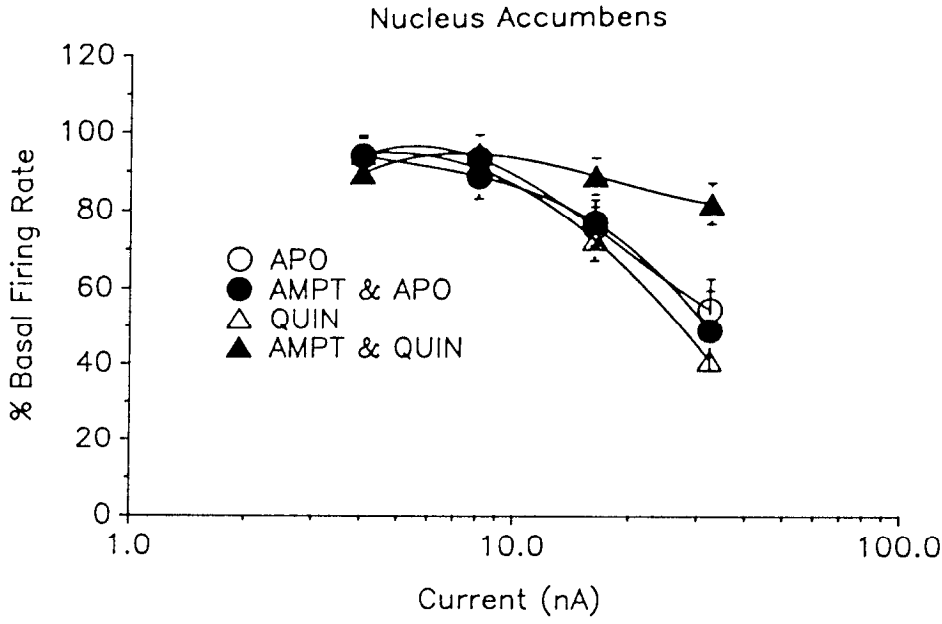

FIGURE 2. *Inhibition of nucleus accumbens neurons by iontophoretically administered apomorphine (APO) and quinpirole (QUIN). Current-response curves illustrate the inability of pretreatment with alphamethylparatyrosine (AMPT) to alter the inhibitory effect of APO, whereas the effect of QUIN was attenuated. Each point represents the mean±SEM(APO control,* n = *10; AMPT + APO,* n = *15; QUIN control,* n = *9; AMPT + QUIN,* n = *8). (From Wachtel et al. 1989.)*

Paralleling our results from studies of the striatal complex are the findings of Walters and colleagues (1987), who demonstrated D_1-D_2 interactions with rat globus pallidus neurons after intravenous (IV) injections of selective D_1- and D_2-receptor agonists (Carlson et al. 1987*b).* Acute depletion of DA with AMPT was found to attenuate the partial (as compared with APO) excitatory effects of IV QUIN but not the marked excitatory effects of APO. Subsequent administration of SKF 38393 to AMPT-pretreated rats that had received QUIN produced marked increases in pallidal firing equivalent to those produced by IV APO or by the combination of SKF 38393 and QUIN in normal rats (Walters et al. 1987). Recently, this group has also demonstrated that the excitatory effects of IV APO on pallidal neurons are attenuated by ipsilateral quinolinic acid lesions of the rostral striatum (Pan et al. 1987; Pan and Walters 1988),

suggesting that the excitatory effects of APO on pallidal neurons result from disinhibition subsequent to inhibition of striatopallidal caudate-putamen neurons, which are thought to be mostly GABA-ergic in content (Fonnum et al. 1978).

Our findings obtained during recordings of caudate-putamen neurons suggest that the permissive role of D_1 receptor stimulation for D_2 agonist-induced excitation observed on globus pallidus neurons (Walters et al. 1987) may be the result of direct enabling actions in the caudate-putamen occurring on

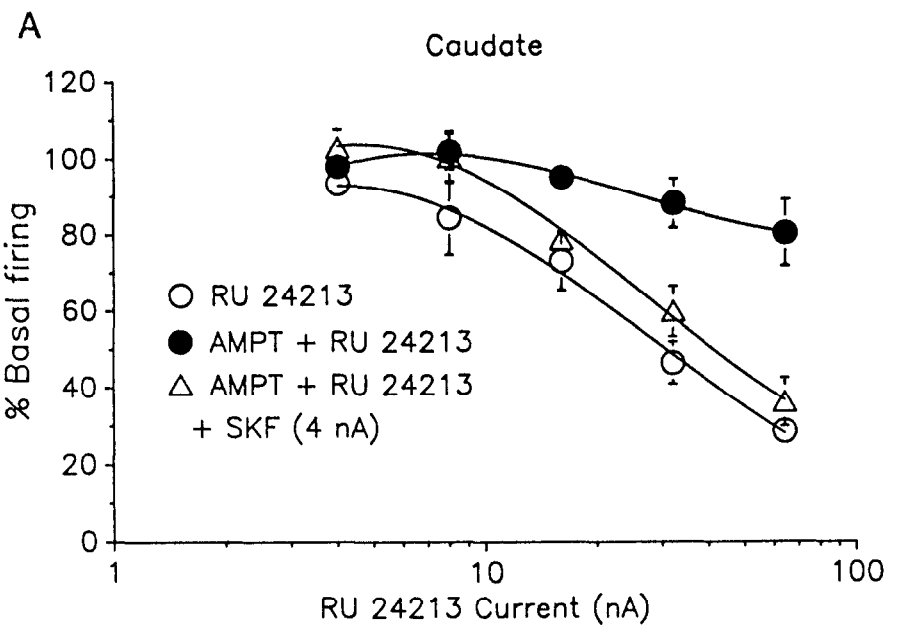

FIGURE 3. *Inhibition of caudate-putamen neurons by iontophoretic administration of RU 242 13. Current-response curves illustrate the attenuation of the RU 24213-induced inhibitory effect on caudate-putamen neurons in rats pretreated with alphamethylparatyrosine (AMPT) and the ability of SKF 38393 (4 nA) to restore the inhibition produced by RU 24213. The attenuation of the RU 24213 effect by AMPT pretreatment is greater in the caudate-putamen than in the NAC (fig. 2). Points represent the mean±SE_M* (n = *10 for each group). (From Wachtel et al. 1989.)*

striatopallidal GABA-ergic neurons. Similarly, Yang and Mogenson (1989) have reported that SKF 38393 enables the ability of QUIN to increase the amount of firing of neurons within the rat ventral pallidum, which receive an inhibitory input from NAC GABA-ergic neurons when the D_1 agonist is infused directly into the NAC before the D_2 agonist.

THE ENABLING ROLE OF D_1 RECEPTORS: BEHAVIORAL STUDIES

In parallel behavioral experiments, we have demonstrated the necessity of combined stimulation of D_1 and D_2 receptors for the full expression of oral stereotyped behaviors (licking and gnawing). As shown in figure 4, combined administration of SKF 38393 and QUIN produced nearly maximal stereotyped gnawing comparable to that observed with the mixed D_1-D_2 agonist APO, whereas QUIN alone, even at extremely high doses (Walters et al. 1987), fails to produce oral stereotypies (figure 4). In addition, we have demonstrated that acute depletion of DA with AMPT completely abolishes the low-level, stereotyped sniffing typically observed in rats following administration of a D_2 selective agonist such as QUIN (White et al. 1988). Administration of SKF 38393 along with QUIN reinstates the behavior and intensifies its quality to oral stereotypies (figure 4). Other investigators have reported similar findings for locomotor activity and oral stereotypy (Arnt et al. 1987; Braun and Chase 1986; Jackson and Hashizume 1986; Longoni et al. 1987*b;* Meller et al. 1988), D_2 agonist-induced yawning (Longoni et al. 1987*a;* Ushijima et al. 1988), and circling in rats with quinolinic acid lesions of the striatum (Barone et al. 1986). As in our electrophysiological experiments, acute DA depletion failed to alter APO-induced stereotyped behavior (figure 4), presumably because APO supplies the necessary D_1 receptor stimulation (White et al. 1988).

D_1-AGONIST-INDUCED FUNCTIONAL EFFECTS DO NOT REQUIRE D_2-RECEPTOR STIMULATION

A remaining unsettled question regarding the interactions between D_1 and D_2 receptors is the extent to which the enabling relationship is reciprocal. Our studies indicate that D_1 receptor-mediated electrophysiological responses do not require D_2 receptor stimulation (Wachtel et al. 1989), because the inhibitory effects of SKF 38393 on both NAC (figure 5) and caudate-putamen cells are unaltered by acute DA depletion. Similarly, the characteristic grooming responses produced by the D_1 agonist SKF 38393 (Molloy and Waddington 1984, 1987*a,* 19876; Starr and Starr 1986*a,* 1986*b)* are still evident in rats acutely depleted of DA (>99.5 percent reductions in striatal DA) by pretreatment

with reserpine plus AMPT (figure 6). Indirect evidence also supports our position, because blockade of D_2 receptors unmasks APO-induced grooming

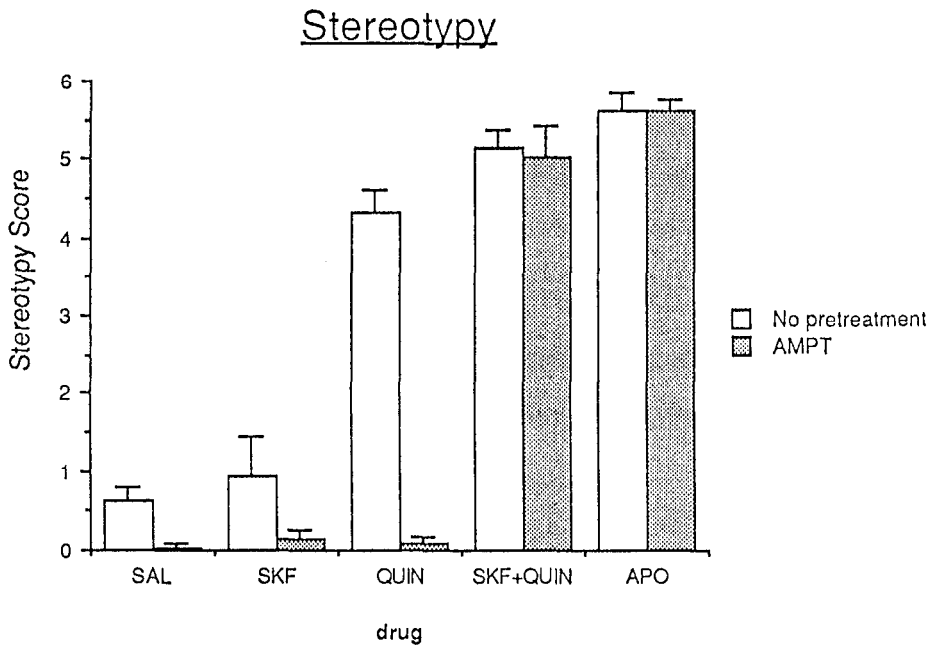

FIGURE 4. *The ability of various drug treatments to induce stereotyped behaviors in normal rats and rats depleted of dopamine by alphamethylparatyrosine (AMPT). Both quinpirole (QUIN) and apomorphine (APO) were administered at a dose of 2.0 mg/kg, whereas SKF 38393* was *administered at 16 mg/kg. Each bar represents the mean±SE_M obtained from eight rats. Stereotypy ratings for QUIN, APO, AMPT + APO, SKF + QUIN, and AMPT + SKF + QUIN were all significantly higher than saline (SAL,* $p < .01$*). The SKF + QUIN treatment was not significantly different from APO, although the APO and QUIN groups were significantly different from one another* ($p < .01$*). The effects of the QUIN + SKF combination were significantly greater than QUIN alone* ($p < .05$*). AMPT pretreatment significantly reduced the effects of QUIN* ($p < .01$*); however, the SKF + QUIN combination reinstated the stereotyped behaviors in the AMPT group. Stereotypy scores were based on a standard 0-6 rating scale. (From White et al. 1988.)*

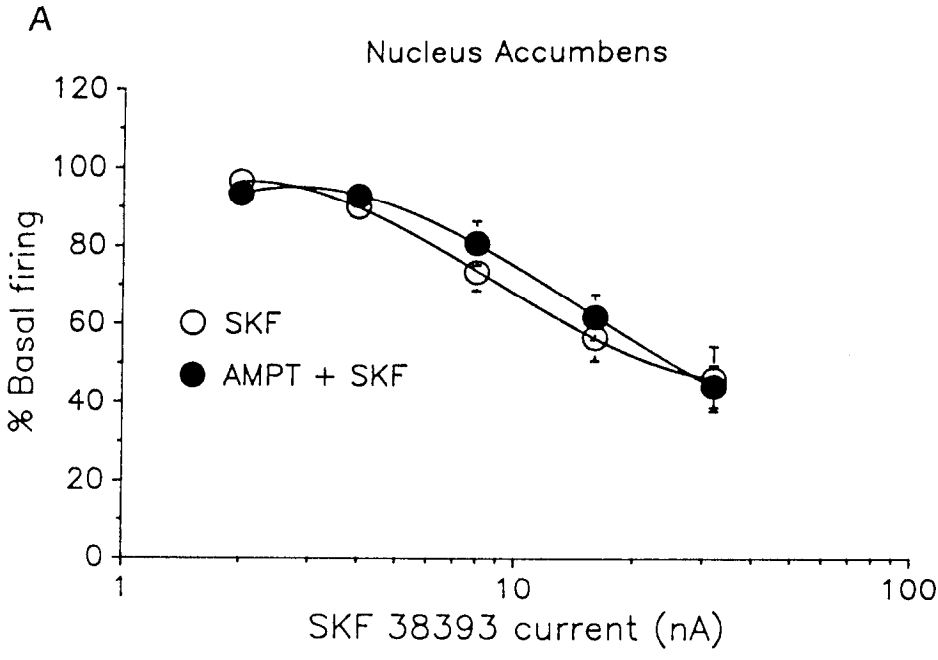

FIGURE 5. *Inhibition of nucleus accumbens (NAC) neurons by iontophoretic administration of the D_1 agonist SKF 38393. Current-response curves illustrating the failure of alphamethylparatyrosine (AMPT) pretreatment to affect the inhibition of NAC neurons by SKF 38393. Each point represents the mean±SE_M (SKF 38393 control,* n = *10; AMPT + SKF 38393,* n = *11). (From Wachtel et al. 1989.)*

responses, suggesting that D_1 stimulation produced grooming in the absence of D_2 tone (Molloy and Waddington 1984; Starr and Starr 1986*a;* Starr et al. 1987). Although some (not all) D_2 selective antagonists have been reported to attenuate SKF 38393-induced grooming (Molloy and Waddington 1987*b), we* believe that such a finding may be due to competing behavioral effects of the antagonist, e.g., catalepsy.

D_2 DA AUTORECEPTORS DO NOT REQUIRE ENABLING

Unlike postsynaptic D_2 DA receptors within the striatal complex, impulse-regulating somatodendritic autoreceptors on midbrain DA neurons are not responsive to D_1 receptor activation. For both A10 (White and Wang 1984) and A9 nigral (Carlson et al. 1987*a)* DA neurons, D_2, but not D_1, agonists inhibit

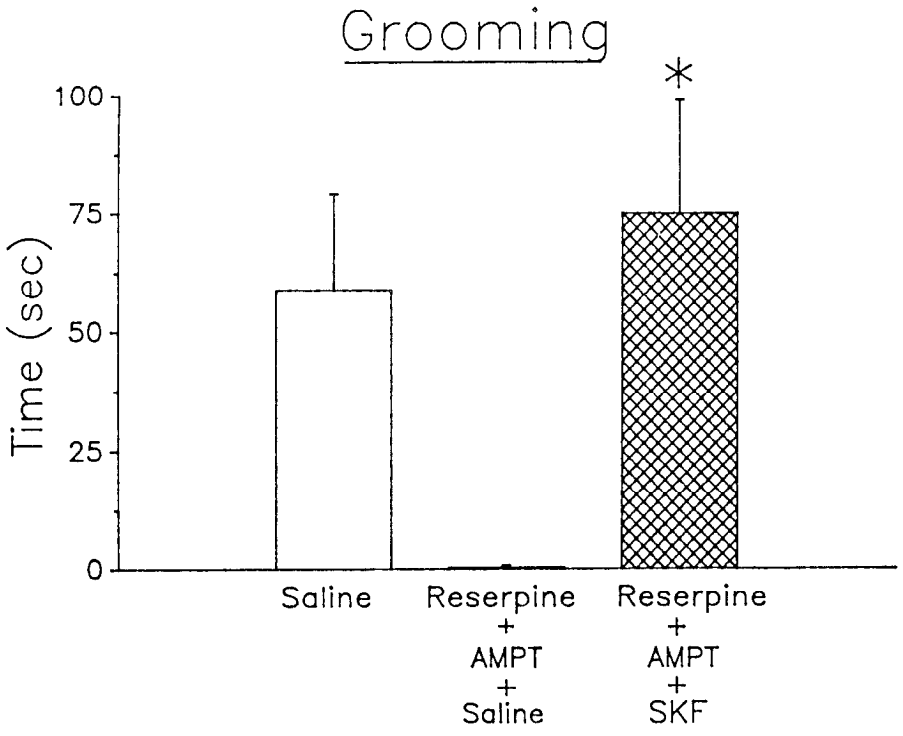

FIGURE 6. *Grooming behavior induced by a 16-mg/kg dose of SKF 38393* (n = 8) versus saline (n = *5) in rats pretreated with a combination of reserpine (5 mg/kg and alphamethylparatyrosine (AMPT, 250 mg/kg) and in nontreated controls* (n = *8). In rats that received reserpine and AMPT, SKF 38393 induced a significant increase in grooming versus saline (p < .001). Data indicate the amount of time the rats engaged in grooming behavior during a 30-min observation period. (From White et al, 1988.)*

neuronal activity. The lack of effect of SKF 39383 is not due to the partial agonist characteristic of this drug because the full D_1 agonist SKF 81297 (Andersen et al. 1987) produced similar results (Wachtel and White, unpublished findings). Given that SKF 38393 can potentiate QUIN-induced inhibition of NAC cells that are not directly inhibited by the D_1 agonist (White 1986; 1987), such "silent" effects of D_1 receptor stimulation might also occur on DA neurons to modulate the effects of D_2 agonists at impulse-regulating autoreceptors. SCH 23390, however, fails to alter the inhibitory effects of D_2 agonists on either A9 or A10 DA cells (Carlson et al. 1986; Wachtel et al. 1989).

In addition, acute DA depletion does not alter the effects of D_2 agonists, because neither IV nor iontophoretic administration of SKF 38393 alters QUIN-induced inhibitions of midbrain DA cells (Wachtel et al. 1989). Thus, it is apparent that impulse-regulating somatodendritic DA autoreceptors are exclusively of the D_2 subtype (White and Wang 1984). Similar manipulations of nerve-terminal, synthesis-modulating receptors also fail to reveal D_1 receptor involvement in (or enabling of) D_2 receptor-mediated inhibition of DA synthesis (Wachtel et al. 1989).

CHRONIC DA DEPLETION RELIEVES D_2 RECEPTORS OF THE NECESSITY FOR D_1 STIMULATION

In contrast to their actions in normal rats, D_1 receptor antagonists fail to block the effects of both mixed D_1-D_2 agonists and selective D_2 agonists in behavioral models using rats with supersensitive DA receptors (Arnt 1985*a,* 1985*b;* Arnt and Hyttel 1985; Breese and Mueller 1985). Whereas both D_1 and D_2 agonists elicit rotation in rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway, these effects are blocked only by antagonists selective for those receptors (Arnt and Hyttel 1984, 1985). Similarly, the hyperactivity produced by D_1 or D_2 agonists in rats with bilateral 6-OHDA lesions (Arnt 1985*b* Breese and Mueller 1985) or in rats treated repeatedly with reserpine (Arnt 1985*a)* is blocked only by the appropriate receptor-selective antagonist. These findings suggest that the necessity of D_1 receptor stimulation for D_2 receptor-mediated functional responses may be relieved after chronic DA depletion.

Despite this apparent lack of interaction between D_1 and D_2 receptors in rats with supersensitive DA receptors, several investigators have reported synergistic rotational responses to D_1 and D_2 DA agonists in rats with unilateral 6-OHDA lesions of the nigrostriatal tract (Koller and Herbster 1988; Robertson and Robertson 1986, 1987; Rouillard and Bedard 1988; Sonsalla et al. 1988). In addition, synergistic interactions between D_1 and D_2 selective agonists have also been reported with respect to the self-mutilating biting observed in rats that had received 6-OHDA as neonates (Breese et al. 1985). Thus, despite the lack of an enabling relationship between D_1 and D_2 receptors, these studies clearly indicate that D_1 and D_2 receptors may still work in concert to regulate behavior in DA-denervated rats.

We have recently investigated these relationships using our single-cell electrophysiological techniques (Hu et al. 1990). In striking contrast to the

effects of acute DA depletion, long-term depletion of DA produced either by repeated reserpine administration or by 6-OHDA lesions of the nigrostriatal DA system did not attenuate the inhibitory effects of iontophoretic QUIN on caudate-putamen neurons. In fact, the inhibitory potency of QUIN was enhanced (supersensitive) compared with that observed in the intact caudate-putamen (figure 7). The inhibitory effects of QUIN in the denervated caudate-putamen could not be attributed to residual DA stimulating supersensitive D_1 receptors (and thereby enabling D_2 receptor-mediated inhibition), because further DA depletion produced by additional acute administration of AMPT failed to reduce the effects of QUIN (figure 7). In addition, the lack of D_1 receptor-mediated enabling in the 6-OHDA rat cannot be attributed to the loss of DA terminals and possible cell-cell D_1-D_2 interaction, because similar findings were observed with repeated reserpine treatment (Hu et al. 1990).

These electrophysiological results, indicating that D_2 receptor-mediated functional effects are relieved from the requirement of D_1 receptor stimulation, provide a single-cell correlate of previous behavioral findings indicating that D_1 receptor stimulation is not required for D_2 agonist-induced behaviors in rats with chronic DA depletion. Taken together, these behavioral and electrophysiological findings indicate that D_1 and D_2 receptors located in the caudate-putamen become functionally uncoupled after long-term depletion of DA. Whether D_1 and/or D_2 receptor supersensitivity or both are required for the uncoupling of these receptor subtypes is a question currently under investigation in our laboratory. It has been reported that selective upregulation of D_1 receptors produced by repeated administration of the D_1 antagonist SCH 23390 potentiates the behavioral effects of D_2 agonists (Hess et al. 1986), suggesting that selective D_1 receptor supersensitivity may cause enhanced enabling rather than functional dissociation of D_1 and D_2 receptors.

CONCLUSIONS

The findings reviewed in this chapter demonstrate a close parallel between the effects of D_1 and D_2 DA receptor agonists at the behavioral and cellular levels. Although the data were not obtained from the same animals, the similarities between the interactions of D_1 and D_2 receptors observed on unconditioned DA-dependent behaviors and those seen on single neurons within the striatal complex (caudate-putamen and NAC) clearly suggest that the behavioral effects may be the reflection of D_1-D_2 receptor interactions occurring at the cellular level within the striatum. Not only have these findings generated

considerable interest in the underlying mechanisms involved in D_1-D_2 receptor interactions at the molecular level, but they also have many important clinical implications.

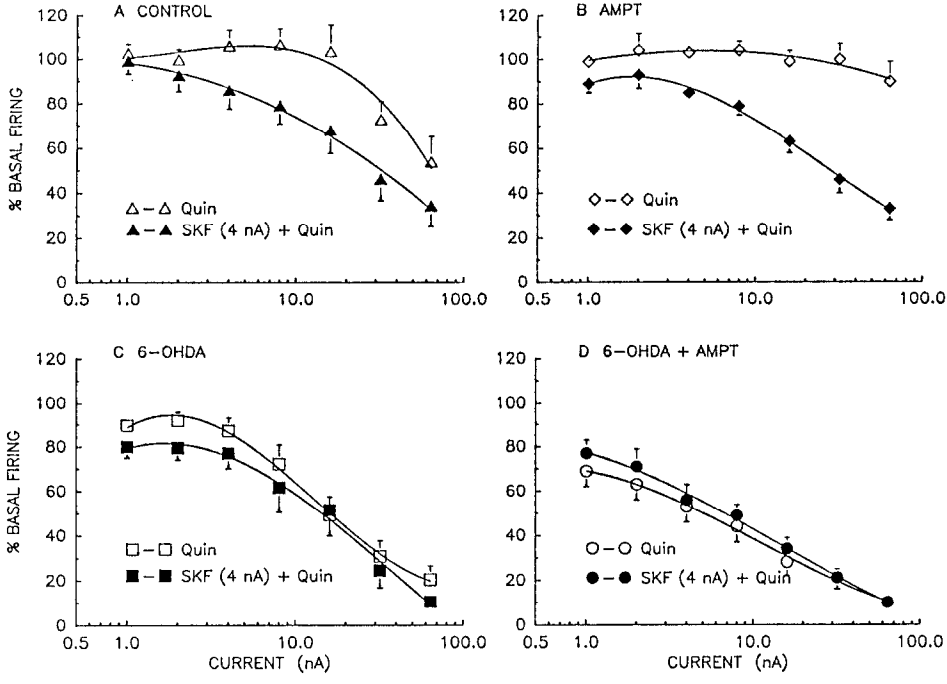

FIGURE 7. *Comparison of responses of caudate-putamen neurons to coiontophoretic administration of D_1 and D_2 selective agonists in control rats* (n = *10 cells) (A); rats acutely depleted of dopamine (DA) with alphamethylparatyrosine (AMPT)* (n = *15 cells) (B); rats chronically depleted of DA with 6-hydroxydopamine (6-OHDA)* (n = *23 cells) (C); and rats that received both 6-OHDA and AMPT* (n = *10 cells) (D). (A) In control rats, low (subinhibitory) currents of SKF-38393 potentiated the inhibitory effects of quinpirole (QUIN) on the same caudate-putamen neurons* ($F[1, 18] = 4.65$, $p < 05$). *(B) In rats acutely depleted of DA by AMPT, WIN-induced inhibition was attenuated without simultaneous stimulation of D_1 receptors* ($F[1, 28] = 47.73$, $p < .0001$). *(C) In rats chronically depleted of DA by 6-OHDA lesions, the requirement of D_1*

One of the most important implications of the enabling relationship between D_1 and D_2 DA receptors is its potential relevance for the treatment of parkinsonism (see Clark and White 1987; Waddington and O'Boyle 1987). With the exception of Ldopa, currently available antiparkinsonian drugs (bromocriptine, pergolide) are selective D_2 agonists. With the discovery that D_1 receptor stimulation is required for certain behavioral effects of D_2 agonists, many investigators suggested that mixed D_1-D_2 agonists or combinations of D_1 and D_2 agonists would be more effective in alleviating parkinsonian symptoms (Clark and White 1987). The more recent finding that D_1 receptor stimulation is no longer necessary for D_2-mediated responses in the DA-denervated striatum appears to argue against the need for simultaneous stimulation of D_1 and D_2 receptors in patients with parkinsonism. Synergistic behavioral effects of selective D_1 and D_2 agonists, however, have been reported in the unilateral 6-OHDA rotational model (Koller and Herbster 1988; Robertson and Robertson 1986, 1987; Rouillard and Bedard 1988; Sonsalla et al. 1988) and in neonatal 6-OHDA-treated rats (Breese et al. 1985). In view of these findings, it was somewhat surprising that, in our electrophysiological study, DA denervation not only relieved the requirement of D_1 receptor stimulation for D_2 agonist-induced inhibition (enabling), also abolished the D_1-mediated potentiation of D_2 agonist-induced inhibition (a quantitative synergistic effect). This failure to observe either quantitative or qualitative synergism on caudate-putamen neurons was not due to a maximal "floor effect" produced by QUIN at supersensitive D_2 receptors, because additional DA depletion produced by AMPT resulted in greater inhibition of caudate-putamen neurons by QUIN (figure 7). These findings suggest that the behavioral synergism reported in previous studies of DA-denervated rats is not due to synergistic actions at adjacent D_1 and D_2 DA receptors located in the caudate-putamen.

FIGURE 7. (continued) *receptor stimulation for QUIN-induced inhibition was abolished, as was the potentiating (synergistic) effect of SKF-38393 on QUIN-induced inhibition* (n = *15). (D) Additional depletion of DA with AMPT in 6-OHDA-lesioned rats not only enhanced the inhibitory effect induced by QUIN* ($F[1, 29] = 8.54$, $p < .01$) *but also failed to produce a synergistic inhibition on caudate-putamen neurons, indicating that (1) the supersensitive response observed in 6-OHDA-pretreated rats was not maximal, thereby masking possible potentiation by SKF-38393 and (2) the ability of D_2 receptor stimulation to inhibit caudate-putamen neurons in 6-OHDA rats* was *not due to residual DA stimulating supersensitive D_1 receptors (enabling). (From Hu et al. 1990.)*

Robertson and Robertson (1986, 1987) have proposed that the synergistic rotational responses produced by submaximal doses of D_1 and D_2 selective agonists may be due to the combined actions at D_2 receptors within the caudate-putamen and D_1 receptors located on the terminals of striatonigral neurons within the substantia nigra pars reticulata. Such systems-level (distant) D_1-D_2 receptor interactions could explain the observation of behavioral synergism in the absence of adjacent D_1-D_2 interactions at the single-cell level. Electrophysiological studies have also provided a possible correlate of this behavioral interaction. Thus, Weick and Walters (1987) found a synergistic inhibition of substantia nigra pars reticulata neurons produced by combined IV administration of D_1 and D_2 selective agonists only in rats with 6-OHDA lesions. These findings, combined with the previous behavioral and electrophysiological studies, provide new evidence for mechanisms through which the behavior of patients with parkinsonism might be influenced. Although it appears that DA receptor supersensitivity relieves D_2 receptors from the requirement of D_1 receptor stimulation (i.e., the loss of adjacent D_1-D_2 receptor interactions on these neurons), additional distant (perhaps compensatory) functional interactions between D_1 and D_2 receptors are evident at a systems level. Thus, the possible utility of combined D_1-D_2 stimulation in the treatment of parkinsonism cannot be ruled out.

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Psychomotor Stimulant Effects on Single Neurons in Awake, Behaving Rats

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INTRODUCTION

The current quest to understand the neural mechanisms of drug abuse requires the use of methods that provide instantaneous measures relating the brain to behavior. Several such methods are reviewed in this volume. This paper describes the application of an established technique, electrophysiology, in a relatively uncharted area: drug administration to awake, freely moving animals.

Combining electrophysiology, pharmacology, and behavior in one preparation is a valuable but challenging endeavor. Neuronal activity varies with behavior, and drug effects on brain activity and behavior vary as a function of complex, but quantifiable, behavioral factors (Johansen 1988). (See also "Importance of Behavioral Controls in the Analysis of Ongoing Events" and "Cocaine Self-Administration: Pharmacology and Behavior.") In the preparation described, such variables are evaluated in terms of conscious neuronal processing involved in and responding to seeking drugs of abuse. Certain compromises are necessary, however, when electrophysiology, pharmacology, and behavior are combined. It is not possible to adhere to the rigorous protocols that strengthen each individual approach (see "Neurophysiological Approaches to Receptor Dynamics"). A thorough review of highly relevant theoretical and methodological issues has appeared elsewhere (Deadwyler 1986).

This chapter focuses on strategies for studying single-unit responses to psychomotor stimulants in freely moving rats. Of particular interest are the effects of stimulants on cellular processing related to motor function and reinforcement. First, with respect to stimulant effects on striatal activity, we describe a protocol that accounts for a combination of variables, including the behavioral effects of the drug and the behavioral correlations of recorded units.

Second, with the intent of studying the neurobiological mechanisms underlying the reinforcing properties of cocaine, we describe an extension of the first protocol to a recently developed method for recording single cell activity during cocaine self-administration. Each strategy is illustrated with results obtained from specific experiments. In spite of certain compromises, the strengths of a combined approach are manifested by demonstrating drug effects on specific patterns of neuronal activity related to particular behaviors and neurotransmitters.

MEASURES OF UNIT ACTIVITY APPROPRIATE FOR DRUG STUDIES

In addition to a neuron's tonic (spontaneous) firing rate, its phasic firing patterns serve as useful substrates for assessing drug effects on neural circuits. Phasic patterns are transient neuronal signals related to specific behaviors, sensory and motor events, electrical stimulation of afferent pathways, agonist-antagonist interactions, and so on. Phasic neuronal patterns that have been used effectively in studying dopamine (DA) function include evoked auditory unit discharges (Rolls et al. 1984), accumbens discharges activated by stimulation of limbic afferents (Yim and Mogenson 1982; Yang and Mogenson 1984; DeFrance et al. 1985*b;* Hakan and Henriksen 1989), and DA receptor agonist-antagonist interactions in the accumbens (White and Wang 1986). The better characterized the phasic pattern in terms of the synaptic input or receptor subtype involved, the more meaningful is the interpretation of drug effects (Bloom 1974). In turn, drug studies can contribute to the basic understanding of synaptic events that mediate particular phasic neuronal patterns.

In the rat striatum (caudate-putamen), striking differences can be demonstrated between the behavioral correlates of neurons in medial subregions and of those in lateral subregions. Whereas medial units show only general relationships to movement-e.g., increased firing during whole-body movements such as locomotion (West et al. 1987)—a substantial percentage (25 percent to 35 percent) of lateral striatal units fire during specific sensorimotor activity of a particular part of the body (West et al. 1990). Virtually all body parts are represented in the lateral striatum, including limbs, individual vibrissae, snout, chin, neck, shoulder, trunk, and so on (Carelli and West 1991). The location of these units corresponds with the location of termination of projections from somatic sensorimotor cortex (Wise and Jones 1977; Cospito and Kultas-Ilinsky 1981; Donoghue and Herkenham 1986).

These phasic, sensorimotor firing patterns provide excellent substrates for (1) assessing the response of these cells to drugs and (2) studying basal ganglia function. An important neurobiological question addressed in this chapter is, how do DA and psychomotor stimulants modulate the activity of DA target cells involved in sensorimotor function?

CONTROLLING BEHAVIORAL VARIABLES IN STUDIES OF PSYCHOMOTOR STIMULANT EFFECTS ON NEURONAL ACTIVITY

Measures of both tonic and phasic neuronal patterns are influenced by behavioral variables. Therefore, when neural activity is compared between predrug and postdrug conditions, the animal's behavior must be comparable across conditions. The following studies of psychomotor stimulants illustrate that this comparability can be accomplished by controlling for drug-induced behaviors. One approach is to produce these behaviors experimentally in the predrug control period. Drug-induced behaviors also can be avoided either by administering low doses that have minimal effects on behavior or by applying drugs microiontophoretically.

Use of Forced Treadmill Locomotion in Conjunction With a Locomotion-Inducing Dose of Amphetamine

The study of amphetamine's effects on striatal-unit activity in awake rats has required the development of a detailed strategy, the description of which is a useful illustration of some of the issues involved in electrophysiological study of drug effects in behaving animals. A critical issue concerns the conditions under which the baseline (control) firing rate is measured. Spontaneous firing rates of striatal neurons vary widely across conditions. They have been shown to be at or near zero in numerous studies performed in anesthetized animals. In marked contrast, we have found that the majority of neurons in the central and medial portions of the striatum of the awake rat are spontaneously active during resting behavior and show robust increases in overall firing rate during general, whole-body movements such as locomotion (West et al. 1987). Therefore, to measure the effects of a psychomotor stimulant on unit firing, it is inappropriate to obtain a control firing rate during resting behavior. Control measures must be taken during motor behaviors comparable to those observed following drug administration (Trulson and Jacobs 1979).

Control firing rates were obtained during alternating 30-s periods of treadmill locomotion versus rest. Animals were given a dose of dexamphetamine

(1.0 mg/kg intraperitoneally) that is known to produce locomotion in rats. Neuronal activity was compared between control (saline) and experimental epochs of treadmill locomotion. Across conditions, no changes were observed in locomotor pace or posture.

We have studied the effects of amphetamine on both types of striatal unit described above, i.e., those related to specific body parts and those related to general body movement. Medial striatal units related to general movement uniformly showed increases (range, 5 percent to 300 percent) in locomotor-related firing 5 to 30 min following amphetamine injection (compared with locomotion following saline injection). Firing recovered after 60 to 120 min; that is, units fired faster after drug injection than they did during the same behavior before injection.

Amphetamine also enhanced the timelocked firing of lateral striatal units related to specific movements (Peoples et al. 1991). A forelimb unit showed a greater likelihood of firing and a shorter latency to respond to footfall after amphetamine injection, although no drug-induced changes were observed in the rate or length of individual strides. The movement-related discharges of two units timelocked to stereotyped, vertical head movement were increased 10-fold after amphetamine injections, with no measurable change in the amplitude or velocity of head movement. The time course of increased unit firing coincided with an increased frequency of stereotyped head movements.

These data suggest a potential correlation between the level of striatal firing and the frequency of a stereotyped movement during psychomotor stimulation. One possible interpretation is that the increased firing represented enhanced responsiveness of striatal neurons to cortical or thalmic inputs carrying sensorimotor information. This could involve facilitation by amphetamine of DA transmission at nigrostriatal synapses, which appears to be a critical substrate mediating the stimulant effects of amphetamine on the motor system. In any case, our data indicate that psychomotor stimulant activation of motor output may be linked to an increased excitability of striatal neurons during the processing of sensorimotor information.

Use of Low Stimulant Doses To Minimize Confounds Resulting From Motor-Related Neural Firing

The response of striatal neurons to the psychomotor stimulant cocaine has also been examined by means of a different strategy to control for possible

motor-related neural activity. In these studies, a low dose (1.0 mg/kg intraperitoneally) of cocaine was used that did not produce any observable changes in motor behavior. Thus, following drug injection, animals walked normally during treadmill epochs and rested normally during intervening 30-s rest periods. The result of these experiments was that drug-related changes in neuronal firing were observed only during locomotor periods, but not during rest. In every case, units showed increased firing rates (compared with locomotion following saline injection) that recovered within 60 to 120 min (Shimizu et al. 1987).

In view of cocaine's mechanism of blocking DA reuptake (Heikkila et al. 1975; Ritz et al. 1987), an interesting interpretation of these findings is that the increases were selective for locomotion because DA release was greater during this behavior than during rest. Indeed, there is substantial literature showing correlations between dopaminergic transmission and movement (Ungerstedt et al. 1977) (as well as DA antagonism and poverty of movement). It follows that the present locomotor-related increases in striatal firing rate may have been mediated, in part, by increased striatal DA release. These data provide further support for the idea-derived from the amphetamine studies described-that DA agonists may increase striatal responsiveness to inputs related to movement. The cocaine study further emphasizes the importance of using a context that allows motor behavior: cocaine's effects on striatal firing would not have been observed if the animal had remained at rest.

Microiontophoretic Application of Drugs Directly Onto Recorded Neurons

A definitive means of preventing drug-induced behavioral changes that might alter neuronal activity independent of direct drug action is the iontophoretic application of drugs directly onto recorded neurons (West and Woodward 1984; West et al. 1986). Preliminary studies using this technique have further implicated striatal DA release in locomotor-related increases in striatal firing. Of sixty striatal units studied, direct iontophoretic application of DA excited 50 percent and inhibited 23 percent of the units. Of the units that showed increased activity during locomotion, 80 percent were also excited by DA. Furthermore, iontophoretically applied trifluoperazine (DA receptor blocker) attenuated the increased striatal firing correlated with locomotion (Shimizu et al. 1987). These findings suggest that DA participates in neuronal processing in the striatum during locomotion and that its effect in freely moving rats can elevate striatal firing, either through direct or modulatory actions (Woodward et al. 1979).

Moreover, the studies illustrate certain significant features of the iontophoretic method in awake animals. The method not only eliminates changes associated with systemic drug administration (e.g., changes in behavior and in multiple neurotransmitter systems) but also, by means of systematic manipulations of behavioral variables, makes it possible to use selective agonists and antagonists to relate a particular transmitter to a particular behavior.

INTERPRETATION OF STRIATAL RESULTS

Despite the variability that is presumably inherent in studying drug effects on neuronal activity in awake animals, results have shown a reasonable degree of uniformity (most likely attributable to efforts to control behavioral variables) in suggesting that striatal firing rates are enhanced by DA and related compounds. The findings are consistent with the hypothesis that DA agonists may modulate the responsiveness of striatal neurons to synaptic inputs carrying sensorimotor information (Freund et al. 1984). The increased excitability of striatal neurons appears to be linked in some way, perhaps causally, to stimulation of motor output by psychomotor stimulants.

ACCUMBENS UNIT RESPONSES TO SELF-ADMINISTERED COCAINE

Psychomotor stimulants are potent reinforcers in drug self-administration paradigms (Aigner and Balster 1978). Stimulants both activate motor behavior and reinforce drug-seeking behaviors; some combination of these properties apparently underlies the abuse potential of these drugs (Wise and Bozarth 1987). In order to study the as yet unknown neuronal mechanism or mechanisms involved in psychomotor stimulant reinforcement, methods for intravenous (IV) self-administration of cocaine have recently been combined with chronic electrophysiological techniques. The focus of initial studies has been on the nucleus accumbens septi (NAS), which is strongly implicated in the neural circuitry that mediates psychomotor stimulant self-administration (Dworkin and Smith 1988; Koob and Hubner 1988). The approach used was an extension of the one used in the above-mentioned studies of stimulant effects on striatal activity. The activity of accumbens cells in response to cocaine was interpreted in light of processing involved in producing drug-seeking behavior versus “nonspecific” motor behaviors. A detailed strategy was used to isolate movement-related variables that may covary with unit activity in order to evaluate the extent to which they contribute to the overall variance in neuronal activity during self-administration.

Four animals were prepared with a chronic catheter implanted in the jugular vein for drug infusion, as well as recording electrodes (microwires 25 to 60 μm in diameter) in the NAS for recording single-neuron activity during behavior. A bipolar electrode implanted in the ipsilateral fimbria was used to stimulate subicular afferents and activate recorded neurons (n = 6) at a monosynaptic latency (5 to 10 ms) in order to identify them as NAS neurons (DeFrance et al. 1985*a).* In the home cage, animals were infused with heparinized saline every 2 hr to maintain the patency of the catheter. One week was allowed for recovery from surgery. Animals were placed in the experimental chamber daily and connected to a harness that led to a fluid and electronic swivel. Baseline firing rates were recorded during resting behavior and during treadmill locomotion to obtain a measure of each unit's general correlation with movement. The lever for self-administering cocaine was then inserted into the chamber.

Before and after drug administration, behavior was videotaped by a computerized process in which each video frame (30 frames per second) as well as each unit discharge was time stamped. In subsequent analysis, individual frame numbers corresponding to a repeated movement (e.g. rearing, turning, nose poking, head bobbing) were entered into the computer as nodes around which perievent histograms were constructed, depicting neural activity timelocked to each particular behavior (Carelli and West 1991).

Figure 1 shows a graph of unit firing rates as a function of behavior and initial cocaine infusions. During the first and second self-administration training sessions of rat 1, the firing rates of four NAS neurons were recorded during (1) a 3-to 4-min period in which the subject rested, i.e., sat in place; (2) a 2-min period of treadmill locomotion; and (3) a 6-to 10-min period in which the rat roamed the chamber freely and received the first three infusions (0.2 mL of 1.65 mg/mL [0.33 mg] cocaine per infusion). These infusions were administered as part of the shaping procedure. All units showed small increases in firing during locomotion (compared with rest) and then further increases in response to initial infusions of cocaine. These results suggest that the increased firing following cocaine infusion was not simply related to drug-induced motor activity.

Figure 2 shows the results of the entire second cocaine self-administration training session (2.5 hr) for the same rat. The following dependent measures are plotted as a function of 15-min blocks: (1) cumulative number of IV infusions (top left panel); (2) number of lever presses (top left panel); (3) number of nose pokes into any of the four corners of the operant chamber (bottom left panel); (4) number of 180" turns made by the animal (bottom left panel); and (5) mean

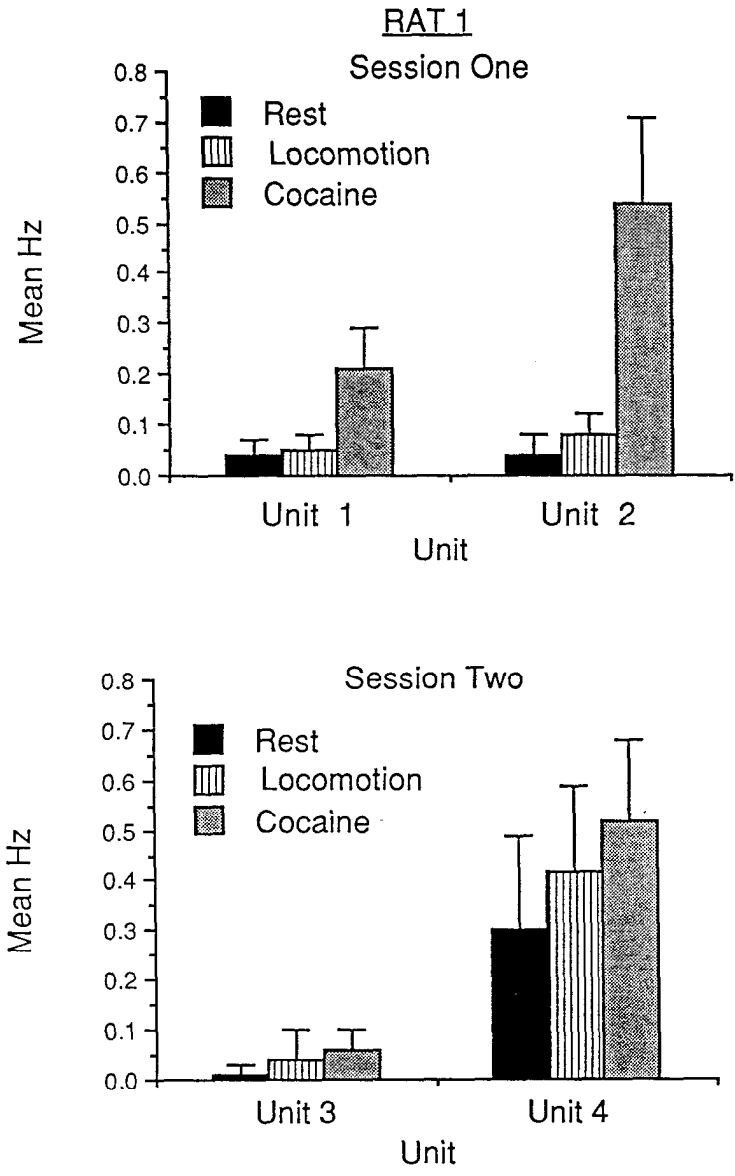

FIGURE 1. *Firing rates (mean Hz + SE_M) of four accumbens units during rest and locomotion and following the first three infusions of cocaine. See text for details.*

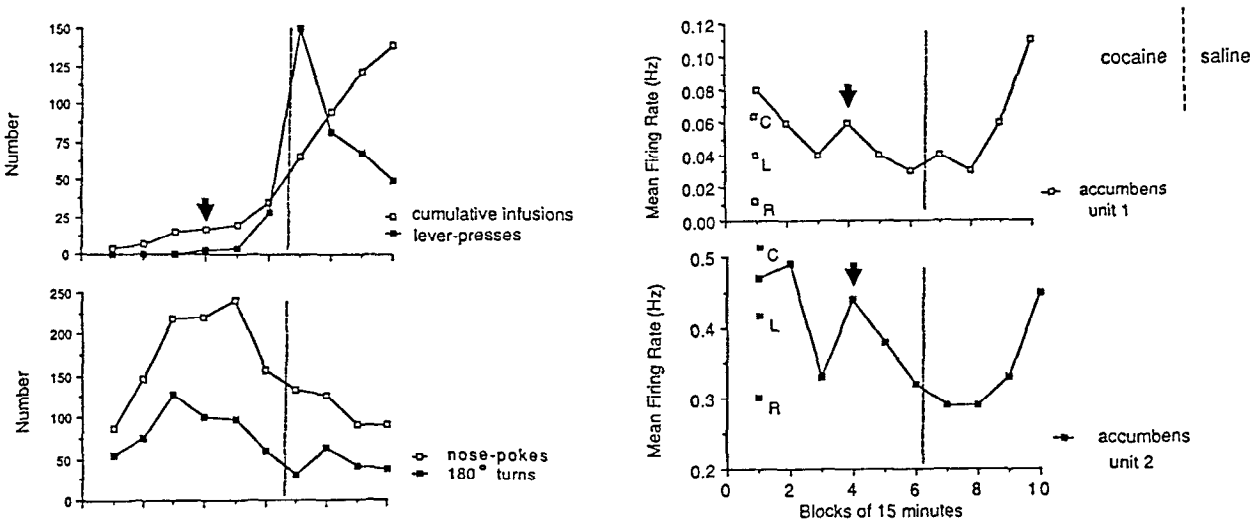

FIGURE 2. *Motor behaviors and accumbens firing rates plotted as a function of 75-min blocks during a 2.5-hr session of cocaine self-administration. Top left panel: Cumulative infusions, white squares; number of lever presses, black squares. Bottom left panel: Number of nose pokes, white squares; number of 180° turns, black squares. Right panels: Mean firing rates (Hz) of two accumbens neurons recorded simultaneously. Arrows indicate onset of lever presses made by animal. Firing rates of these neurons during rest (R) and locomotion (L) and following the first three cocaine infusions (C) are taken from figure 1. Dashed vertical lines indicate time when saline replaced cocaine in infusion syringe.*

firing rate recorded from each of two nucleus accumbens neurons recorded simultaneously (right panels). Note that during the first six 15-min blocks, each lever press was followed by an infusion of cocaine; during the last four 15-min blocks, each lever press was followed by a 0.2-mL infusion of isotonic saline (extinction).

The shaping procedure ended after three 15-min blocks and 13 infusions. After that point (arrows), the animal lever-pressed at a steady rate ranging from 0.25 to 0.5 presses per minute. Thus, the cumulative number of infusions increased linearly to 30. The stimulant effects on behavior were exhibited as increases in both nose pokes and locomotion back and forth across the chamber (i.e., 180° turns). Both behaviors, however, exhibited inverted U-shaped curves, reflecting the fact that the animal became ataxic during the sixth 15-min block (cumulative dose = 29.7 mg/kg cocaine).

In the right panels of figure 2, unit firing rates from figure 1 are inserted for reference. During the first 15-min block, firing rates were elevated after the first three cocaine infusions, relative to rates observed during rest or locomotion. Firing rates of both units decreased throughout the remainder of the period of cocaine availability. Note that both units showed transient increases during the top left block in which the animal lever-pressed (arrows). Within an hour after saline replaced cocaine in the infusion syringe, both units recovered to firing rates observed at the beginning of the session.

Using the data from figure 2, Pearson Product Moment Correlations were calculated between the mean firing rate of each unit and the (1) cumulative number of cocaine infusions, (2) number of nose pokes, (3) number of 180” turns, and (4) number of lever presses (table 1).

TABLE 1. *Pearson Product Moment Correlations from data in figure 2*

	Mean firing rate	
Measure	Unit 1	Unit 2
Cumulative cocaine infusions	-.847*	-.793*
Nose pokes	-.500	-.076
180° turns	-.341	-.090

NOTE: Correlations between mean firing rate and cumulative number of cocaine infusions were calculated across the period of cocaine availability; other correlations were calculated across all 10 blocks of 15 min.

Correlational analysis revealed that the firing rate of each accumbens unit correlated significantly with the cumulative number of cocaine infusions but not with any recorded motor behavior. That is, relative to elevated firing rates after the first three cocaine infusions, continued cocaine infusions significantly reduced the firing rates of both units tested to precocaine levels. Thus, preliminary findings suggest that cocaine may initially excite accumbens neurons, whereas subsequent exposure to the drug appears to produce no further activation but might produce a depression of firing or a return to precocaine levels. Furthermore, the lack of correlation with any measure of motor behavior suggests that these results were not due to unit correlations with drug-induced motor activity. Finally, perievent time histograms depicting unit activity with respect to lever pressing have thus far shown no patterns indicative of neuronal firing related to drug-seeking behavior.

CONCLUSION

To characterize the effects of a particular drug on neuronal activity in an awake animal, it is essential to use appropriate behavioral controls. Doing so involves both accounting for the behavioral effects of the drug and characterizing each unit's behavioral correlates. Although advances have been made in understanding the functions of striatal neurons during behavior, little is known about the behavioral correlates of accumbens neurons. Initial studies have demonstrated emphatically the need for a better understanding in this regard. Thus far, the most reliable correlation observed is that 90 percent of accumbens units (n = 224) increase firing rate during general movement, such as locomotion, (relative to rest). Correlations with more specific behaviors have not yet been observed. Because cocaine produces motor simulation, accumbens activity in response to cocaine could potentially be related to motor processing. In fact, substantial evidence suggests that stimulant activation of motor output is mediated by accumbens and striatal neurons, neural substrates apparently directly influenced by cocaine's effect at dopaminergic synapses (Gershanik et al. 1983). Therefore, the responses of these cells to cocaine must be interpreted in light of sensorimotor processing involved in producing drug-seeking behavior. It is not yet clear to what extent explanations in these terms will contribute to an understanding of the neural mechanisms mediating reinforcement. The approach described in this chapter, and the continued refinement of it, might be suitable for addressing such issues and for making significant advances in understanding the neurobiological effects of psychomotor stimulants.

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Brain-Stimulation Reward: A Model for the Study of the Rewarding Effects of Abused Drugs

Conan Kornetsky and George Bain

During the past decade, there has been increased acceptance of the hypothesis that the reinforcing effects of abused substances are related more to their ability to enhance neuronal activity of the brain reward systems than to ameliorate withdrawal symptoms or reverse some underlying psychological or somatic pathology. Much of the change in attitude about bases of the reinforcing effects of abused substances has been shaped by the experimental work of investigators who, during the past 18 years, have studied the action of these drugs on behavior maintained by rewarding intracranial stimulation. Most abused substances increase the sensitivity of animals to (i.e., increase the rewarding value of) this intracranial stimulation. This model of drug-induced euphoria is not only predictive of abuse liability, but it also has the further advantage of lending itself to the study of the neuronal events that underlie the reinforcing effects of abused substances. James Olds (1977, p. 4), in commenting about the discovery of self-stimulation, described it as “a new window on the brain.”

The phenomenon that animals would work to obtain intracranial stimulation was first described by Olds and Milner (1954), who stated, “It is clear that electrical stimulation in certain parts of the brain, particularly the septal area, produced acquisition and extinction curves which compare favorably with those produced by conventional primary reward.”

An early report of the effects of pentobarbital and d-amphetamine on brain-stimulation reward (BSR) (Killam et al. 1957) suggested that the method might be a useful technique for studying the rewarding effects of abused substances. With few exceptions, however-notably a series of experiments of the effects of d-amphetamine on BSR by Stein and associates (Stein and Ray

1960; Stein and Seifter 1961; Stein 1962*a,* 1962*b;* Stein 1964; Stein and Wise 1969)—BSR was not considered a useful mode for the study of abused drugs. Among the reasons for this lack of interest was that the effects of drugs on BSR were not significantly different from those of natural reinforcers such as food. Starting, however, in the early to mid-1970s approximately 20 years after the original report by Olds and Milner, there was renewed interest in the procedure because of the potential it offered for understanding the neurobehavioral bases of drug-induced euphoria, reward, and subsequent reinforcement.

METHODOLOGY

The experimental paradigms of BSR studies and behavioral studies that use other reinforcers such as food are similar. Most investigators use an operant procedure employing a lever manipulandum in one wall of the experimental chamber. The critical factor in selecting a manipulandum is that the response required of the animal should not be so great that completing a response is difficult for the animal or so easy that any random movement would result in the delivery of a rewarding stimulation.

Electrode Implantation and Stimulus Parameters

Both monopolar and bipolar stereotaxically implanted electrodes are used in brain-stimulation studies. In the monopolar configuration a single wire is implanted and a screw anchored to the skull serves as the indifferent electrode or collector. This monopolar arrangement leads to a greater spread of current and has been reported to require higher stimulus intensities to obtain response rates similar to those found with bipolar electrodes (Valenstein and Beer 1961). These differences in effective intensities were attributed to the alternation of the cathode and anode during biphasic stimulation between the neural site at the tip of the electrode and the indifferent surface electrode. The bipolar configuration consists of two straight wires cemented together or two wires twisted together and implanted so that their uninsulated tips alternate as cathode and anode during the stimulation to the neural site.

The parameters of the actual electrical stimulation employed by the several groups who are conducting brain-stimulation studies vary to some degree. Some investigators employ brief trains of constant-current, cathodal, monophasic, rectangular pulses and vary the frequency (e.g., Fouriezos and Nawiesniak 1987; West and Wise 1988). In many laboratories the current intensity is varied and trains of either sine waves or rectangular pulses of

alternating polarity with each positive or negative rectangular pulse lasting approximately 0.2 ms are typically used. The train durations generally are 0.5 ss; the fixed frequency of pulse pairs is usually between 60 and 160 Hz. Current intensities for effective stimulation may vary from approximately 50 to 300 μA; the lower current intensities are characteristic of bipolar electrodes and the higher intensities are more characteristic of monopolar configurations. The lower the frequency used for stimulation, the greater the amount of current needed before for the stimulus reaches a rewarding level; thus the effectiveness of the stimulus is probably a function of the total charge (coulombs), which is resultant of frequency, current intensity, and stimulus duration.

Figure 1 shows an example of the relationship between the probit of the percent of responding and the current intensity at two different frequencies in the same animal. In this figure the curve for the lower frequency is to the right of the curve for the higher frequency, indicating that at the same intensity the lower

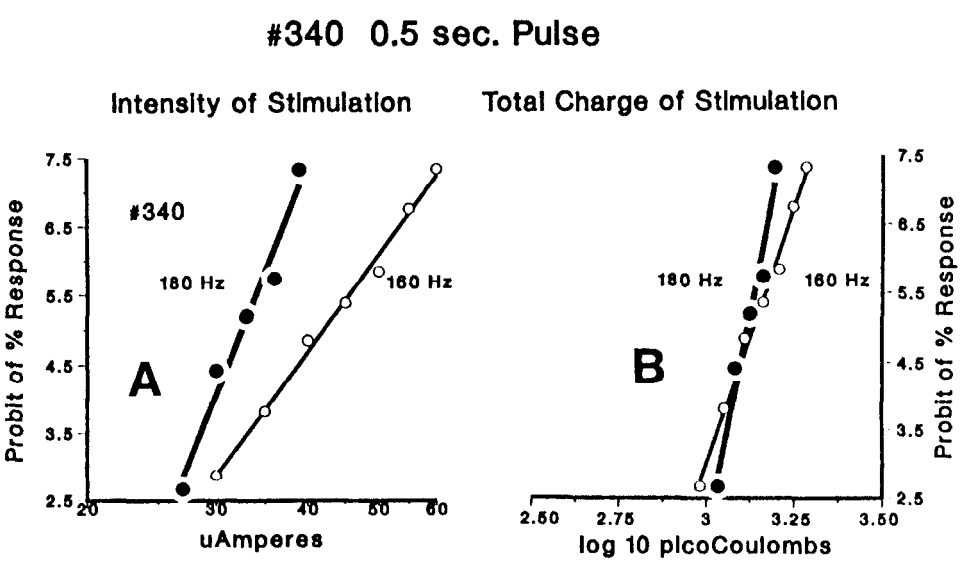

FIGURE 1. *Relationship between probit of percent response and current intensity. Two frequencies (160 Hz and 180 Hz) are shown for a single subject on two different days, respective/y. A. Probit of percent response versus intensity μA) in log representation. B. Probit of percent response for the* same *two frequencies versus electrical charge (picocoulombs) in log representation.*

frequency is less rewarding. However, when the same data from the two experiments are presented as a function of the electrical charge (coulombs), the curves are almost superimposed. Thus, the effective stimulus is the power (electrical charge) of the stimulus and not the components of the electrical charge.

Lever-Pressing-Rate Methods

The most commonly used BSR method has been to use the simple rate of lever pressing (i.e., rate of response) as the dependent variable. For the most part, investigators have used continuous reinforcement or low fixed-ratio reinforcement schedules. When rate of response is used, there is a direct relationship between the intensity of stimulation and the rate of response. This increase in response rate will asymptote at some level and increasing the intensity further will result in a decrease in rate (figure 2). The example shown is a single animal tested on two successive days for 5 minutes at each indicated intensity. On day 1, the order of intensities was increasing; on day 2, the animal was started at the previous day's ending intensity, and a descending order of intensities was used.

In the simplest paradigm, a level of intensity is selected that generally allows for a high rate of responding. An increase in rate of response after a drug is administered is usually interpreted as the result of the animal becoming more sensitive to the stimulation (the stimulation is more rewarding) whereas a decrease in responding is interpreted as the result of the animal becoming less sensitive to the reinforcing stimulus (the stimulation is less rewarding). The major problem in using rate of response as the dependent variable in drug or lesion studies employing BSR is the lack of specificity of the mechanisms involved. An increase in rate of response may be the result of a general level of psychomotor stimulation, and a decrease may be the direct result of treatment on the ability of the animal to press a lever at the high rates usually obtained when the reinforcer is BSR. In an experiment in which each animal was prepared with two stimulating electrodes bilaterally implanted and each of two levers was wired to one of the electrodes, Hodos and Valenstein (1962) found no relationship between the rate of response and the preference for a stimulation site.

Despite the problems of interpretation when the dependent variable is rate of response, it is still extensively used to determine the effects of drugs on the reinforcing value of the stimulation. Trying to measure changes in the reward

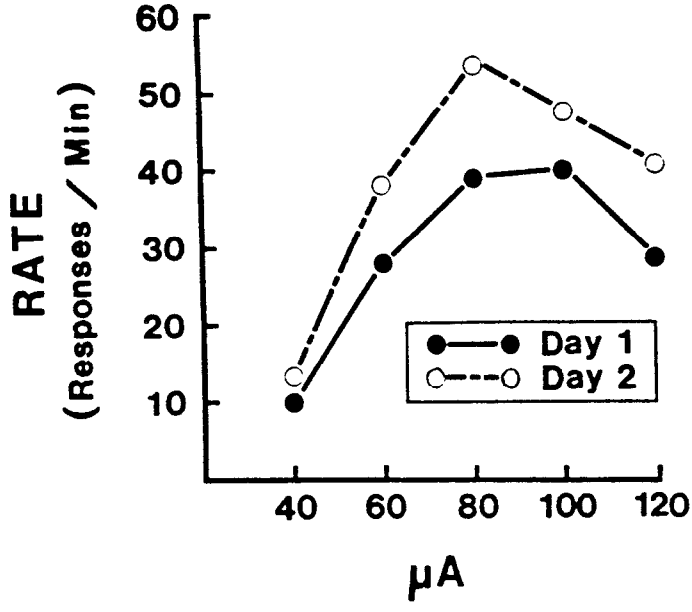

FIGURE 2. *An example of a rate-intensity function for a single animal. The solid line reflects rates of response on* day *1 when the order of reinforcement current* was *ascending. The dashed line reflects rates of response on* day *2 when descending order of reinforcement current* was *used. Note that* at *the higher intensities the rate of response was less than maximal. (From Payton 7986.)*

value of the stimulation more directly, a number of investigators have used a range of stimulus intensities. Using this paradigm, investigators look for shifts in the intensity-rate function after administering of drugs.

An example of testing at a full range of intensities (rate-intensity method) was the method used by Leith and Barrett (1976), who demonstrated tolerance to the facilitatory effects of d-amphetamine on BSR as well as a poststimulation depression in response rate (figure 3). The current intensity was adjusted to an intensity that resulted in maximal response rates for each animal. Then, within each session, the intensity was attenuated every 5 s by one-twentieth of the

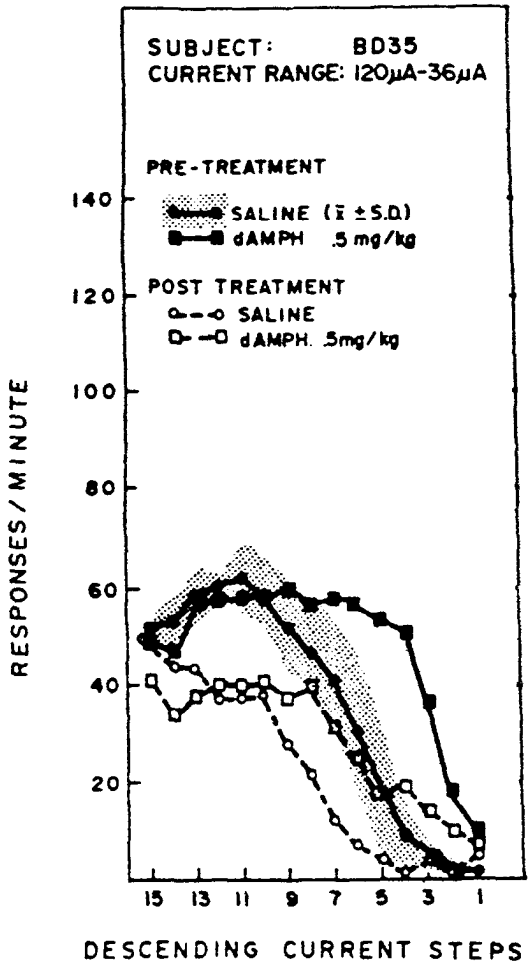

FIGURE 3. *Effects of* d*-amphetamine (0.5 mg/kg) on the rate of response for BSR before and after chronic (2 wk) treatment with high doses (20 mg/kg per day) of the drug in a single subject. The current intensity is decreased by one-twentieth of the initial value every 5 s. After the initial 0,5-mg/kg dose of d-amphetamine, a clear shift of the response-intensity curve to the right of saline is shown for the subject prior to chronic administration. A similar shift of lesser magnitude is shown after chronic d-amphetamine administration of the same dose. (From Leith and Barrett 1976.)*

starting intensity until 15 levels of stimulation had been tested. At this point, the intensity was reset to the starting level, and the procedure was repeated throughout the length of the 60-min session. Figure 3 illustrates the effects of 0.5 mg/kg of damphetamine in a single animal. Facilitation in response rate after *d*-amphetamine is clear from the shift of the response intensity curve to the right of the saline curve. After 2 weeks of daily 20-mg/kg doses of d-amphetamine, animals were again tested after 0.5 mg/kg of *d*-amphetamine or saline. As shown, there was not only evidence of tolerance to *d*-amphetamine, but also a depression in the rate of responding even after saline. This depression is indicated by the shifi of the curve to the left. Other investigators (e.g., West and Wise 1988) have used multiple intensities in the study of drug effects. In these studies, rate of response is expressed as a function of frequency (rate-frequency function). A shift in the rate-frequency function is called a phase shift; a shift to the right after the drug is administered is interpreted as a decrease in the reward value; a shift to the left is interpreted as an increase in reward value. Failure to reach the same asymptotic rate of response after the drug is obtained following saline administration is interpreted as evidence that the drug causes **a** performance deficit. The phase shift method has been used in which the dependent variable was speed of running combined with a lever press (Gallistel et al. 1974). Figure 4 illustrates the effects of various doses of pimozide using this technique (Stellar et al. 1983). In this experiment the animal was required to negotiate a one meter long alley to a goal box where a lever was available. A single lever press resulted in the delivery of the intacranial stimulation.

Because the rate-intensity and rate-frequency methods also use rate of response or running speed as an integral part of the procedure, the effects of drugs on the reward value and on performance might still be confounded. And because threshold and asymptotic values are at the two extremes of the curve (the asymptotic low and high rate points) there is greater chance for error than when the threshold is in the mid-range of the rate-frequency or current curve.

Alternative Operant Manipulandum

To avoid the possible confounding effects of drugs on motor performance with the reinforcement value of the stimulation, many investigators have used an alternative manipulandum (Liebman 1983). Despite the simplicity of the lever press, it is not a response that is programmed into the repertoire of the rat. Schiff (1976) clearly demonstrated that not all lever pressing is equivalent.

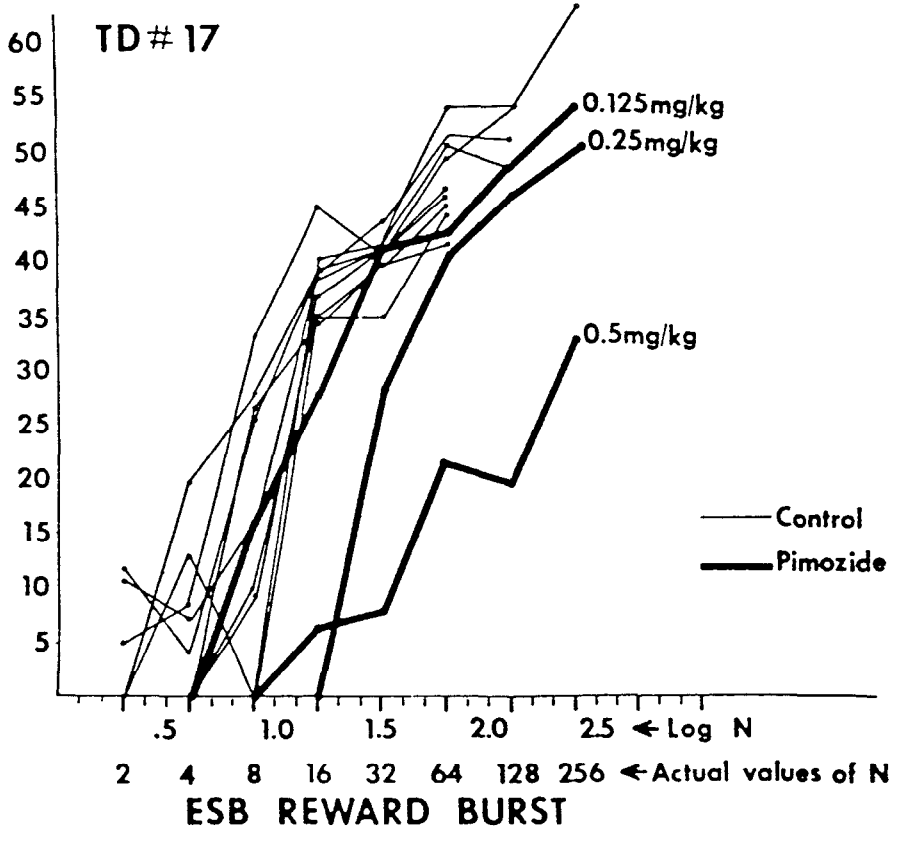

FIGURE 4. *Shift in the baseline running speed (cm/set) (reward summation functions) for a single subject after 0.125, 0.25, and 0.5 mg/kg of pimozide. Then a 0.25-mg/kg dose produced a clear shift of the curve to the right without a significant decrement of asymptotic running speed (as indicated along the ordinant). The 0.5-mg/kg dose, however, not only shifted the curve but also decreased the asymptotic running speed. N = the number of current pulses per burst. (From Stellar et al. 1983.)*

Animals with caudate lesions had greater decreases in rate of response when the lever was 64 mm versus 10 mm above the chamber floor.

An operant response that is indigenous to the rat and requires little motor effort is the nose poke. Rats will readily stick their noses into small apertures in the

floor or wall of an experimental chamber. By use of a photocell beam in the aperture, whose interruption results in the delivery of the stimulus, this natural and easily made response can be exploited. This manipulandum has been used in both lesion and pharmacologic studies (Gerhardt and Liebman 1981; Ettenberg et al. 1979; Simon et al. 1979). From these experiments, it became clear that if the performance demands of a manipulandum become too great, then changes in response rate might well be a function of the effects of the lesions or drugs on the motor performance of the animal and of any change in the reward value of the stimulation.

Rate-Independent Instrumental Response Methods

Despite attempts to circumvent the motor response effects without circumventing the reward value of the stimulation, Liebman (1983) argued that using other manipulanda did not solve the problem, because the effects on reward might be confounded with the operant rate per se. In an attempt to separate performance effects from rewarding effects, a number of investigators have employed rate-independent procedures. Valenstein and Meyers (1964) used a “place preference” procedure; Gallistel and colleagues (1974) used a procedure that incorporated running speed to a goal box, where a lever press resulted in delivery of the stimulation (see figure 4). Other investigators have employed a shuttle box procedure (Levitt et al. 1977; Atrens et al. 1976; Liebman et al. 1982; Liebman 1983; Wauquier et al. 1983). Many of these investigators also have criticized these procedures because of the difficulty in interpreting the specificity of the drug effects and because these methods are more cumbersome than using operant rate as the dependent variable. A further problem (rarely mentioned when there is a high density of stimulation, as when rate is the dependent variable or is an integral part of the procedure) is the marked oral stereotypy seen in the animals. A great deal of mouthing behavior is observed; subjects often press the lever not only with a paw but also with the mouth simultaneously. Similar behavior is seen when animals receive direct injections of dopamine agonists into the ventral lateral striatum (Kelley 1988). This indication of marked dopamine nigrostriatal stimulation suggests that the effects of drugs on BSR that are seen with using rate-dependent procedures might be combined effects of the action on reward systems and on the nigrostriatal motor system.

Threshold Measures

Very early in the investigations of BSR effects of drugs, the term *threshold* change often was used to define the effect of a drug. In these studies, *threshold* was defined as the intensity that caused a particular defined rate of responding or the lowest intensity at which any responding took place. Often, when a range of intensities are used, the threshold is defined as the intensity at which the animal responds at 50 percent of the maximum response rate. This definition is similar to what has been referred to as the "locus of rise" by some investigators (Edmonds and Gallistel 1977). Despite the usefulness of these procedures, they are by definition rate dependent. To circumvent this problem some investigators have developed alternative procedures for determining threshold.

Two-Lever Reset Titration Method. The first attempt at a threshold procedure that was independent of response rate was the two-lever reset method (Stein and Ray 1960). This procedure makes use of **a** chamber with a two-lever manipulandum. Pressing the left lever, for example, results in the delivery of a suprathreshold stimulation. Each subsequent response (or a fixed number of responses) on this lever results not only in the delivery of a rewarding stimulation but also in the attenuating of the stimulus intensity. A response on the other lever resets the stimulus level to its original intensity. The average intensity at which the animal resets the stimulus intensity is defined as the threshold.

Although this procedure has a number of advantages over a simple rate procedure, it does have some problems in interpretation. Fouriezos and Nawiesniak (1987) have pointed out that the higher the starting level, the higher the reset value. Among other criticisms is that there may be habituation to the stimulation; that is, the animals may reset at higher levels as **a** function of experience with the procedure. Also, the differences observed after drug may be confounded with the rate of response. A major advantage of the procedure is the relative ease in the control and delivery of stimulation. Among the investigators who recently have employed this procedure in the study of drug effects are Gardner et al. (1988) and Van Wolfswinkel and Van Ree (1985).

Psychophysical Discrete Trial Method. In attempting to determine the effects of drugs on BSR without the contamination of motor effects and independent from rate of response, we have developed a psychophysical discrete trial method for determining the threshold for rewarding intracranial stimulation (Marcus and Kornetsky 1974; Esposito and Kornetsky 1977;

Kornetsky 1985). This procedure consists of discrete trials, each requiring a single response by the subject to receive the stimulation. Figure 5 shows a cartoon of an animal in the experimental chamber and a schematic representation of the experimental procedure.

A trial begins with an experimenter-delivered (noncontingent) stimulation (S1), which signals the animal that the appropriate response (turning a wheel manipulandum within 7.5 s) results in a second (contingent) stimulation (S2). This contingent stimulation is identical to the noncontingent stimulation. If the animal does not respond within the 7.5-s available response time, the trial is terminated; the only consequence is that the subject does not receive the second stimulation. In most of our experiments the inter-trial interval varies randomly between 7.5 s and 22.5 s (mean, 15 s). To preclude responding during the intertrial interval, any response during the interval postpones the onset of the next trial for 15 s. In the final form of the procedure the current intensities are varied in a stepwise fashion of descending and ascending order, and the S2 intensity is exactly the same as the S1 intensity.

An example of the methods we have used for collecting the data and determining the threshold is given in figure 6. The simplest method we have used to determine the threshold is to average the column thresholds, which are arbitrarily defined as the midpoint intensity between the two lowest steps in which there are three contingent responses and fewer than three contingent responses, respectively. More recently, we have used a more exact estimate of the threshold, which allows the slope of the response-intensity curve to be determined. In this method, the percentage of times the animal responded at each intensity is determined. When plotted against the intensity of stimulation, this percentage is expressed as a sigmoid curve (figure 7A). When percentage is converted to probit and plotted against intensity expressed on a log scale (figure 7B), the data fit a straight line. With this procedure the threshold is the intensity of stimulation corresponding to probit 5. If the slope of the regression line not significantly different from zero, the animal is not under stimulus control.

Using the psychophysical discrete trial method, Markou and colleagues (1989) demonstrated the specificity of this threshold procedure and the independence of the threshold measure from motor effects. In their experiment, they found that increasing the force necessary to turn the wheel manipulandum had no effect on the threshold but did increase the latency of response. Additional evidence that the discrete trial procedure is independent from motor effects is the failure of rate of response for rewarding brain stimulation to correlate with

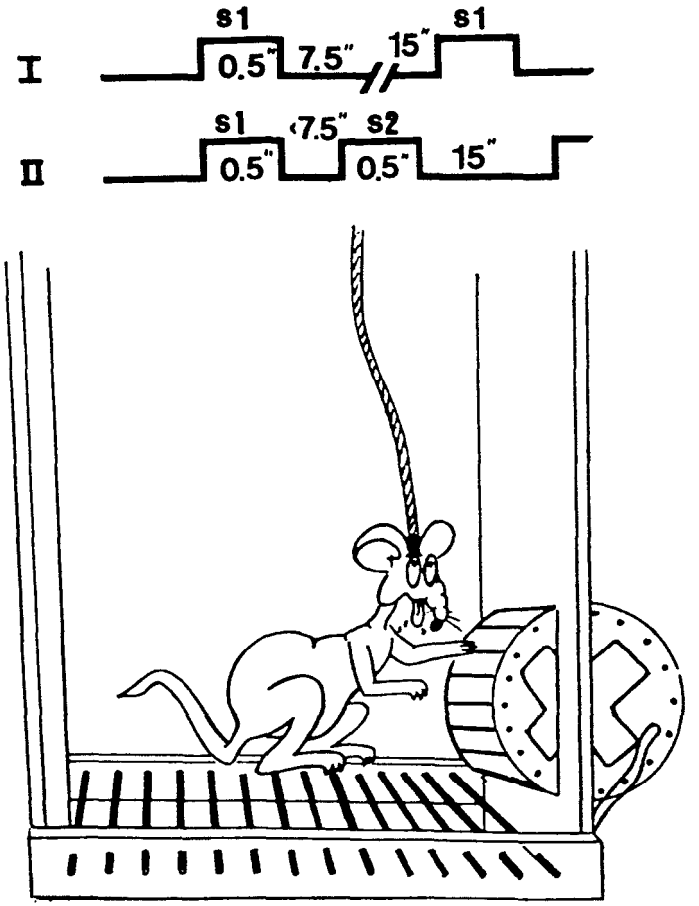

FIGURE 5. Schematic *of the two possible outcomes during a sing/e trial of the psychophysical discrete trial method. In example I, the rat is presented a noncontingent stimulus (S1), affer which if does not respond during the indicated 7.5-s response interval. Failure to respond has no scheduled consequences, and a new trial begins affer a 15-s interval. In example II, the rat is presenfed a noncontingent stimulus, responds by turning the wheel manipulandum within 7.5 s, and receives a second stimulus (S2) of the same intensity. The 7.5- by 75-cm cylindrical manipulandum is mounted in one wall of a plastic chamber 25- by 25- by 35-cm. A four-cog cam attached to one end of the manipulandum triggers a microswifch for* each *quarter-rotation.*

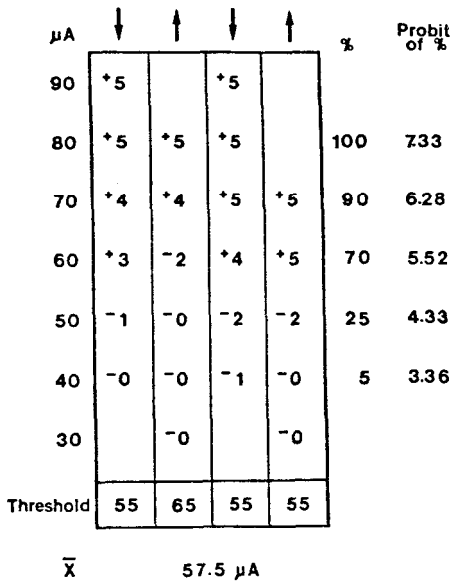

FIGURE 6. *Shown are the number of responses made at each intensify by a single animal in an experiment in which the step size was 10 μA and five trials were given at each intensity before moving to the next intensify. In this experiment, the stimulation started with five trials at 90 μA, followed by five trials at each of the lower intensities until the animal made fewer than two responses out of the five trials at two consecutive intensities (in this case, 50 μA and 40 μA). These are indicated by a minus sign. The next five trials were started at one step size lower (30 μA), and the ascending intensity trials were begun, When three or more* responses out *of the five* trials were made at two consecutive *intensities, indicated by a plus sign, a descending series was begun starting at one step size higher. This procedure* was *repeated until four columns were completed. Percentage of responses at each intensity were then computed and converted to probif. Because a probif of 100 does not exist, the probit for 99 percent is used. These percentage and probif values are used for computation of the power functions shown in figure 4. Threshold can also be approximated by faking the midpoint in each column between the step in which the animal made three or more and* two *or fewer responses. The threshold for the session* is *the mean of the column thresholds.*

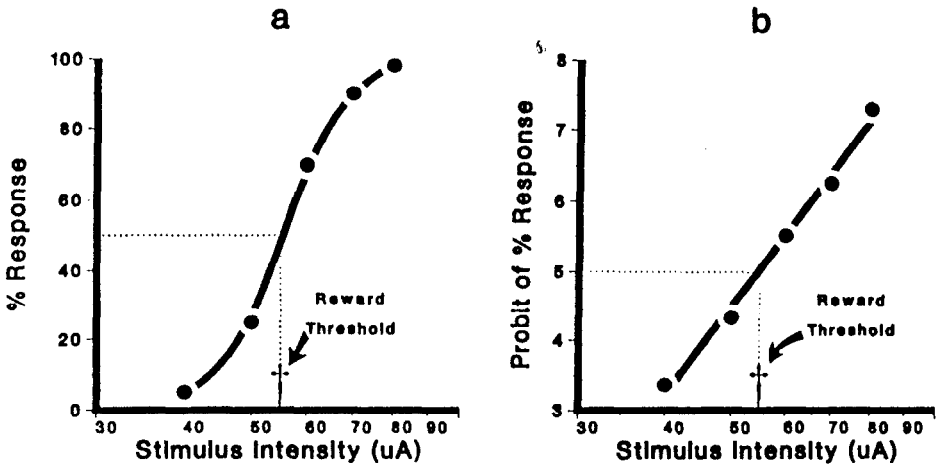

FIGURE 7. *Power functions from data shown in figure 6. a. Percentage response-intensity function in log representation. b. Probif-intensity function in log representation.*

the threshold obtained in the same group of animals *(r*= –.24, 31 *df)* (Kornetsky 1985; Payton 1986).

EFFECTS OF DRUGS ON BSR

Using the psychophysical discrete trial method, we have found that all drugs that lower the threshold for rewarding brain stimulation (table 1) are abused, have the potential for abuse as determined by studies in human subjects, or will be self-administered by animals. Although we have not been able to demonstrate that 9-tetrahydrocannabinol (9-THC) or the barbiturates lower the threshold for BSR, Gardner et al. (1988) have reported that by the psychophysical discrete trial method 9-THC will lower the threshold when the two-lever reset method is used, but only in the Lewis rat. Although results are somewhat mixed, some investigators have found that barbiturates as well as the benzodiazepines (Liebman 1985) will increase response rates at low doses.

The rate-independent threshold method often gives results similar to those in the rate-response method. There are, however, some marked differences, especially with opiate drugs (Kornetsky and Bain 1990). Most investigators have failed to find clear evidence that BSR will be facilitated the first time the

TABLE 1. *Effects of various drugs on brain-stimulation reward threshold*

Lowers	No change	Raises
Morphine	THC	Haloperidol
6-acetylmorphine	LSD	Pimozide
Buprenorphine	Naloxone	Chlorpromazine
Nalbuphine[a]	Naltrexone	Imipramine
Methamphetamine	Cyclazocine	Atropine
Amfonelic acid	EKC	Scopolamine
Tripelennamine[b]	Nisoxetine	
MDMA	Apomorphine	
Heroin	U50,488	
Cocaine	Pentobarbital	
Pentazocine[a]	Procaine	
d-amphetamine		
PCP		
Bromocriptine		
Nicotine		
Ethanol[c]		

NOTE: The drugs listed are only those that have been tested in our laboratory using the rate-independent threshold procedure. Some of those that cause no change, especially pentobarbital, might lower the threshold under drug self-administration conditions, as observed with ethanol.

[a]Especially in combination with tripelennamine.
[b]Especially in combination with pentazocine or nalbuphine.
[c]Only under conditions of self-administration (Moolten and Kometsky 1990).

animal is given morphine (Reid 1987). This has not been the case using the psychophysical discrete trial procedure. Figure 8 shows the threshold-lowering effects of the first exposure to 4.0 mg/kg of morphine (Izenwasser and Kornetsky 1987). We have consistently obtained threshold lowering at the first exposure to morphine (e.g., Marcus and Kornetsky 1974). The major difference between our finding and those of others in regard to the first exposure to morphine is probably related to the independence of the rate-independent threshold method of motor effects. The motor-depressant effects of morphine are probably paramount the first few times a rat receives morphine; this effect translates into decreases in rate of response. Changes in rate of response after drug are, as in most operant procedures, subject to rate dependency. Motor effects of a drug are often reflected in changes in rate of response in animals

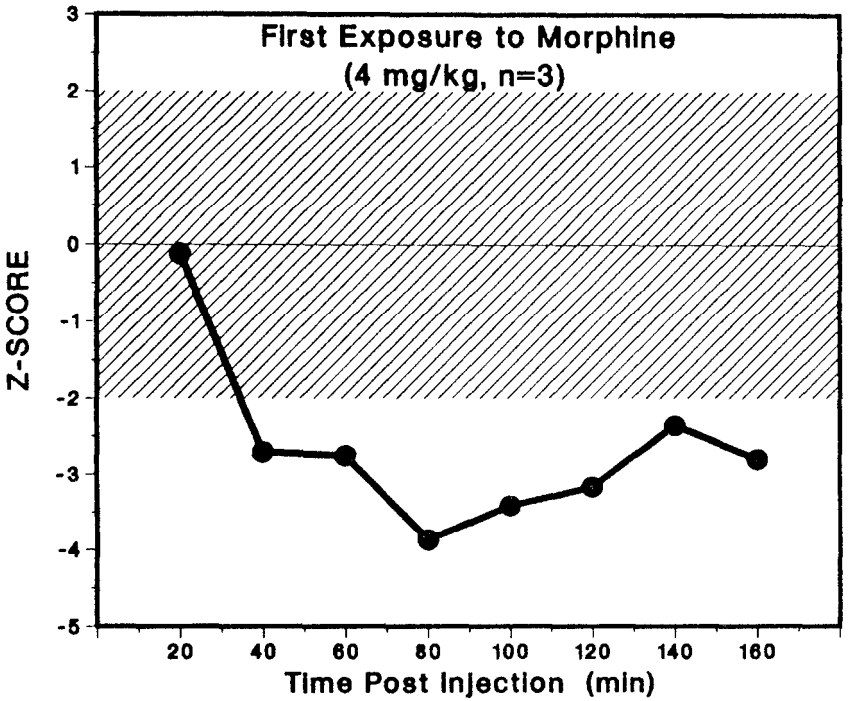

FIGURE 8. *Mean z scores of first-time administration of 4 mg/kg of morphine to five rats. The shaded area indicates the 95 percent confidence limits. (Adapted from Izenwasser and Kornetsky 1987.)*

that are working for natural reinforcers such as food, so that increases in response rate may be due to psychomotor stimulation-a phenomenon often seen after low doses of sedative-hypnotics-and decreases in response rate may reflect motor impairment.

The results we have obtained-as well as those of other investigators using different methods-indicate that BSR is a powerful model for studying of the rewarding effects of abused substances. Another major use for the technique is in studying the neurobehavioral bases of the reinforcing effects of such drugs. Although the model is not homologous with drug use in humans, as the drug self-administration model is, it has the advantage of being a direct measure of the action of drugs on the brain-reward system. The understanding of the

anatomy and the neurochemistry of this system and of how drugs change this system tells us much about the bases for the rewarding effects of the abused substances. For a review of the neurochemical bases of the reward system and the effect of drugs on this system see Phillips and Fibiger (1989).

SUMMARY

The robustness of the findings of the BSR effects of abused substances indicates that almost any technique used to measure these effects will be useful. The simplest procedure, however, of selecting a single intensity of stimulation and determining the effects of drugs on the rate of response for that selected intensity is fraught with difficulty in interpretation. More than 25 years ago the interpretation of changes in response rate as a reflection of changes in the reward value of the stimulation was challenged (Hodos and Valenstein 1962). Today it is rare to see a single stimulation intensity used in a published manuscript. However, most studies still use procedures of which rate of response is an integral part. Because animals will press a lever more than 80 times per minute and-on a continuous reinforcement schedule-receive as many as 80 stimulations a minute, conclusions about the specificity of the effects of drugs are difficult.

The results of BSR experiments on mechanisms of action of abused substances clearly indicate that the reinforcing effect of most, if not all, such substances is probably the result of activation of a reward system that originates in the cell bodies of the ventral tegmental area and courses rostrally to the limbic and frontal projection sites of the mesocortical system. Thus, we believe, the technique has clearly fulfilled its promise as a “window on the brain” (Olds 1977).

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New Neuroimaging Techniques for Investigating of Brain-Behavior Relationships

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INTRODUCTION

Neuroscientists using brain-imaging techniques to study human behavior have characteristically relied on methods that demonstrate anatomic structure to understand the neural substrates of cognitive function. Inferences about the relationship between structure and function are drawn by correlating of lesion locations with their effects on behavior. Characteristic methods for demonstrating lesion location have included neurological examination, neuropathological study at autopsy, and anatomic imaging techniques such as pneumoencephalography, angiography, and x ray computed tomography (CT). Studies of this sort have formed the basis of much of our present-day understanding of human cortical function and have provided important insights into the cerebral organization of language, memory, attention, and emotional processes in humans.

Within the past decade, new techniques for the imaging of brain function have been developed. These techniques, positron emission tomography (PET) and single photon emission computed tomography (SPECT), use radiation-detecting instruments (tomographs) to provide three-dimensional images of the distribution of injected radiotracers. By labeling a variety of molecules with radionuclides, researchers can study a variety of physiological processes.

This chapter reviews the recent developments in brain imaging. In particular, the use of functional imaging techniques to study a specific illness, Alzheimer's disease (AD), is discussed to demonstrate potential applications of these methods. The use of magnetic resonance imaging (MRI) for studying the relationship of brain structure to function is also discussed.

BASIC PRINCIPLES OF EMISSION TOMOGRAPHY

All emission tomographic techniques use an injected radioactive tracer that is detected by a tomograph. The tracer consists of a molecule of interest, for example, a metabolic substrate such as deoxyglucose, which is labeled with a radioactive isotope, such as ^{11}C or ^{18}F, that can be detected by the tomograph. The tomograph may use different principles to map accurately the distribution of the tracer (depending to a great extent on the nature of the emitted radiation), but the final result is a series of tomographic brain "slices" that provide three-dimensional information about the distribution of the tracer. The ultimate goal of these techniques is not simply the production of the image, but rather the quantitation of physiological processes. Models that rely on a priori information about the behavior of the tracer in vivo (such as the transport and metabolism of the molecule), in conjunction with time-varying measurements of the tracer in the circulation and the brain, are necessary for measuring these physiologic processes quantitatively. Once such information is available, the mathematics of the modeling is relatively straightforward, although assumptions about the behavior of the tracer must be carefully evaluated in each experimental situation.

The essential difference between PET and SPECT is in the types of radioactive isotopes used to label the molecules of interest. PET uses isotopes in which the decay process results in two high-energy photons that are always oriented 180" opposite each other. SPECT uses isotopes that decay by emitting single photons in random directions. Most of the PET isotopes have shorter half-lives than the SPECT isotopes. These differences have profound consequences for the radiochemical synthesis methods as well as for the nature of the tomographs and measurements that can be made.

PET tomographs consist of a radial array of crystal detectors that are electronically coupled to detect the simultaneous excitation of two opposing crystals by the simultaneously emitted high-energy photons. Thus, the positron emission can be localized to a line, and the reconstruction of an image from these multiple projection lines results in a map of the distribution of the tracer. The intrinsic property of electronic collimation leads to high sensitivity (generally defined as counts per millicurie of activity per second) for detecting of radionuclides. The consequence is better statistics describing tracer location than SPECT provides, because PET resolution is several millimeters higher than SPECT resolution. Also, the high sensitivity of PET translates into the

ability to acquire many counts in a short time and thus allows dynamic, time-varying measurements to be made quickly.

In SPECT imaging, the tracer map is ascertained by using collimation to localize the line along which a decay event occurred. Collimation results in restricting of the field of view of each detector element. Consequently, the decrease in sensitivity is greater with SPECT than with PET, which collimates electronically. The decrease in sensitivity of SPECT scanners is greater as the resolution is improved; the practical limitation is at resolutions less than about 10 mm. In addition, the problems of radiation scatter and attenuation by brain and skull are more difficult to solve with SPECT imaging than with PET, resulting in somewhat less accurate measurements of brain radioactivity. A more detailed analysis of the physics and instrumentation of these two techniques has been published by Budinger (1987).

The isotopes used in SPECT and PET obviously differ as well. These isotopes, and some of the compounds of interest that have been labeled with them, are shown in tables 1 and 2. The commonly used PET isotopes, particularly carbon and oxygen, are natural constituents of organic molecules. Therefore, use of these isotopes in synthesizing tracers frequently, preserves the biological behavior of the molecule of interest. The use of ^{18}F is also advantageous, because its relatively longer half-life makes it easier to work with. The breadth of possibilities for synthesizing tracers chemically is not as great for SPECT as for PET, because the radionuclides used in are not generally natural constituents of the biological molecules, e.g., iodine and technetium versus carbon and oxygen. The result is that SPECT tracers for human brain studies have been widely used only for measuring regional cerebral blood flow (rCBF). Many potential SPECT tracers for studying the dopamine, serotonin, and adrenergic systems are in various stages of development, however. New tracers for both PET and SPECT are being developed in many laboratories; the enterprise is important but time-consuming, because the in vivo behavior of a tracer must be understood entirely before mathematical modeling and quantitating of the physiological process of interest can be done.

PET and SPECT differ considerably in their applicability to neuroscience research questions. The short half-life of many PET isotopes makes using them simpler for repeated measurements and permits sequential studies to be made in the same patient. Because the variability of PET data appears to increase with the test-retest time period, studying the same subject at one sitting minimizes the problem of differences in the subject's physiological states at

TABLE 1. *Some PET tracers that have been used to study the human brain, the radionuclides that have been used to label them, and the physiological processes that they quantitate*

Isotope	Tracer	Physiological process
^{18}F	FDG (fluorodeoxyglucose)	Glucose metabolism
	Fluormethane	Blood flow
	N-methyl spiperone	D-2 dopamine receptors
	Fluoro-DOPA	Dopamine uptake and metabolism
	Haloperidol	D-2 dopamine receptors
^{15}O	O_2	Oxygen metabolism
	H_2O	Blood flow
	CO_2	Blood flow
^{11}C	CO_2	Blood volume
	Deoxyglucose	Glucose metabolism
	DMO	Tissue pH
	N-methyl spiperone	D-2 dopamine receptors
	Carfentanil	Mu-opiate receptors
	Diprenorphine	Mu-, delta-, and kappa-opiate receptors
	Leucine, methionine	Protein synthesis
	Methyl-bromo-LSD	S-2 serotonin receptors
	N-methyl nicotine	Nicotinic receptors
^{68}Ga	EDTA	Blood-brain barrier permeability

TABLE 2. *Some SPECT tracers that have been used to study the human brain, the radionuclides that have been used to label them, and the physiological processes that they quantitate*

Isotope	Tracer	Physiological process
^{123}I	IMP	Blood flow
	HIPDM	Blood flow
	QNB	Muscarinic cholinergic receptors
	IBZM	D_2 dopamine receptors
^{99m}Tc	HM-PA0	Blood flow
	ECD	Blood flow

different test times and decreases errors in repositioning the patient in the scanner. The longer half-life of SPECT isotopes generally requires that test-retest paradigms be performed on different days. Although many PET studies can be done using the longer lived tracer ^{18}F, which can be generated by a regional cyclotron for delivery to several local PET centers, using the shorter lived PET tracers such as ^{15}O and ^{11}C necessitates locating a cyclotron very near the PET scanner; additional expense and technical support are therefore involved. Improvements in the technology of compact cyclotrons specifically designed to produce radionuclides of interest for PET studies will lessen the technical support and space requirements for PET in the future. Because of the nature of the quantitative studies usually carried out by PET centers, only small numbers of subjects can practically be studied in a particular investigative protocol. SPECT is less technically demanding and consequently enables larger patient series to be studied. Tissue radioactivity can be measured more accurately with PET than with SPECT, mainly because the actual radioactivity-concentration measurement in brain structures requires an instrument resolution smaller than the structures of interest. PET resolution is close to the size of most small cerebral structures. A second advantage of PET is the ease with which dynamic studies can be performed because of its high sensitivity.

PET IN STUDIES OF ALZHEIMER'S DISEASE

Dementia is a progressive loss of cognitive abilities that interferes with function. It is most commonly caused by AD, which accounts for between 50 percent and 70 percent of all cases of adult dementia in numerous series (Tomlinson et al. 1970). Multiple cerebral infarctions (multi-infarct dementia, or MID) accounts for the second most common cause of dementia. Besides being a medical problem of enormous social and economic importance (Katzman 1976), AD presents a clinical problem as well, because no direct laboratory marker can confirm its presence during life. Thus, AD is a diagnosis of exclusion, which can be arrived at only after ruling out numerous other causes of dementia (some of which are curable) and can be confirmed only at autopsy. Although some aspects of AD have been reproduced in a variety of laboratory animals, there is no complete animal model of the illness; therefore, studies of the pathophysiology of the disease must be performed in humans.

Anatomic imaging studies have not generally been helpful in diagnosing AD; results are nonspecific. CT has confirmed pathological findings that gross atrophy is seen in dementia, with smaller brain size and enlarging sulci and

ventricles (Zatz et al. 1982). This atrophy is not specific for AD, because it occurs in other dementias as well as in normal aging. Although studies that use volumetric CT measurements generally show better separation of AD and control groups than linear measurements do, studies that use volumetric CT measurements usually find overlap among groups on these measures and have not been useful diagnostic criteria for individual cases (McGeer 1986). As a research tool, CT has demonstrated correlations between neuropsychological measures of dementia severity and brain atrophy (Chawluk et al. 1987) and has shown differences in the rate of progression of atrophy between AD and age-matched control subjects (Luxenberg et al. 1987). At present, however, CT scanning has been used clinically to exclude other dementia-causing illnesses rather than to diagnose AD directly.

The application of PET to the study of AD has delineated changes in regional cortical physiology and thus expanded our understanding of this disease. Frackowiak and colleagues (1981), using the isotope ^{15}O as elemental O_2 to study oxygen metabolism and as H_20 to study CBF, found diminished flow and metabolism in temporal and parietal cortex in AD. Concurrent measurement of flow and metabolism permits the cerebral oxygen extraction ratio (OER) to be evaluated. Frackowiak and colleagues found OER to be normal-evidence that the diminished flow is a consequence, and not a cause, of the metabolic deficit. Other laboratories have used glucose metabolic tracers and also found generally diminished glucose metabolism in temporal and parietal cortex (Benson et al. 1983; Duara et al. 1986).

In our laboratory we have used the glucose metabolic tracer fluorodeoxyglucose (FDG) in conjunction with the PET-280, a single-slice tomograph with 8-mm resolution (full width at half maximum) and a 10-mm slice thickness. More detailed characteristics of this instrument have been published previously (Derenzo et al. 1981). Initial studies of ten AD patients (Friedland et al. 1983) showed significant decreases in relative FDG uptake in temporoparietal cortex. Further investigation of this phenomenon in our laboratory was designed to quantitate these differences and attempt to understand their mechanisms.

Glucose metabolic rates may be estimated by two methods. The first, an autoradiographic method developed by Sokoloff and colleagues (1977) for use in animals, uses an operational equation subsequently modified by Phelps and colleagues (1979) for use with PET in humans. The technique permits the quantitation of regional cerebral metabolic rate for glucose (rCMRglu), with the

measurement of brain radioactivity at only one time point approximately 45 min following tracer injection. In addition, the time course of the tracer in plasma must be known and is generally obtained by multiple sampling from an artery or arterialized vein. Assumed values of rate constants (which may be measured separately or obtained from the literature) are then used in a correction term to account for the small amount of the tracer in the brain that remains unmetabolized. This technique has the advantages that it requires only one PET measurement to estimate rCMRglu and is relatively insensitive to small errors in the rate constants. Two shortcomings of this technique are that large errors in the rate constants may cause errors in the calculation of rCMRglu (Hawkins et al. 1981) and that the pathophysiology of the diminished glucose metabolism remains unknown when the operational equation is used, because diminished glucose metabolism may result from either diminished transport or phosphorylation.

The second method for quantitating rCMRglu uses dynamic PET data, that is, sequential PET data beginning immediately following injection of the tracer and continuing for 40 min or more. The simultaneous acquisition of a time-versus-activity curve describing the tracer time course in blood permits iterative least-squares fitting for the individual parameters of the Sokoloff model: k_1 and k_2 for forward and reverse transport of glucose, respectively, and k_3 for phosphorylation by the hexokinase enzyme. Estimating of these individual parameters might further elucidate of the mechanism of decreased glucose utilization.

We performed PET studies in 16 patients who met current research criteria for AD (McKhann et al. 1984) and in 7 age-matched controls. Subjects in both groups were free of significant medical illnesses and were taking no medication. The AD group averaged 65.5 years of age (SD = 6.6) and the control group averaged 63.0 years (SD = 3.0). The AD patients were mildly to moderately demented, and disease onset had occurred before age 65 in 12 of them. A subset of these patients (11 with AD and 6 controls) underwent dynamic PET scanning. A more detailed report of this study can be found in a recent publication (Friedland et al. 1989).

The dynamic PET experiments were designed to acquire time-activity curves for the PET data and the time course of the activity in blood. Immediately after the tracer was injected, blood was withdrawn from a heated hand vein, at short intervals at first, then at progressively longer intervals. Simultaneously, PET data were acquired, initially every 2.5 s or 5 s, then at increasingly longer

aintervals. Regions of interest were then drawn in PET images, and the time-activity curves for each brain region were calculated. These data were then fit with the blood time-activity curves by use of an iterative least-squares fitting technique to derive the rate constants for glucose transport and phosphorylation. Figure 1 shows sample PET data from a single region in one patient, in conjunction with blood data and the fit. Values for each rate constant were averaged in each patient group and then applied with the operational equation to determine metabolic rates for the patients who did not have dynamic studies and to determine metabolism in tomographic levels at which dynamic data were not obtained.

Although dynamic studies did not reveal differences between controls and AD patients for any of the model parameters K_1 through K_3, the variability of the data was large; coefficients of variation ranged from 18 percent to 45 percent. When this variability was reduced by normalizing the data with ratios of the parameter in a given brain region to the value in the entire cortex, we found that AD patients showed relatively reduced K_3 in the temporal cortex, the brain region that has been consistently most abnormal in AD.

Results using the operational equation also confirmed our earlier results, and those of others, that glucose metabolism is significantly decreased in temporal and parietal cortex. We also reduced the variability of these data by normalizing regional values for glucose metabolism by dividing the rCMRglu for a given region by the glucose metabolism in relatively normal brain regions, the thalamus and striatum. When this was done, relative reductions in glucose metabolism were apparent in all neocortical regions (frontal, parietal, temporal, and occipital cortex) but not in cerebellum or white matter.

These PET studies demonstrate a disturbance in neocortical glucose metabolism that, while involving all neocortical regions, is most prominent in temporal and parietal cortex and appears to be due to abnormalities of the hexokinase step of glucose metabolism and not to glucose transport. Nevertheless, it remains possible that the disturbed glucose transport is a result of loss of neuronal number in the image volume rather than a primary disturbance of glucose metabolism. Friedland and colleagues (1985*a)* have noted that the regions showing diminished glucose metabolism are also the sites of the most severe neuropathological involvement (Brun and Englund 1981).

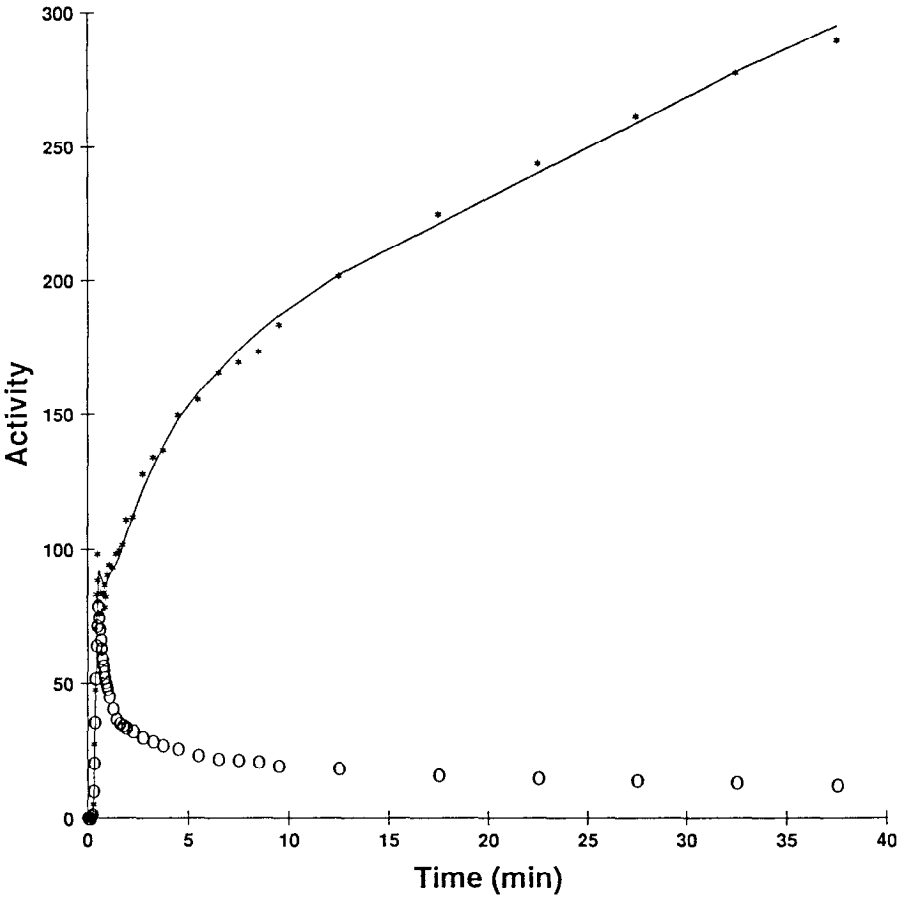


FIGURE 1. *Blood and brain time-activity curves from a dynamic positron emission tomography (PET) study of fluorodeoxyglucose (FDG) uptake. The (*) symbols represent the brain data obtained from sequential PET scans, the (O) symbols represent the time course of the blood radioactivity, and the solid line is the result of the model fit.*

Although the pathophysiology of glucose hypometabolism in AD is still not completely resolved, the technique has utility both for diagnosing AD and for understanding the production of disease symptoms. Several laboratories have shown that the neocortical glucose hypometabolism occurs early in the illness

when memory loss is the only significant abnormality (Haxby et al. 1986; Kuhl et al. 1987; Cutler et al. 1985) raising the possibility that the pattern of hypometabolism can predict which amnesiac patients will subsequently develop AD. In addition, Pick's disease, a dementia marked by frontal and temporal lobe neuropathology, shows a metabolic pattern different from that of AD (Kamo et al. 1987), with prominent frontal lobe hypometabolism. Whereas other dementias, including MID (Kuhl et al. 1985) and normal-pressure hydrocephalus (Jagust et al. 1985), have not generally shown the same metabolic pattern as AD, the pattern is clearly not entirely specific; it also has been seen in Parkinson's disease (Kuhl et al. 1984) and in one patient with Creutzfeldt-Jakob disease (Friedland et al. 1984).

The metabolic abnormalities in AD have been found to correlate with neuropsychological functioning and are thus clearly related to the disease symptoms. Patients frequently demonstrate asymmetries of glucose metabolism, and the nature of neuropsychological impairment is related to the hemisphere that is more severely hypometabolic (Friedland et al. 1985*b;* Haxby et al. 1985). AD patients with prominent disturbances in language, visuospatial relations, or other cognitive functions also demonstrate glucose metabolic abnormalities in brain regions known to correspond to the localization of these functions. Evidence suggests that early metabolic abnormalities may predict later neuropsychological deficits that will develop as the disease progresses (Haxby et al. 1987). It appears that as the disease progresses the frontal lobes eventually become affected and asymmetries tend to diminish (Jagust et al. 1988).

PET has also been used to investigate the blood-brain barrier in AD. Neuropathological evidence showing the frequent deposition of amyloid in leptomeningeal vessels suggests that abnormalities of the blood-brain barrier may play a role in the development of AD. PET studies using ^{68}Ga-labeled ethylenediamine tetra-acetic acid (EDTA), a large molecule normally excluded by the blood-brain barrier, have shown no transport of the tracer into brain tissue from the vascular compartment (Friedland et al. 1986; Schlageter et al. 1987).

Higher resolution instruments will be used in future PET work on AD. Our new tomograph, the Donner PET-600, has an in-plane resolution of 2.6 mm and an axial thickness of 5 mm (Derenzo et al. 1987). Images from a control and an AD subject are shown in figure 2. Improved resolution provides the ability to quantitate physiologic processes in small brain regions, particularly the mesial

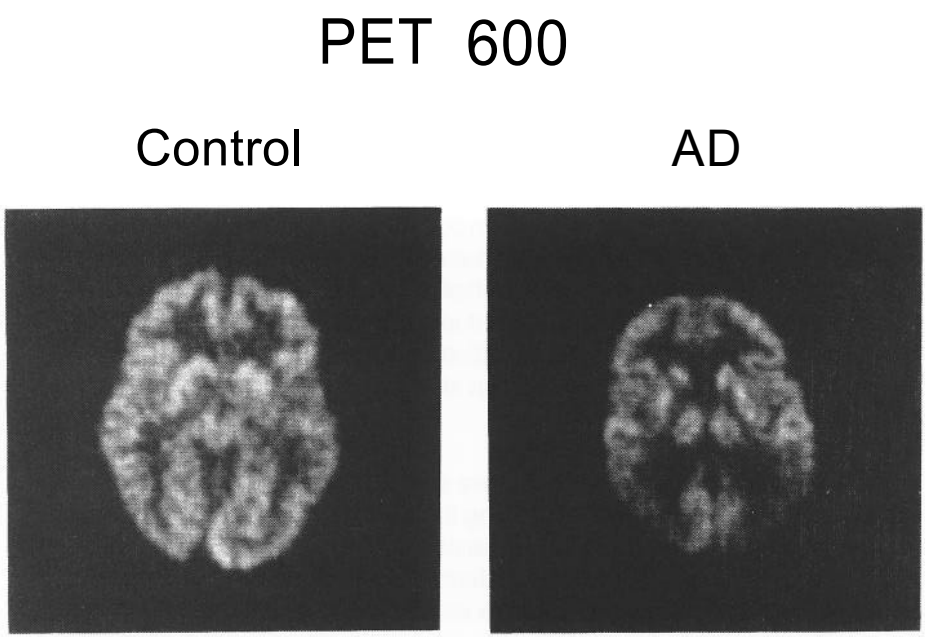


FIGURE 2. *Results of PET-FDG studies using the new PET-600 tomograph with 2.6-mm resolution. The subject with Alzheimer's disease shows bilateral decrements in tracer uptake in temporal cortex.*

temporal lobe structures, which we suspect are the earliest to be involved in AD. The potential use of PET with tracers to study human brain neurochemistry in AD will also provide a model for examining the effects of neurochemical lesions on behavior.

SPECT IMAGING IN AD

Initial findings using PET with FDG suggested that the abnormal pattern of glucose metabolism in AD could have diagnostic utility. The technical factors that have been described, however, largely limit the availability of PET to tertiary care university centers. SPECT imaging technology is immediately accessible to most community hospital nuclear medicine departments, and tracers for studying rCBF are now commercially available. Findings that rCBF appears normally coupled to regional metabolism in AD have supported the use of this technique in investigations of demented patients.

Studies of rCBF in AD patients have used both ^{123}I-labeled *N*-isopropyl-piodoamphetamine (IMP) and ^{99m}Tc-labeled hexamethyl propyleneamine oxime (HM-PAO) with similar results (Johnson et al. 1987; Sharp et al. 1986; Cohen et al. 1986; Gemmell et al. 1987). Prominent temporal and parietal hypoperfusion is seen when both tracers are used. Methods of data analysis have included visual inspection and within-patient ratios of regional radioactivity distribution and generally show some overlap between AD and control subjects. In addition, the frequent asymmetry of the temporoparietal lesions has resulted in the misclassification of AD patients as having MID, a problem that can be avoided by using an anatomic imaging technique in conjunction.

In our initial series (Jagust et al. 1987) we studied nine AD patients, five controls, and two patients with MID using the tracer IMP. Because of the technical problems limiting accurate measurements of tracer uptake, we did not model and quantitate rCBF in these patients but rather used a ratio of the radioactivity in a region of interest to the activity in the entire tomographic slice. Initial results showed that the temporoparietal-to-whole-slice ratio completely discriminated the AD from the control and MID subjects. In addition, this ratio strongly correlated with dementia severity as measured using the Mini-Mental State Examination (MMSE) (Folstein et al. 1975).

Other groups also have noted relationships between tracer uptake and dementia severity (Johnson et al. 1988). Studies of patients with non-Alzheimer types of dementia, particularly those that clinically appear to affect the frontal lobes, have shown abnormalities of frontal lobe structures rather than the temporoparietal abnormalities of AD (Jagust et al. 1989).

SPECT series of AD patients also show relationships between regions of diminished tracer uptake and cognitive performance (Perani et al. 1988). These results are quite similar to results obtained by PET and demonstrate the pathophysiological basis for the disease's heterogeneity. Indeed, a considerable advantage of SPECT over PET is that its relative low cost and ease of use allow larger subject groups to be studied. This point is important in illnesses like AD, in which biological differences between individuals may be large.

The study of larger patient groups has also indicated that the technique is neither completely sensitive nor specific for the diagnosis of AD. We recruited patients in the earliest stages of AD to evaluate the sensitivity of SPECT and

IMP for diagnosing the cause of dementia (Reed et al. 1989). We studied 21 patients, all of whom had SPECT scans and neuropsychological testing. Nine were mildly demented, with scores on the MMSE in the normal range (mean = 26.6, SD = 1.7), although all complained of memory loss and were felt to have AD; 12 were moderately demented (MMSE mean score = 15.0, SD = 5.4). Thirty-six healthy, age-matched control subjects were studied; 14 received SPECT scans and 22 underwent neuropsychological testing, but no control subject received both. We quantitated SPECT data by calculating an rCBF ratio defined as the ratio of regional radioactivity normalized to occipital cortical radioactivity. We studied four cortical rCBF ratios-orbitofrontal and dorsolateral frontal cortex-and temporal and parietal cortexand found considerable overlap among patients (both mild and moderately demented) and control subjects in all brain regions. Furthermore, when SPECT and neuropsychological abnormalities were defined as scores outside the 95 percent confidence intervals for the control-group means, all AD patients showed memory abnormalities, whereas only 55 percent of the mildly demented patients and 83 percent of the moderately demented patients showed temporal or parietal perfusion deficits. Temporal or parietal SPECT abnormalities, however, were seen in only 7 percent of control subjects, suggesting that the presence of abnormalities might indicate dementia more reliably than does the presence of abnormal memory function, which was seen in 27 percent of control subjects. Although other methods of SPECT data analysis might be more sensitive for diagnosing AD (such as visual inspection by a trained observer), it is clear that a normal SPECT scan does not rule out the presence of AD.

These preliminary results with SPECT provide general guidelines for both the clinical and research uses of the technique. As a diagnostic tool in dementia, SPECT is clearly not a definitive tool. It may be useful, however, in difficult diagnostic cases, such as patients with early symptoms, because findings of temporal and parietal hypoperfusion support the diagnosis of AD. SPECT may also be useful in diagnosing patients with unusual dementia symptoms, such as the apathy and depression seen in frontal lobe dementia syndromes. As a research tool, SPECT is useful for studying clinical problems in which biological heterogeneity limits PET studies of small patient groups.

MRI IN DEMENTIA

A primary advantage of MRI over traditional methods of anatomic brain imaging is its inherent contrast sensitivity. The use of different pulse sequences to

image the brain allows the distinct visualization of grey and white matter and the delineation of both from cerebrospinal fluid (CSF). The sensitivity of the technique to small changes in water content is also useful in detecting small lesions in grey or white matter.

An important application of MRI to the study of dementia has been its use to define the extent of cortical atrophy, because sulcal detail is not obscured by the beam-hardening effects of CT. When MRI measurements are compared with CT measurements, they show similar estimates of ventricular size but different estimates of sulcal size; MRI provides a better measure of cortical atrophy (Chawluk et al. 1987).

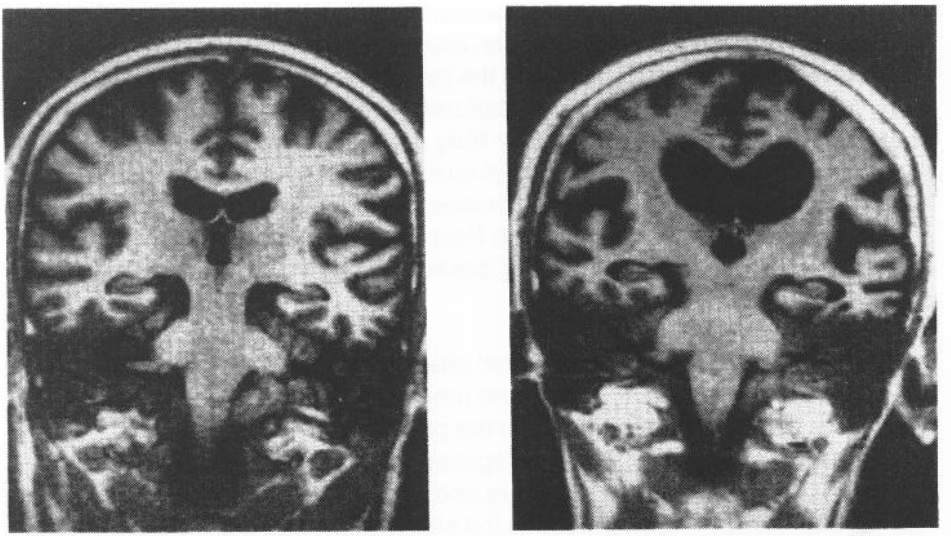
MRI has also been used to evaluate changes in the white matter in dementia. Several studies have demonstrated that white-matter lesions (WMLs) occur in both the nondemented and demented elderly; similarities exist between groups in both the configuration and extent of the lesions except for the presence of large, confluent WMLs in MID (Hershey et al. 1987). These lesions have not correlated with dementia severity, and some AD patients have not had any WMLs at all. Although finding these lesions on MRI frequently guides the physician toward the diagnosis of MID or Binswanger's disease, these abnormalities may be misleading, because their pathological significance is not well understood.

Recent work from our laboratory has made use of the greater contrast sensitivity of MRI to evaluate specifically the brain region that is most implicated in AD-the hippocampus (Seab et al. 1988). We used the IBM-MIT-LBL 0.5 T imager and a phase-corrected inversion-recovery sequence. By taking scans 5 mm thick at 8-mm intervals (figure 3), we were able to localize the slice containing the hippocampus and model its size as an elongated ellipse. The comparison of AD patients and controls demonstrated that hippocampal size (normalized to the size of the lenticular nucleus) completely differentiated all ten AD subjects from seven controls. Although this is a preliminary study of a small patient group, it demonstrates that anatomical imaging may be useful in studying of a specific brain structure in AD.

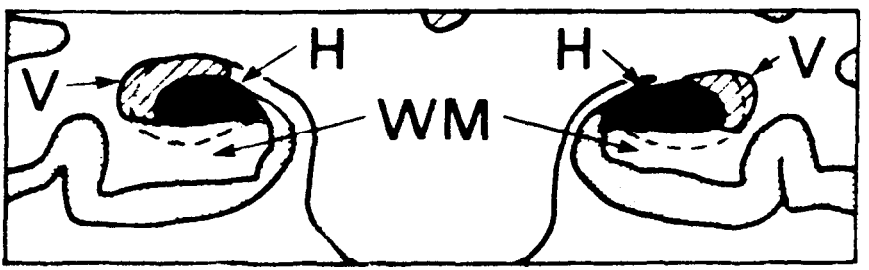
NEUROIMAGING STUDIES OF BRAIN AND BEHAVIOR

The imaging studies reviewed here infer relationships between brain physiology and behavior through correlations, such as the relationship between metabolism in a given brain region and performance on a neuropsychological

NMR



Normal Alzheimer's Disease



Pattern of hippocampal atrophy: Solid area represents atrophic hippocampus. Dashed line is outline of normal hippocampus. H=hippocampus. WM=white matter. V=ventricle.

FIGURE 3. *Nuclear magnetic resonance imaging of a patient with Alzheimer's disease compared with that of a control subject, demonstrating hippocampal atrophy. Studies used a phase-corrected inversion-recovery pulse sequence (TR = 200, TI = 500, TE = 33 ms) with 5mm-thick sections spaced at 8-mm intervals.*

test in a patient group. Such methods have obvious drawbacks, including the difficulty in concluding a causal mechanism because of the possible eff acts of an unknown and unmeasured variable. Studies that use behavioral tasks to activate specific brain regions during the performance of a PET scan have many advantages for studying the relationships between brain and behavior. These techniques are demanding, for they generally involve the use of short-lived tracers and performance of multiple cognitive tasks at a single sitting. Several reviews of these experimental procedures and their results have been published (Petersen et al. 1988; Posner et al. 1988), demonstrating the utility of the technique for uncovering functional consequences of cognitive activity in humans.

PET tracers designed to study regional changes in neurochemistry also have wide potential application to behavioral research. Changes in neurotransmitter receptor number may be important in the production of disease symptoms in schizophrenia (Seeman 1987) and depression (Mayberg et al. 1988). The use of ligands to evaluate opiate receptors and dopamine receptors in the brain also has obvious potential applications to the study of drug abuse. Similar ligands will undoubtedly also become available for SPECT imaging.

The advantages of MRI in localizing and quantitating the size of different brain structures will enhance our ability to relate structure to function in normal subject populations and in illnesses such as AD, in which no discrete brain lesion is present. Thus, high-resolution MRI studies of patients with amnesia of unknown cause have demonstrated bilateral structural changes in the hippocampus (Press et al. 1989). Gazzaniga (1989) has noted that magnetic resonance images of the corpus callosum in monozygotic twins provide strong evidence of genetic factors influencing brain structure.

The study of behavior by means of these new imaging techniques has important implications for neuroscientists. It is now possible to image human metabolism and blood flow in three dimensions and to quantitate such physiologic processes accurately. Measurements of neurotransmitter receptor number and affinity are also possible. The ability to make these measurements noninvasively and safely allows them to be repeated over time, so that the effects of mental activation, drugs, treatments, or disease can be evaluated. The coupling of such measurements with the use of MRI to image anatomy in detail holds great promise for understanding the neural mechanisms underlying human behavior.

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Studying Psychoactive Drugs With Positron Emission Tomography: Relationships Between Mood and Metabolic Rate

Harriet de Wit, John Metz, and Malcolm Cooper

INTRODUCTION

The study of neural mechanisms underlying the effects of abused drugs has, for practical and ethical reasons, been limited largely to studies using laboratory animals (Snyder 1984; Koob and Bloom 1988). Most current techniques in neuroscience require invasive procedures that cannot be conducted with human subjects. The use of animals as subjects, however, requires inferences across species, and it is sometimes difficult to determine which of a drug's multiple pharmacologic effects are related to its potential for abuse in humans. Recently, techniques that permit us to study the effects of psychoactive drugs on neural activity in normal, awake human subjects have become available. These techniques eliminate the need for cross-species inferences and allow us to study neural events in relation to other drug effects that are known to be related to drug abuse, such as the drugs' subjective effects. The subjective, or mood-altering, effects of drugs (such as euphoria) are considered to be central to the initiation and maintenance of drug-seeking behavior; therefore, a better understanding of the relationship between the subjective experiences and corresponding neural events will greatly advance our knowledge of the biological basis of drug abuse.

In the present study, positron emission tomography (PET) was used with a radioactively labeled glucose analog-18-F-2-deoxy-2-fluoro-D-glucose (FDG) as the tracer. This technique provides a measure of regional cerebral glucose uptake, which is thought to be an indicator of neuronal activity. Two placebo-controlled studies were conducted in normal, awake volunteers, examining the effects of (1) ethanol (ETOH) and (2) diazepam (DZP). We

studied the metabolic changes induced by the drugs and the relationship of these metabolic effects to the drugs' effects on mood and subjective state.

An important feature of these studies was that the subjective effects of the drugs were monitored at the same time the metabolic data were obtained. It is known that many effects of drugs, including subjective effects, vary both across individuals and across settings (Galizio and Maisto 1985). Even drugs that are usually considered to be reliable euphorigenic agents, such as amphetamine and alcohol, produce dysphoric effects in some individuals (de Wit et al. 1986, 1987, 1989), and the environmental settings in which drugs are administered are known to alter profoundly their mood-altering effects (McCarty 1985). Therefore, in studying the neural substrates of subjective drug effects, it is important to verify that the drugs are actually producing their expected subjective effects.

The studies described here were initiated to explore the biological basis of individual differences in responses to ETOH in normal volunteers. It had been observed in previous studies (de Wit et al. 1987, 1989) that normal social drinkers varied widely in their subjective responses to a low dose of ETOH: some subjects experienced stimulantlike effects, whereas others experienced sedation. These differences in subjective effects corresponded to differences in ETOH preference in a choice test: the subjects who experienced ETOH as stimulantlike chose it more often than a placebo, whereas those who experienced ETOH as sedativelike chose the placebo more often than ETOH. One goal of the present studies with PET was to explore possible physiologic bases of individual differences such as these. The more immediate goals were simply to assess the effects of ETOH and DZP on regional cerebral metabolic rate for glucose (CMRglu) and to determine the relationship between the metabolic and the mood-altering effects of the drug.

STUDY 1: ETHANOL

Study 1 consisted of two phases: a behavioral phase and a PET phase. The behavioral phase was a seven-session preference experiment conducted under seminatural conditions to assess the subjective and reinforcing effects of ETOH in a recreational setting. After completing the behavioral phase, subjects were tested in the PET phase, which consisted of three brain scans-one with placebo, one with 0.5 g/kg of ETOH, and one with 0.8 g/kg of ETOH. The behavioral phase provided a "natural" referent against which to assess the

generality of the subjective responses to ETOH that were obtained in the highly unnatural conditions of the PET setting.

Procedure

Subjects. Eight normal, healthy males participated in study 1. All were students aged between 21 and 29 years, and they were light social drinkers (mean, 6.0 drinks per week; range, 1-20 drinks per week). Most subjects reported having tried marijuana at least once but had little experience with other drugs. They were screened for physical and psychiatric health before participating, and they had read and signed a consent form that outlined the procedures to be used.

Behavioral Phase. Subjects participated in seven sessions, which were conducted one or two evenings a week from 7 p.m. to 11 p.m. The sessions were conducted in a recreational environment consisting of two comfortably furnished rooms with television, radio, movies, and a variety of games. Subjects were tested in groups of four individuals to create a natural social situation. During the sessions when no other events were scheduled, subjects engaged in leisure activities of their choice but were not allowed to work or study. The first four sessions were sampling sessions, during which either ETOH (0.5 g/kg) or placebo was administered in color-coded cups. The last three sessions were choice sessions, during which subjects chose whichever beverage they preferred. Subjects were told that they might receive a tranquilizer, a stimulant, alcohol, or a placebo, but that the same substance would always be in a cup of the same color. During sampling sessions, ETOH and placebo were administered during alternating sessions in counterbalanced order across subjects. The beverages were administered in divided unit doses of 0.1 g/kg, ingested at 15-min intervals. During the sampling sessions, five unit doses were administered between 7 p.m. and 8 p.m. During choice sessions, subjects could take from 1 to 11 doses of their chosen substance-i.e., a total dose of 1.1 g/kg of ETOH if they chose the ETOH beverage—between 7 p.m. and 10 p.m. ETOH beverages consisted of 10 percent ETOH by volume mixed with tonic and lime (total volume, 450 mL for a 70-kg subject), and placebo beverages consisted of mix with 1 percent ETOH as taste mask. The number of choice sessions during which ETOH was selected over placebo was the measure of drug preference, and the number of unit doses taken was the measure of dose preference.

During each session, subjects completed mood and drug effects questionnaires before ingesting the beverages and at regular intervals throughout the sessions. They completed the Profile of Mood States (POMS) (McNair et al. 1971), a 72-item adjective checklist consisting of eight primary scales (anxiety, depression, anger, vigor, fatigue, friendliness, confusion, and elation) and two composite scales (arousal and positive mood). At regular intervals, subjects also completed drug-liking and identification questionnaires and tests of psychomotor performance. Several of the questionnaires were also used in the PET phase that followed, to facilitate comparisons of the subjective effects of ETOH across settings.

PET Phase. After the behavioral phase was completed, subjects participated in the PET phase, which consisted of three weekly sessions. A PET scan was conducted during each of these sessions, preceded by administration of a low or moderate dose of ETOH (0.5 g/kg or 0.8 g/kg) or a placebo beverage.

For 12 hours before each session, subjects fasted and abstained from beverages containing sugar, caffeine, and alcohol. They reported to the PET laboratory between 9 and 11 a.m., at which time a transmission scan was conducted for attenuation correction. Intravenous catheters were inserted in each arm, one for injecting the tracer and one for blood sampling. After the catheters had been inserted, subjects completed a predrug POMS and practiced a visual monitoring task (VMT). The VMT consisted of bright and dim light flashes presented at 4-s to 7-s intervals. Subjects were instructed to press a handheld button after each dim flash but not after the bright flashes, and their reaction times and accuracy were recorded. Subjects performed this task throughout the scanning period, except for a brief pause to complete the first postdrug POMS. The function of the VMT was to stabilize cognitive activity across sessions and across individuals; in the absence of such a task there is wide variability in the level of cognitive activity (e.g., wakefulness) during scans, which could obscure the effects of drug treatments. After practicing the VMT and completing the presession POMS, subjects ingested (double-blind) a beverage containing 0.5 g/kg ETOH, 0.8 g/kg ETOH, or placebo. The beverages, which were similar to those administered in the behavioral phase but smaller in volume, were administered in five equal portions at 1-min intervals. Immediately after ingesting the beverage, a subject was positioned in the scanner (this took about 5 min), began the VMT, and was then injected with the FDG. The subject remained in the scanner for the next 40 min, continuously performing the VMT except for a brief pause after 20 min to complete subjective-effects questionnaires. The POMS and a drug-liking questionnaire

were presented on a Macintosh computer placed in front of the subject while he remained positioned in the scanner. At the end of the scanning period, the subject completed a final POMS and drug-liking questionnaire.

PET studies were performed in "dynamic" mode (Huang et al. 1980), although the data reported here were reconstructed using the autoradiographic method (Phelps et al. 1979; Reivich et al. 1979). Data were obtained by use of **a** three-ring PETT VI scanner (Ter-Pogossian et al. 1982) which provides five transaxial image slices with an interslice spatial resolution of 8 mm (full width at half maximum) and an interslice distance of 14 mm. The PET images integrated metabolic activity occurring during the period from 10 to 50 min after the ETOH was ingested. Subjects were injected intravenously with 5 to 10 mCi of FDG within 5 min of ingesting the ETOH beverage. Periodically throughout the scanning period 1-cm3 blood samples were taken for use in converting radioactive counts to metabolic rates as well as to verify the stability of glucose levels after ETOH administration and to determine blood ETOH level.

CMRglu was assessed bilaterally in seven regions of interest: four **cortical** regions (temporal, parietal, occipital, and frontal), basal ganglia, thalamus, and cerebellum. These regions were identified on each reconstructed image by **a** technician who was blind to the experimental conditions. The regions of interest were relatively large so that they remained well within the limits of resolution of the scanner (8 mm). A measure of global CMRglu was obtained in each PET session by averaging all 14 regions for each subject. To compare across conditions, relative CMRglu in each region of the brain was calculated as a ratio of the average (whole-brain) rate.

Results

Behavioral Phase. Seven of the eight subjects chose the ETOH-containing beverage in at least two of the three choice sessions and reported liking the ETOH beverage more than the placebo. The remaining subject chose the placebo during all three sessions and reported liking ETOH less than the placebo. Most of the subjects correctly identified ETOH during sampling sessions, except for the subject who chose the placebo and labeled ETOH a "tranquilizer." The average dose of ETOH ingested during choice sessions was 0.62 g/kg. In sampling sessions, blood ETOH level reached an average peak level of 0.028 g/dL at approximately 17 min after the last dose. In choice sessions, blood ETOH level varied according to the self-administered dose. The POMS data obtained on the sampling sessions indicated that most subjects

experienced typical, ETOH-like subjective effects from the drug, including increases on the vigor, friendliness, and elation scales and decreases on the anxiety and fatigue scales (figure 1). Subjects' ratings of drug liking during the sampling sessions were positively correlated with the number of doses they subsequently ingested during the choice sessions (R = .73). Thus most, but not all, subjects showed positive mood responses to ETOH in the naturalistic situation, and their subjective responses to ETOH were consistent with their choice of the drug.

PET Phase. On average, ETOH produced subjective effects in the PET setting similar to those produced in the naturalistic setting, e.g., increases in friendliness and elation (figure 1). There was considerable variability in responses across individuals, however, and the POMS scores of individual subjects after ETOH were not significantly correlated across the two settings. Ratings of drug liking for individual subjects, however, were moderately correlated across the two settings, indicating some stability of responses within individuals. The one subject who did not choose the ETOH in the behavioral phase was also the only subject to rate his liking of ETOH lower than his liking of placebo in the PET phase, and to misidentify both doses of ETOH (he labeled it "tranquilizer" at the low dose and "stimulant" at the higher dose).

Blood ETOH level reached a mean peak level of 0.044 g/dL at 46 min following the 0.5-g/kg dose of ETOH and 0.062 g/dL at 58 min following the 0.8-g/kg dose. The fact that blood ETOH level was higher in the PET phase after the 0.5-g/kg dose than after the same total dose in the behavioral phase can be accounted for by differences in rate of drug administration (5 min vs. 1 hr) and possibly by the fact that subjects fasted before drug ingestion in the PET phase but not in the behavioral phase. ETOH did not affect either reaction times or accuracy on the VMT, nor did it change plasma glucose levels.

Average global metabolic rates were 8.88 mg/100 g per minute during the placebo sessions, 8.55 mg/100 g per minute after the low dose of ETOH, and 8.35 mg/100 g per minute after the moderate dose of ETOH. Only the difference between the higher dose and placebo approached statistical significance ($p < .06$). At the 0.5-g/kg dose, global CMRglu decreased in five of the eight subjects relative to placebo, and at the 0.8-g/kg dose, CMRglu decreased in seven of the eight subjects. In one subject (the same individual who showed an idiosyncratic response in the behavioral phase) average CMRglu increased after each dose of ETOH.

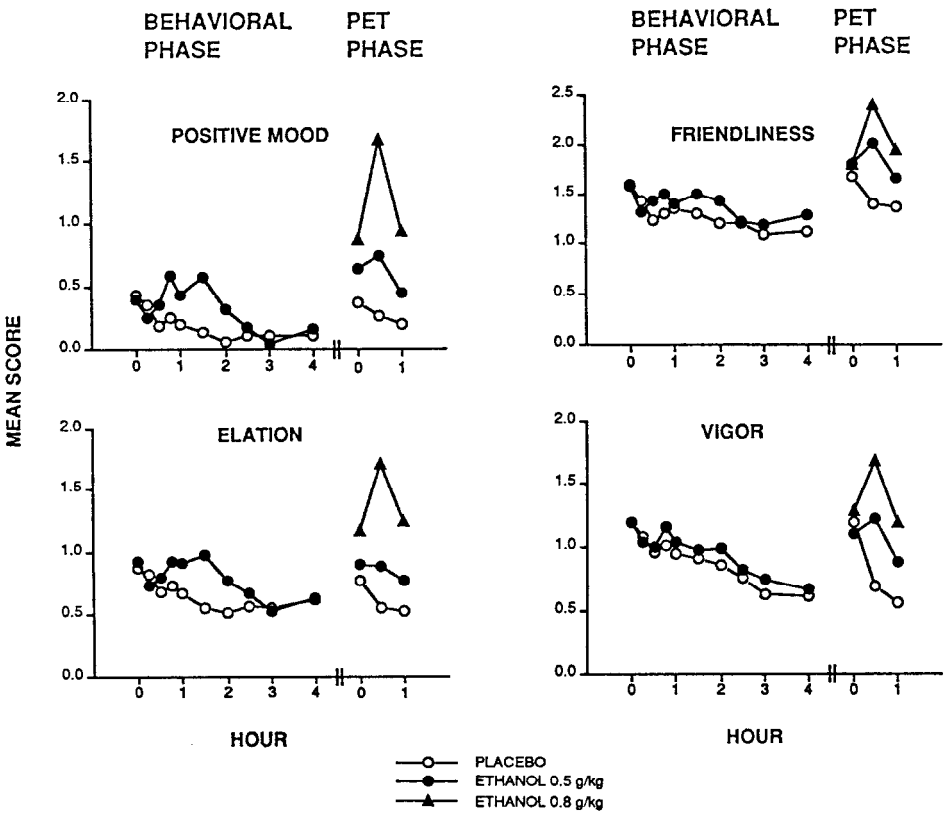

FIGURE 1. *Mean scores* on *four Profile of Mood States scales after administration of placebo and ethanol during the behavioral phase and the position emission tomography (PET) phase of study 1. Behavioral phase data are based on the mean scores from the two sampling sessions; the PET phase data are* based *on the mean of the three scores (1) before beverage administration, (2) 20, and (3) 40 min into the scanning period.*

The effects of ETOH on CMRglu (figure 2) appeared to be fairly uniform across all the regions of interest, although at both doses the thalamus was relatively less affected than other parts of the brain. During all sessions, regardless of drug administration, CMRglu was higher in the left hemisphere than in the right-a difference that has been observed previously (Wagner et al. 1986)—but relative CMRglu in different regions of the brain remained the same. Neither global nor regional changes in CMRglu were related to blood ETOH

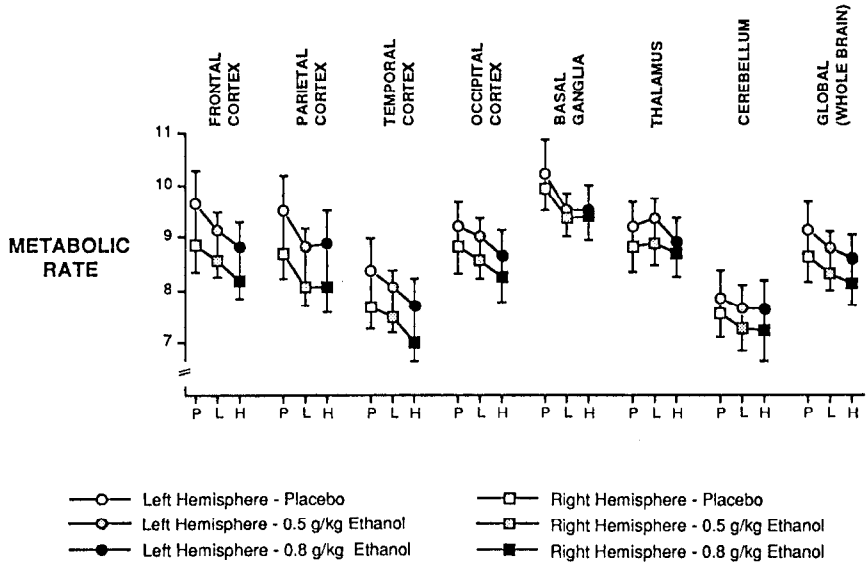

FIGURE 2. *Mean (and SEM) cerebral metabolic rate for glucose in each region of interest after administration of placebo, and 0.5 g/kg of ethanol (ETOH) and 0.8 g/kg of ETOH in eight healthy males. Whole-brain CMRglu was calculated by averaging the values for regions of interest for each subject.*

level, reaction times, or plasma glucose level. Regional changes in CMRglu after ETOH were examined by calculating ratio scores of particular brain regions relative to the rest of the brain. The ratio of regional CMRglu to whole-brain CMRglu during placebo sessions was subtracted from the ratio of regional CMRglu to whole-brain CMRglu during ETOH sessions. These regional-change scores were then correlated with mood changes after ETOH. Mood changes for each POMS scale were calculated by subtracting predrug scores from the first postdrug score (20 min into the scan) and then subtracting the placebo-difference score from the ETOH-difference score. The effects of ETOH on regional CMRglu were then correlated with its effects on mood. The results of this exploratory correlational analysis are presented in tables 1 and 2, which show correlations that exceeded an arbitrarily selected cutoff value of $r = .70$ (.71 is the level that would be significant at the $p = .05$ level if only two variables were being compared). In view of the small number of subjects and the large number of dependent variables, these correlations can only be

TABLE 1. *Summary of correlations between changes in mood and CMRglu after administration of low dose of ethanol (0.5 g/ks)*

Region of interest	Negative moods[a]		Positive moods[a]			
	Co	Dp	El	PM	Vg	Fr
Left parietal	—	-.71	.82	.85	.83	.89
Left basal ganglia	-.86	—	—	—	—	—
Right occipital	.71	.71	—	—	—	—

NOTE: Co = confusion; Dp = depression; El = elation; PM = positive mood; Vg = vigor; Fr = friendliness; — = correlation < .70.

[a]POMS scales.

TABLE 2. *Summary of correlations between changes in mood and CMR after administration of moderate dose of ethanol (0.8 g/kg)*

Region of interest	Negative moods[a]			Positive Moods[a]		
	Co	Fa	Ag	El	PM	Ar
Left temporal	-.77	-.94	—	—	—	.86
Left thalamus	—	.79	—	—	—	—
Left cerebellum	-.78	—	—	—	—	—
Right parietal	—	—	.80	—	—	—
Right occipital	—	—	—	-.74	-.72	—

NOTE: Co = confusion; Fa = fatigue; Ag = anger; El = elation; PM = positive mood; Ar = arousal; — = correlation < .70.

[a]POMS scales.

considered exploratory. The lack of consistency in the patterns of correlations between CMRglu and mood across the two doses suggests, however, that no systematic relationships exist between the mood-altering effects of ETOH and its effects on regional CMRglu.

STUDY 2: DIAZEPAM

In study 2, a separate group of eight subjects participated in three PET sessions—one with placebo, one with 0.07 mg/kg of DZP, and one with 0.14 mg/kg of DZP. (These doses of DZP are equivalent to about 5 mg and 10 mg for a 70 kg person.) (Although six of the eight subjects also participated in a natural-choice experiment with DZP before the PET scans, data from that study are incomplete and are not reported here.)

Procedure

Eight normal, healthy males similar to those described in study 1 participated. Their average age was 24.5 years (range, 21-29 years), most were students, and they drank on average 6.25 alcoholic drinks per week (range, 2-20). Use of other drugs was minimal. The subjects were screened for physical and psychiatric health.

The three PET sessions were conducted according to the same procedures described for ETOH, except that capsules containing DZP or placebo were administered 30 min before the FDG was injected. To further blind the drugs, a placebo beverage similar to that used in the ETOH experiment was also administered immediately before the FDG was injected; subjects were told that they might receive a stimulant, a tranquilizer, alcohol, or a placebo. Subjective-effects questionnaires were administered before subjects ingested the capsules and 20 and 40 min after scanning had begun (i.e., 50 and 70 min after subjects had ingested the capsules). All other procedures were as described in study 1.

Results

Relative to placebo, average global CMRglu decreased significantly *(t* tests, $p < .04$) after both doses of DZP. The means were as follows: (1) placebo, 8.25 mg/100 per minute; (2) 0.07 mg/kg DZP, 7.36 mg/100 per minute; and (3) 0.14 mg/kg DZP, 7.42 /100 per minute.) In seven of the eight subjects, global CMRglu decreased after both doses of DZP, whereas in one subject, CMRglu increased after both doses. CMRglu decreased to about the same level in all regions of the brain after DZP (figure 3), except in the thalamus and basal ganglia, which were relatively less affected by the drug, particularly at the higher dose. Although DZP decreased accuracy in the VMT task (96 percent correct after placebo and 86 percent correct after both doses of DZP), it did not

change reaction times. Subjects most often thought they had received a tranquilizer, even when they had received a placebo. Accordingly, POMS arousal scores decreased over the course of all sessions, i.e., from predrug to 20 and 40 min after FDG injection, regardless of whether subjects received DZP or placebo (figure 4). The only measure that clearly differentiated DZP from placebo was the POMS anxiety scale; scores decreased after the higher dose of DZP. Liking ratings were higher after DZP was ingested at both the low dose and the moderate dose than after placebo was ingested. The one subject whose metabolic rate increased after ingesting DZP was also the only subject to label the higher dose of the drug "placebo," and he was the only subject who rated his liking of DZP lower than placebo.

Correlations between regional CMRglu and mood were conducted using the same formula described in study 1 (tables 3 and 4). The consistently high correlations between CMRglu and measures of sedation (fatigue and arousal)

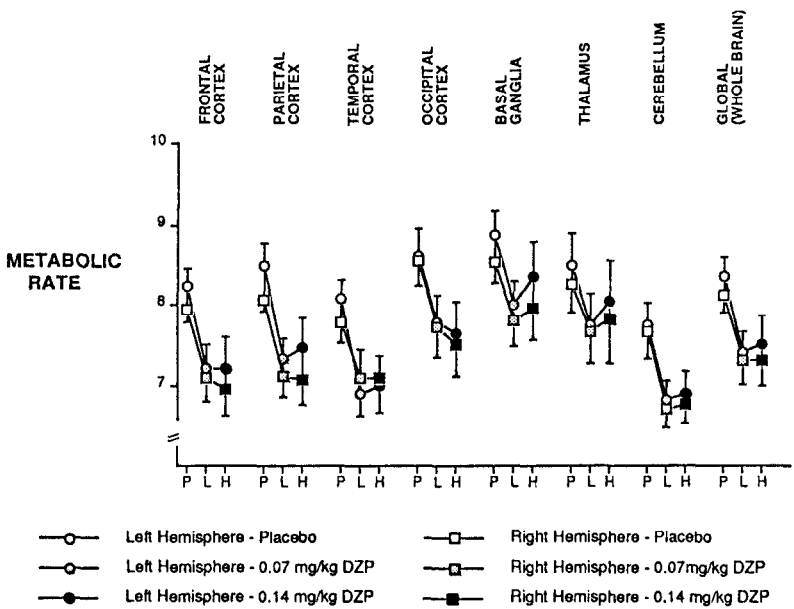

FIGURE 3. Mean *cerebral metabolic rate for glucose (CMRglu) in each region of interest after administration of placebo, and 0.07 mg/kg of diazepam (DZP) and 0.14 mg/kg of DZP in eight healthy males. Whole-brain CMRglu* was *calculated by averaging the values for regions of interest for each subject.*

indicate that subjects who experienced the strongest sedative effects also showed the largest decreases in CMRglu. The correlations between measures of sedation and left temporal cortex responses were also observed in the previously described ETOH experiment. Neither global nor regional CMRglu was correlated with other measures obtained in the experiment (i.e., subjects' demographic characteristics or drug-use histories, VMT performance, or blood levels of DZP).

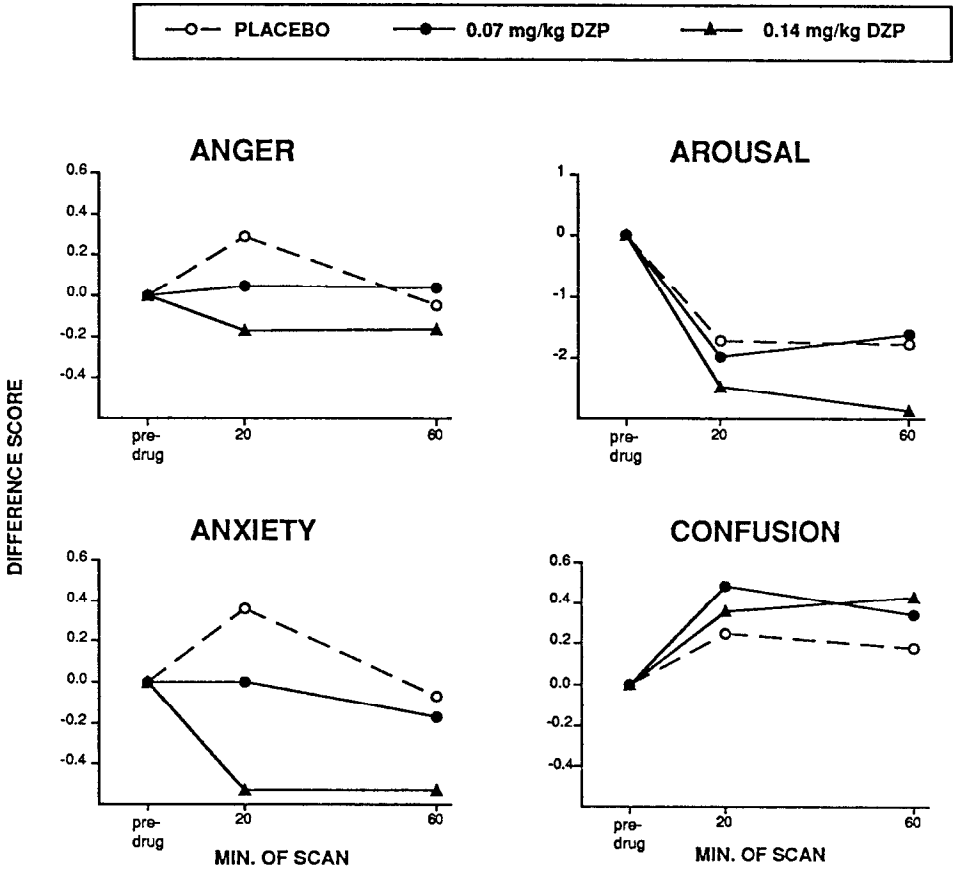

FIGURE 4. *Mean scores on four Profile of Mood States scales: after placebo, 0.07 mq/kg of diazepam (DZP), and 0.14 mg/kg of DZP; before drug administration; and 20 and 40 min into the scanning period (50 and 70 min after drug administration).*

TABLE 3. *Summary of correlations between changes in mood and CMRglu after administration of a low dose of diazepam (0.07 mg/kg)*

Region of Interest	Depression[a]	Arousal [b]
Left temporal	—	**.76**
Left basal ganglia	**.86**	—

[a]POMS scales, negative moods.
[b]POMS scales, positive moods.

TABLE 4. *Summary of correlations between changes in mood and CMRglu after administration of moderate dose of diazepam (0.14 mg/kg)*

Region of interest	Negative moods[a]		Positive moods[a]	
	Co	Fa	Ar	Vg
Left frontal	—	—	—	**.74**
Left temporal	—	-.79	**.71**	—
Left occipital	—	—	**.74**	—
Left cerebellum	—	-.76	**.72**	—
Right frontal	—	—	**.70**	—
Right occipital	—	.79	**.75**	—
Right thalamus	-.71	—		
Right cerebellum	—	-.72		
Left mean	—	-.73		
Right mean	—	-.72	—	—
Global mean	—	-.72	—	—
Global s.d.	-.73	—		

NOTE: Co = confusion; Fa = fatigue; Ar = arousal; Vg = Vigor.

[a]POMS scales.

DISCUSSION

These studies demonstrate the feasibility of using PET to study drug-induced changes in cerebral metabolic rate and of exploring the relationship between these metabolic changes and the drugs' mood-altering effects. The studies

illustrate both strengths and limitations of the PET methodology in the study of brain mechanisms underlying drug actions. This discussion focuses on these strengths and limitations, using the above studies as examples.

One of the primary advantages of PET is that it can be used with normal, awake human subjects. It is safe and relatively noninvasive and yet provides a direct measure of brain activity in both cortical and deeper, subcortical structures of the brain. There are several advantages to using humans as subjects: (1) Because a primary goal of many of the studies is to improve our understanding of human drug use, the use of human rather than nonhuman subjects eliminates the need for inferences across species. (2) Human subjects, unlike laboratory animals, can describe the quality and magnitude of their subjective responses to the drugs (e.g., euphoria), which are thought to be closely related to the drugs' likelihood of being abused (Martin et al. 1971). (3) Although this study used only normal, healthy volunteers, the technique is well suited to studying individual differences in drug effects among different subgroups of individuals, such as those with presumed biological predispositions (e.g., family history of alcoholism), extensive drug-use histories (e.g., ex-addicts), or other presumed risk factors (e.g., antisocial personality disorder). Even within a relatively homogeneous population of subjects such as those used here, the correlations between metabolic changes and subjective effects may be informative. For example, in each group of eight subjects in the ETOH and DZP studies, one individual exhibited unusual metabolic changes after drug administration relative to the rest of the group, and in both cases those individuals also showed unusual subjective responses to the drugs. Although little can be concluded from these isolated instances, it is possible that individual variations in metabolic responses to drugs are meaningfully related to variability in their mood-altering effects.

In these studies, the subjective effects of ETOH and DZP were measured at the same time and in the same setting as the metabolic effects. In addition, in the ETOH study the subjective and reinforcing effects were also assessed in the same subjects in a more natural setting. Because it cannot be assumed that drugs produce the same subjective (or other pharmacologic) effects in different environmental settings and in all individuals, concurrent assessment of the metabolic effects and the mood effects was a critical feature of this study. Indeed, it was found that some subjects did not exhibit the expected subjective effects of either ETOH or DZP in the PET studies. A related question is whether the subjective drug effects reported by subjects in the relatively unnatural PET setting are representative of the effects they might experience in a more

natural, recreational setting. The data from the ETOH study indicate that although drug-liking scores of individual subjects were correlated across the two settings, the quality of their mood responses as measured by subjective-effects questionnaires varied across the two settings. These findings suggest that generalizations from laboratory drug studies to the drugs' presumed effects outside the laboratory should be made cautiously.

Another important feature of our methodology was the use of the VMT to stabilize the subjects' cognitive activity. If cognitive activity is left uncontrolled (resting state), wide variability is observed both across and within subjects. Duara and colleagues (1987) have shown that this variability can be reduced greatly by engaging subjects in a uniform cognitive task during the scanning period. The vigilance task used in the present studies was used in a previous study by Wagner and colleagues (1986), who obtained highly stable metabolic data from the same subjects across two repeat scans when they performed the VMT. Despite the improved control achieved by such a behavioral "clamp" on mental activity, however, the possibility exists that performance of the task may mask or inhibit other potential drug effects. In our studies, for example, either the drugs' effects on metabolism or their effects on mood might be altered by performance of the task. This possibility will be explored in future studies.

Among the limitations of the current FDG-PET technology is the relatively poor spatial and temporal resolution. The regions of interest in these studies were defined to be relatively large so that they would be well within the limit of resolution of the PETT VI scanner (8 mm). The inability to study smaller brain regions is clearly a limitation of the current technique and may account for the absence of regional changes in the present studies. Improvements in PET technology, including an improved scanner and improved image-correlation techniques (Pelizzari et al. 1989) and improved methods of analyzing existing data, have been and are being developed to improve the spatial resolution of PET. Similarly, the relatively long period required for FDG uptake (40 min) may obscure changes of shorter duration, such as those expected to occur as short-lived effects of abused drugs. Alternative PET techniques, such as the use of oxygen-15 to measure oxidative metabolism or blood flow, will provide greater temporal sensitivity.

Another limitation of studies such as those described here is that they are correlational. Without convergence of data from other sources, it is not possible to determine whether systematic relationships between the two measures (i.e., metabolic rate and mood) reflect causal relations (and, if so, the direction of the

causality) or coincidental relationships. Changes in regional CMRglu may reflect the initial sites of drugs' actions, or they may reflect neural events at some point beyond the initial site of action. Correlations between CMRglu and mood might either reflect effects of the drugs directly or the consequences of the mood-altering (or other) effects of the drugs. These possibilities can be explored in future studies-including, for example, investigations of other (similar and dissimilar) drugs and manipulations that affect mood without drug administration.

The assumptions underlying the PET methodology are highly complex (Phelps et al. 1979), and the models for reconstructing images have been controversial. Although the methods used in our studies are well accepted (Nelson et al. 1987), the complexity of the models increases the risk of error in data handling and interpretation. In particular, when drugs are administered as an experimental treatment, the possibility exists that the drugs may alter brain chemistry or neural function in a way that invalidates the model. For example, alcohol is known to have widespread effects on the characteristics of cell membranes (Tabakoff and Hoffman 1981; Goldstein et al. 1982), which might invalidate the rate constants used in reconstructing the images. Fortunately, data are available to rule out some of these possibilities (rate constants, for example, can be calculated from data collected in the dynamic mode), but caution is clearly necessary in interpreting drug-induced changes in CMRglu.

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Metabolic Mapping Methods for Identifiying the Neural Substrates of the Effects of Abused Substances

Linda J. Porrino

The administration of an abused substance to an organism, whether self-administration or passive administration, can result in a variety of pharmacologic actions that may include both central and peripheral effects. These physiological and behavioral responses are more likely to be the product of multiple processes at a number of anatomic sites than the result of a single action in a single location. To determine the neural substrates of the actions of drugs of abuse, it is necessary, therefore, to identify neural events in circuits and pathways throughout the brain, not just at one location. Doing so requires either that we investigate small portions of the brain one at a time (with electrophysiological methods, for example, or that we use methods capable of surveying the entire brain simultaneously. The 2-[^{14}C]deoxyglucose (2-DG) method developed by Sokoloff and colleagues (1977) has provided such a capability. The 2-DG method is a means by which neuroscientists can investigate the functional events in the brain related to various physiological, pharmacologic, and behavioral states. It has been used extensively in neuropharmacology to identify the neural circuits that mediate the effects of a wide variety of pharmacologic agents (for reviews see McCulloch 1982; Porrino and Pontieri, in press). In this chapter, the application of the 2-DG method will be described with particular attention to the advantages and disadvantages of its use in pharmacology.

The 2-DG method measures rates of local cerebral glucose utilization throughout all portions of the central nervous system (CNS). Although the 2-DG method may appear to be essentially an anatomic technique on the basis of autoradiographic images that are produced, it is in fact a biochemical method that measures a biological process: glucose utilization, or the rate at which energy is consumed in neuroanatomically defined regions in the CNS of

conscious animals. The value of measuring rates of glucose utilization stems from the fact that in the brain, as in other tissues that do physicochemical work, the amount of energy used is correlated with the amount of work done in that tissue. Glucose utilization in the brain is a measure of energy use under normal physiological conditions, because glucose is virtually the exclusive substrate for energy metabolism. The basic rationale of the 2-DG method is that functional activity in any given brain region is directly related to energy metabolism in that region. It is possible, therefore, by measuring changes in rates of glucose utilization, to identify brain regions in which functional activity is altered during various experimental manipulations.

There are a number of energy-requiring processes in brain that contribute to basal rates of glucose utilization; for example, transmitter synthesis, release and reuptake, and synthesis of lipids and proteins. The largest proportion of energy in the CNS, however, is used to maintain and restore ionic gradients. In fact, it has been estimated that 80 percent of the energy generated in brain is used for this purpose (Kurumaji et al., in press). It is important to appreciate that the changes in rates of glucose utilization that are evoked by an experimental manipulation are thought to result mainly from increases or decreases in electrical activity or synaptic activity in the CNS. These changes in electrical activity, in turn, produce corresponding increases or decreases in the activity of Na+, K^+-ATPase, the energy-consuming enzyme involved in the restoration of neuronal ionic gradients to their resting state. Experiments with deoxyglucose have shown that the coupling of glucose utilization to functional activity is dependent on Na^+,K^+-ATPase activity, in that ouabain, an ATPase inhibitor, can block the increases in glucose utilization that accompany electrical stimulation (Mata et al. 1980).

One significant issue in the interpretation of changes in glucose utilization is the site of these changes. A number of studies (Schwartz et al. 1979; Kadekaro et al. 1985) have clearly demonstrated that functional activation of glucose utilization occurs mainly in nerve terminals rather than in cell bodies. Terminals undoubtedly make a larger contribution than cell bodies because of the greater ratios of surface area to volume in neuronal terminals (Schwartz et al. 1979). Alterations in energy metabolism in a specific brain area are due to changes in terminals of the afferent inputs to that structure and not in the cell bodies contained within the structure. This is the reason why data from electrophysiological recording studies and 2-DG studies frequently do not agree (Palombo et al. 1990). Different sets of events are being measured by the two met hods.

The measurement of rates of glucose utilization follows the general principles for measuring of rates of any reaction with radioactive tracers (figure 1). The amount of product formed over an interval of time is determined, related to the integrated specific activity (which is the ratio of the labeled precursor to the total precursor pool integrated over the time of measurement), and corrected for kinetic differences between the labeled and unlabeled compounds. To measure rates of local cerebral glucose utilization, a radioactively labelled analog of glucose, 2-DG, is used. Like glucose, 2-DG is transported into cerebral tissue by the same carrier and phosphorylated by hexokinase, but, unlike glucose, it is not metabolized further and is, therefore, trapped within cells. It is this trapping that allows quantitative autoradiography to be used to measure actual rates of glucose utilization in individual brain regions, Glucose, in contrast, is metabolized further via the glycolytic and tricarboxylic acid pathways or the pentose phosphate pathway eventually to carbon dioxide, which is cleared rapidly from cerebral tissue. The use of [^{14}C]glucose as a tracer for the measuring of cerebral glucose utilization can be inaccurate because it is difficult to correctly estimate the loss of labeled product from tissue. The amount of label in tissue as measured autoradiographically with [^{14}C]glucose is not a true reflection of the amount of glucose used in that tissue. Direct comparisons of the two tracers have shown that rates of glucose utilization in animals in which the visual system has been stimulated are underestimated when [^{14}C]glucose is used as the tracer (Collins et al. 1987).

The calculations of rates of glucose utilization with the 2-DG method (figure 1) are made from two measurements. The first is the concentration of radioactivity in the tissue measured autoradiographically and is a measure of the product formed. The concentration of radioactivity in the tissue, however, is composed of both the product formed (in this case, 2-[^{14}C]deoxyglucose-6-phosphate) and unmetabolized precursor (in this case, 2-DG)' The two cannot be distinguished autoradiographically. The percentage of unmetabolized precursor must be subtracted from the total to measure the rate of the reaction. The second measurement is the levels of glucose and 2-DG in plasma during the experimental period, which are required to determine the integrated specific activity of the precursor in tissue. Levels in plasma are used because it is impossible to make direct tissue measurements. Corrections are made for the lag in tissue equilibration with plasma. The rate of glucose utilization is described mathematically by the operational equation of the method (figure 1). The details of the mathematical derivation of the 2-DG method and an extensive discussion of its theoretical basis are beyond the scope of this chapter (see Sokoloff et al. 1977; Sokoloff 1982; Sokoloff and Porrino 1986). In

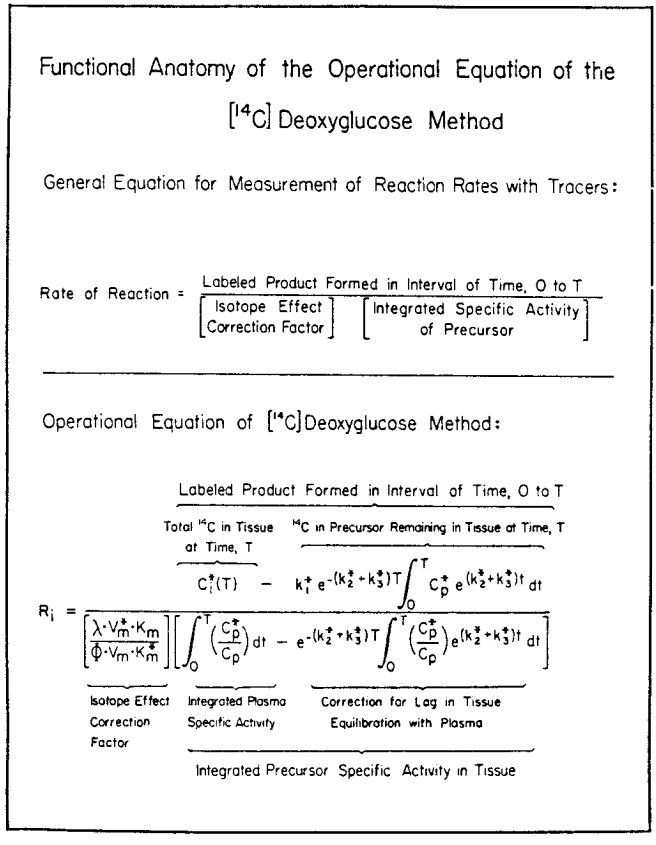

FIGURE 1. *Operational equation of the radioactive deoxyglucose method and its functional anatomy. T represents the time at the termination of the experimental period; C* represents the total ^{14}C concentration in brain tissue; C_p* and C_p represent the concentrations of [^{14}C]deoxyglucose and glucose in the arterial plasma. The constants k_1*, k_2*, and k_3* represent the rate constants for carrier-mediated transport of [^{14}C]deoxyglucose from plasma to tissue, for carrier-mediated transport back from tissue to plasma, and for phosphotylation by hexokinase, respectively. λ equals the ratio of the distribution space of deoxyglucose in the tissue to that of glucose; 0 equals the fraction of glucose, which, once phosphorylated, continues down the glycolytic path; and K_m* and V_m* and K_m and V_m represent the Michaelis-Menten kinetic constants of hexokinase for deoxyglucose and glucose, respectively. (From Sokoloff 1983 with permission.)*

addition, the techniques used for the application of the 2-DG method have been described in detail elsewhere (Porrino and Crane 1990).

ADVANTAGES

A number of the characteristics of the 2-DG method are advantageous for its use in neuropharmacologic as well as in other physiological investigations. One of the primary advantages of the 2-DG method is that it uses autoradiography, which allows the visualization not only of individual structures within the brain, but also of small subnuclei and anatomical subdivisions within a given structure. The resolution of the 2-DG method is approximately 100 microns. The limiting factor is the diffusion and migration of the labeled compound in the tissue during freezing and cutting of the brain. Even though cellular resolution is not possible, autoradiography still provides a considerable degree of anatomic resolution. This point is apparent in 2-DG studies of the visual system of primates, in which a pattern of light and dark striations in the visual cortex can be seen following the occlusion of one eye (Kennedy et al. 1978). These columns correspond to ocular dominance columns identified electrophysiologically and anatomically (figure 2). Even in the rat, it is possible to distinguish subdivisions of a structure as small as the lateral habenula. Glucose utilization in the lateral portion of the lateral habenula is decreased significantly after dopaminergic agonists are administered (McCulloch et al. 1980; Wechsler et al. 1979; Porrino et al. 19846, 1988).

Another advantage is the ability to examine the entire CNS. Autoradiography allows a broad survey of possible effects at all levels of the brain, not limiting investigations to loci in which effects are expected. Table 1 shows representative values for local cerebral glucose utilization in normal conscious rats and primates in a variety of structures. If the whole brain is examined, effects of an experimental manipulation may be seen that would otherwise have gone unnoticed. In a primate study on the effects of lesions of the substantia nigra induced by the infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, alterations in cerebral glucose utilization rates were evident not just within the basal ganglia, as expected, but also in circuits that mediate eye movements (Porrino et al. 1987). By using this kind of an approach it is possible to survey effects in a number of systems without using different sets of animals.

Although originally experiments with the 2-DG method were first conducted in restrained or partially restrained animals, methods have been developed recently that permit the 2-DG method to be used in freely moving, conscious

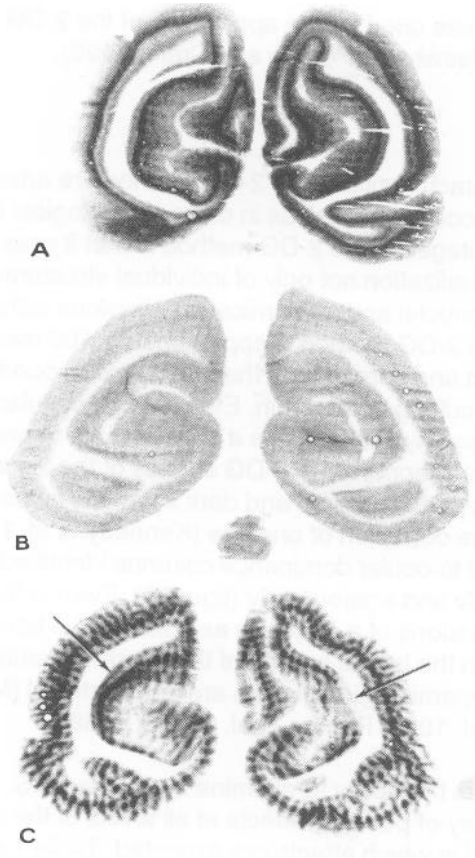

FIGURE 2. *Autoradiograms of corona/ brain sections at the level of the striate cortex. A: Animal with normal binocular vision. Note the laminar distribution of the density; the dark band corresponds to layer 4. B: Animal with bilateral visual deprivation. Note the reduced density and the disappearance of the dark band corresponding to layer 4. C: Animals with right eye occluded. Note the alternate dark and light striations. These columns are most apparent in layer 4 but extend through the entire thickness of the cortex. The arrows point to regions of bilateral asymmetry where the ocular dominance columns are absent. These are presumably areas 'with only monocular inputs. (From Kennedy et al. 1978 with permission.)*

TABLE 1. *Representative Values for Local Cerebral Glucose Utilization in Normal Conscious Rat and Monkey. (µmol/100 g per minute)*

Structure	Rat[a] (n=4)	Monkey[b] (n=4)
Prefrontal cortex	66±3	57±2
Motor cortex	94±1	47±2
Auditory cortex	154±3	73±2
Visual cortex	102±2	69±1
Caudate	107±2	64±4
Nucleus accumbens	87±2	37±2
Globus pallidus	55±1	25 ± 1
Lateral hypothalamus	55±1	28±1
Ventral thalamus	95 ±1	43±2
Medial geniculate	113±4	61±2
Lateral geniculate	92±2	39±2
Substantia nigra compacta	72±2	46±1
Inferior colliculus	167±2	133±6
Superior colliculus	91 ± 1	52±1
Locus coeruleus	71 ± 1	36±3
Cerebellum	58±1	37±2

[a]Values from Porrino et al. 1988.
[b]Values from Porrino et al. 1987.

animals (Crane and Porrino 1989). This approach allows the method to be applied to the study of behavior. Animals that were lever-pressing for electrical brain stimulation have been studied (Porrino et al. 1984*a,* 1990), as have the effects of the administration of psychostimulants on locomotor activity (Porrino et al. 1988; Pontieri et al. 1990). One potential problem with this modification is that the catheters are necessarily rather long. Using catethers requires that a relatively greater amount of blood be drawn to clear the dead space before sampling. Clearing the dead space prevents the sampled blood from mixing with the blood that may be present in the catheter before sampling and assures that the sampled blood actually reflects the internal state of the animal. To eliminate the problems of a large volume of dead space in experiments, an amount of blood equivalent to approximately twice the volume of the catheter is removed and discarded before sampling of blood (Crane and Porrino 1989). In this way, rates of glucose utilization can still be measured accurately in behaving animals without compromising their physiological state.

One problem with the 2-DG method is that the uptake of 2-deoxyglucose is dependent on variables such as blood pressure, cardiac output, and plasma glucose levels. Overall differences in uptake based on these variables could obscure differences among groups that result from changes in functional activity, particularly if non- or semiquantitative methods are used. Because the calculation of absolute rates of glucose utilization with the 2-DG method takes these variables into account, however, valid comparisons can be made among groups; the brain areas with altered functional activity can be identified and the magnitude of the changes can be measured. These capabilities are clearly an advantage in pharmacologic studies in which the physiological status of the animal is frequently altered by drug administration.

DISADVANTAGES

Despite its advantages, the 2-DG method has a number of limitations that should be considered. First, the method is unable to differentiate between direct and indirect effects of a given stimulus. An entire pathway or circuit may be metabolically activated even though the direct action of the stimulus may occur only at the origin of or at some point along the pathway. The 2-DG method, therefore, identifies the sites in the brain that are activated when a drug initiates its actions. Apomorphine (a dopaminergic receptor agonist), for example, has widespread effects on rates of cerebral glucose utilization (figure 3). In a study by McCulloch et al. (1982), the areas in which glucose utilization was altered included brain regions devoid of dopaminergic receptors. In addition, metabolism was unchanged in regions in which high concentrations of dopaminergic receptors are found, e.g., the nucleus accumbens. Although this lack of specificity may be a limitation in some instances, as in the case of determining the primary site of action of a drug, it is an advantage when the goal is to identify the neural circuits or pathways that mediate a specific behavioral response.

Another disadvantage is that it is not possible to identify the specific neurotransmitters, neuromodulators, or receptor types that are responsible for changes in brain electrical activity. This point is illustrated by a study in which both rates of glucose utilization and turnover rates of dopamine, norepinephrine, and serotonin were measured simultaneously in animals receiving electrical brain stimulation to the ventral tegmental area (Smith et al. 1990). The increased rates of glucose utilization observed in the nucleus accumbens of self-stimulating animals were accompanied by increased dopamine turnover but decreased serotonin turnover. In contrast, in the

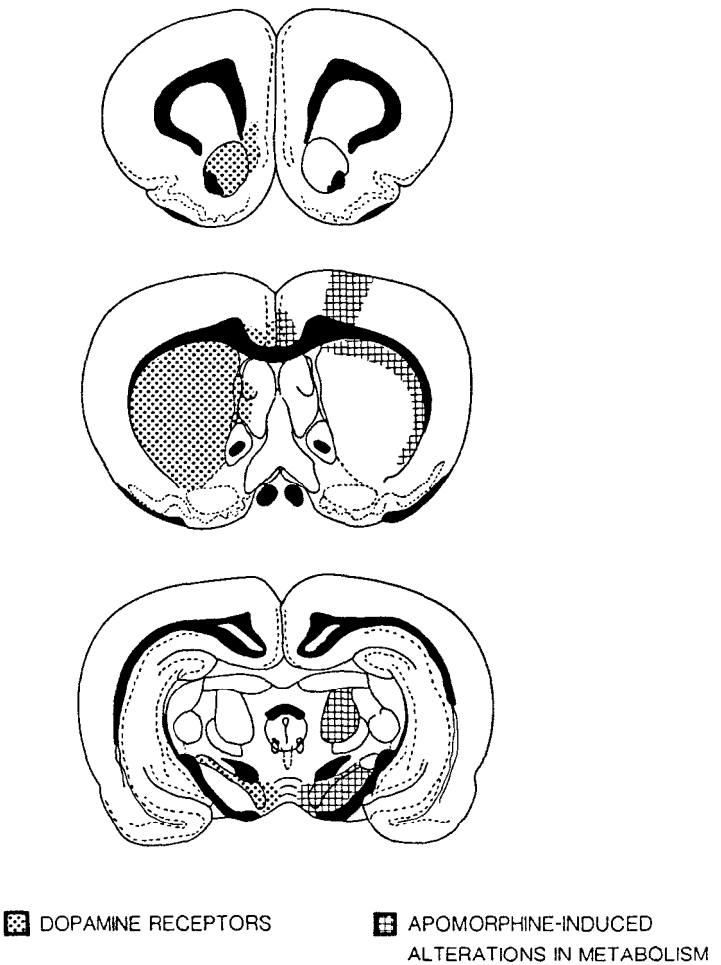

FIGURE 3. *Schematic representations of three coronal sections of rat brain* at *the levels of the nucleus accumbens (top), caudate-putamen (middle), and substantia nigra (bottom). Areas rich in dopamine receptors are shown on the left of each section; regions in which alterations in cerebral metabolism were observed following apomorphine administration on the right of each section. (Data from McCulloch et al. 1982.)*

amygdala-where increased rates of glucose utilization were also found-norepinephrine turnover was altered. It is not possible to predict the nature of neurotransmitter changes on the basis of alterations in glucose utilization.

Another limitation is the inability to distinguish between inhibitory and excitatory processes on the basis of increases or decreases in 2-DG uptake. Both excitation and inhibition involve similar metabolic processes at the neuronal level, in that maintenance and restoration of ionic gradients have similar energy requirements regardless of whether excitatory or inhibitory neurotransmitters are secreted at synaptic terminals. Ackermann and colleagues (1984) demonstrated that when either the fimbria-fornix or perforant paths were stimulated electrically, the resulting increases in 2-DG uptake within the hippocampus were a consequence of the long-lasting recurrent inhibitory processes rather than of the brief excitatory potentials that also accompany the stimulation. The increased 2-DG uptake in this study was the consequence of the increased activity of afferents to the hippocampus. Neither excitation nor inhibition was the critical determinant of the direction of changes in rates of glucose utilization.

Because constant conditions are considered necessary for measuring rates of glucose utilization (Sokoloff et al. 1977) the long experimental time period can be a significant limitation in drug studies. Physiological and behavioral responses to drug administration can vary significantly during the 45 minutes required for applying the 2-DG method. Although the total experimental time is 45 minutes, the uptake and phosphorylation of 2-DG in brain reflect predominantly the metabolism of 2-DG in the first 5 to 10 minutes. It is so because the tissue concentrations of free 2-DG available for metabolism are highest during the first 5 to 10 minutes after it is injected (Sokoloff et al. 1977). Choosing the optimal time for drug administration in relation to the time of injecting the 2-DG is important to ensure maximal sensitivity of the method as well as to ensure that data will be comparable across treatments. Drug administration should be timed so that the maximum drug response coincides with the time of maximum incorporation of 2-DG.

A final disadvantage that should be considered is that repeated measurements are not possible in the same animal. Groups of separate animals must be used to determine the effects of each experimental condition. The use of separate animals increases variability as well as the number of animals necessary.

FACTORS INFLUENCING INTERPRETATION

One of the most important considerations in the interpreting alterations in rates of glucose utilization is the choice of measure. Because it can sometimes be difficult to design experimental procedures that allow for blood sampling of freely moving, behaving animals, the method has been modified to eliminate the necessity for catheterization and blood collection. These modifications have led to the use of alternatives to rates of glucose utilization as measures of functional activity. Some of these modifications, however, compromise the validity of the method and, in effect, produce erroneous results (for additional discussion see Sokoloff et al. 1983). In many of these modifications (Meibach et al. 1980; Gallistel et al. 1982) the radioactive tracer is injected intraperitoneally. This route of administration results in a high proportion of unphosphorylated deoxyglucose as a percentage of the total radioactivity in tissue at the end of the experiment-as much as 20 percent in gray matter and 40 percent in white matter (Kelly and McCulloch 1983*a).* Following an intravenous pulse, in contrast, most of the unmetabolized 2-DG is cleared from the tissue, leaving as little as 5 percent in gray matter and 10 percent to 12 percent in white matter (Kelly and McCulloch 1983*a).* With intraperitoneal tracer injections, the higher proportion of unmetabolized tracer can mask true changes in metabolism.

Three other factors besides route of administration can affect the clearance of unmetabolized 2-DG from brain tissue: plasma glucose concentration, cardiac output, and the rate of glucose consumption in other tissues. These factors may vary significantly with experimental conditions, particularly in pharmacologic experiments, leading to errors. Optical densities, or even tracer concentrations, of given brain regions cannot be compared accurately from animal to animal without taking into account the proportion of unmetabolized tracer.

To circumvent these problems, some investigators (Collins 1978; Sharp et al. 1983) have used relative rates of glucose utilization, or the ratio of optical densities, or tracer concentrations of a given structure to that of white matter. The validity of gray-white matter ratios as a measure of glucose utilization depends on the assumption that optical densities or tracer content in white matter are constant across conditions. This assumption may be valid at times, but many pharmacologic treatments can cause changes that invalidate it. The choice of the particular white matter measured may also be critical; the fornix or the internal capsule, for example, may be more sensitive to drug-induced changes than the corpus callosum or the optic tract. In addition, the relationship between tracer concentration in tissue and optical density is not a linear one,

and it varies with exposure time (Kelly and McCulloch 1983*b).* The exposure times chosen must be on the linear portion of the curve for the particular film type.

Other investigators have used a measure in which the average optical density of a given structure is related to the average optical density of the entire section in which the structure is analyzed (Gallistel et al. 1982). The relative optical density (ROD) measure and the gray-white ratios suffer from the same problems: (1) lack of constancy of the referent; (2) nonlinearity of the relationship of optical density to tracer concentration; (3) failure to take into account differences in the percentage of unmetabolized tracer; and (4) the use of intraperitoneal injections. There is, however, an additional problem with this measure. Because increased or decreased 2-DG uptake may occur in a number of structures sampled in the same section (i.e., the average rate in the entire section is likely to be increased or decreased), it may not be possible with the ROD method to detect changes in some structures if those changes are proportional to the changes in the entire section. If a particular structure happens to be sampled in sections in which tracer uptake has not been altered, changes in that structure are more likely to be detected than if sampling occurred in sections in which many changes occurred.

RODs and local rates of cerebral glucose utilization were measured in autoradiograms of two groups of animals treated either with methamphetamine (2.5 mg/kg, intravenously) or saline vehicle. Identical brain regions on each section were analyzed simultaneously for each measure. Of the structures analyzed, rates of glucose utilization were increased in the caudate-putamen (+62 percent), nucleus accumbens (+39 percent), olfactory tubercle (+42 percent), and anterior cingulate cortex (+18 percent). In contrast, RODs measured simultaneously in the same autoradiograms were increased in the caudate-putamen (+15 percent) and olfactory tubercle (+23 percent), unchanged in the nucleus accumbens (+4 percent), and decreased in the anterior cingulate (-12 percent). The ROD measure not only underestimated the increases in glucose utilization in some structures but also failed to detect changes in others and yielded spurious results regarding the direction of change in still others (figure 4). Clearly, caution must be used in interpreting the results of any study in which this measure is employed.

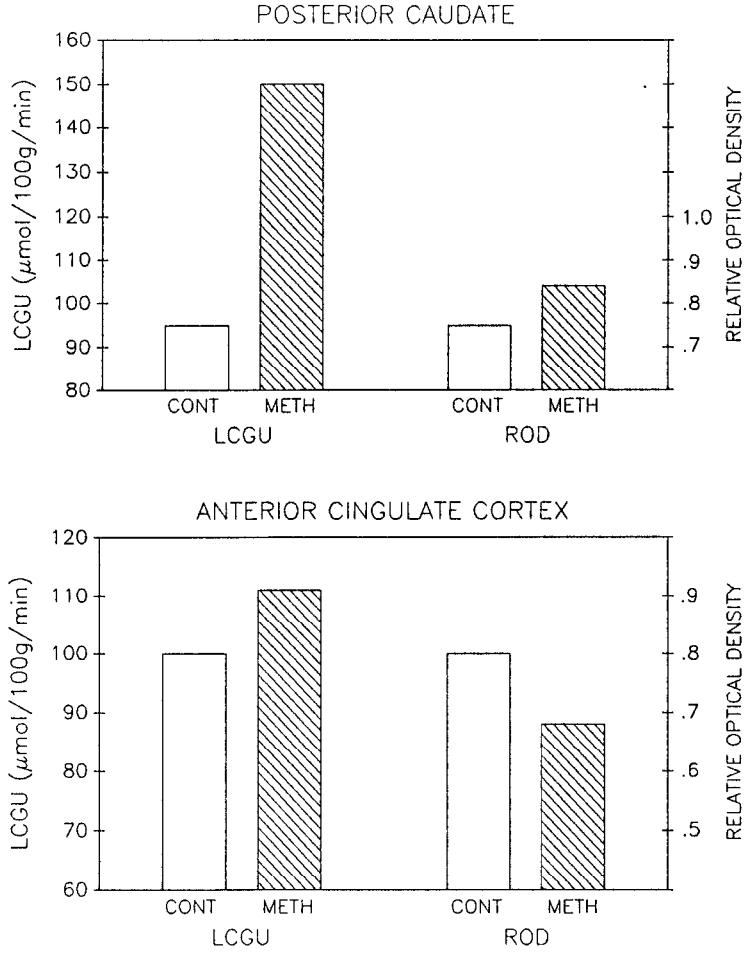

FIGURE 4. *Comparison of changes in rates of glucose utilization and relative optical densities (RODs) in the posterior caudate (top) and the anterior cingulate cortex (bottom) in rats treated with methamphetamine (METH; 2.5 mg/kg, intravenously) and saline vehicle (CONT). Scales have been adjusted so that measures of glucose utilization and ROD of control are equivalent on each scale. Scales of glucose utilization and ROD have been adjusted so that the values of the methamphetamine-treated group illustrate the percentage of change from control for each measure.*

CONCLUSIONS

The 2-DG method provides a number of unique advantages for neuropharmacologic investigations. First, the effects of a drug can be assessed comprehensively because the entire brain can be examined at one time. Second, studies can be carried out in conscious animals that are not restricted. Third, the 2-DG method is a powerful means to relate structure to function, in that a dynamic neuronal process can be measured with the resolution of autoradiography. Finally, since there is a unique pattern of alteration in functional activity associated with any given drug, the effects of a variety of drugs of the same and different pharmacologic classes can be compared and contrasted in a novel and highly informative way.

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Interactions of Neurotransmitters With Drugs and Behavior

Lewis S. Seiden

The behavior of organisms can be considered either a dependent variable or an independent variable. Since the brain affects behavior, behavior can be considered dependent on neural activity. On the other hand, the brain's status (e.g., how it synthesizes neurotransmitters) depends on the amount of ongoing behavior (an independent variable). Consequently, there is interaction among behavioral events, both dependent and independent. In this chapter I review evidence that leads to a concept of brain-behavior interactions and then propose a framework for explaining a way in which drugs, behavior, and neurochemistry interact with one another.

In 1867, Claude Bernard proposed that life forces are geared toward maintaining a constancy of the *internal milieu* and that the behavior and physiology of the organism maintain this balance; this postulate implied that a balance is restored after chemicals are utilized. For example, food deprivation elicits a feeding response to restore nutrient balance. A few years later Bernard put forward the concept of *homeostasis* to designate the maintenance of the internal milieu (Bernard 1927). The concept of homeostasis had profound implications for nervous system function as well as for basic biochemical and cellular processes. Bernard went on to consider stress. A stressor was conceptualized as a massive sensory stimulation that emanates most often from the external environment and alters the physiology of the organism. After a stressor occurs, physiological responses take place that mitigate against the stress-induced response to achieve homeostasis. In humans, it is possible to identify internal stimuli such as anxiety or pain, which could also lead to a physiological stress response. Thus, Bernard, with his notion of the internal milieu, set the conceptual framework for much of the research into central nervous system function that has taken place in the past century.

Later, W.B. Cannon, a major figure in physiological psychology, observed that stress results in an increase in circulating corticosterone (Cannon 1922). This observation became the operational definition of a stress response, and the stimuli eliciting the increase were called stressors. Furthermore, Cannon noted that the stress response is accompanied by an increase in sympathetic tone and a decrease in parasympathetic tone, and so he suggested that stress is mediated by sympathetic nervous system activity. Von Euler later couched this idea in chemical terms by showing that animals with hypertension (which occurs with stress) had alterations in adrenergic tone (von Euler and Sjostrand 1943). Cannon also noted that behavioral changes are part of the response to stress, and this point is consistent with Bernard's ideas of homeostasis. An example of a behavioral concomitant to stress is the increase in an animal's aggressive posturing in response to threat. Avoidance might also occur as a response to threat. Thus, Cannon proposed the famous *flight-or-fight* response (Cannon 1922).

A few years later, Hans Selye became interested in stress and coined the term *general adaptation syndrome* for a group of stereotypic responses to stressors (Selye 1950). Examples of stimuli eliciting the syndrome are extreme heat or cold, infection, muscular fatigue, and large doses of drugs.

A combined definition of stress can be derived from the writings of Selye and Cannon: stress is a group of responses to a specific pattern of stimuli, and the responses include an increase in adrenal cortisol secretions, an increase in circulating epinephrine and norepinephrine (NE), and an increase in the rate of specific behaviors. Responses may be short-term (acute) or long-term (chronic). Over a long period of exposure to stressors, either hypersensitive or tolerant responses may occur. The short-term response is a flight-or-fight response, in contrast to the long-term general adaptation syndrome, which includes habituation or tolerance and in which the flight-or-fight response is often extinguished.

Dopamine (DA) and NE were discovered in the brain in the 1950s. Considerable data supported the notion that the effects of certain psychotropic drugs, as well as the stress response, are mediated by centrally located NE and DA (Gordon et al. 1966). Several investigators reported that NE levels as well as turnover rates were changed by stressful stimulation. Early attempts to determine central nervous system events as a simple function of stress, such as continuous electrical shock, produced variable results (Bliss et al. 1966; Gordon et al. 1966; Modigh 1974). Levels of NE and DA were found to be lower

in brains of individually housed mice than in brains of group-housed mice. Turnover was also increased in isolated animals.

Other work showed altered NE and DA turnover in brains of animals exposed to shock or shock avoidance (Hurwitz et al. 1971). Even without shock in the experiments, once the rat had been trained to avoid shock, there was a change in NE turnover as a result of training on the avoidance schedule. Bliss et al. (1966) noted a change in levels of central NE between self-stimulating and shocked rats; Thierry et al. (1968) noted long-term changes in NE metabolism in response to electroconvulsive shock (ECS) in rats and found that the effects of ECS persisted after animals had received it. This discovery was important not only because the changes were acute but also because the changes could persist. The persistent nature of the changes implies that the history of the animal is an important determinant of the turnover rate of the amines, which, in turn, may become an important determinant of behavior.

Looking at a paradigm that did not involve shock, Draskoczy and Lyman (1967) measured the turnover rate of NE in hibernating and awake ground squirrels. The ground squirrel responds to the lowering of its core temperature by hibernating. During hibernation, NE metabolism decreases; the rate of NE metabolism in the brain decreases to zero, but the rate in the adrenal medulla and in adipose tissue, while very low, is still measurable. When core temperature is increased, the squirrels come out of hibernation and the rate of NE metabolism returns to normal; this rate is comparable to that of other mammals that are awake. The rate of NE metabolism in ground squirrels can, therefore, be manipulated by changing the environmental circumstances.

Changes in catecholamine (CA) turnover in brain can also be observed in an operant response (Schoenfeld and Seiden 1969). Tyrosine hydroxylase inhibition by alpha-methyltyrosine (AMT) engendered a decrease in fixed-interval (FI) and fixed-ratio (FR) responding, and when tyrosine hydroxylase was inhibited, the rates of responding under the FR schedules showed a decrease proportional to the FR requirement. This change in behavior was a function of the ratio size and suggested that ongoing behavior itself was an important mediator of the drug effect. We concluded from these data that the initial rate is an important factor in determining the depletion of CAs resulting from tyrosine hydroxylase inhibition because the rates were higher in FR schedules, but there were only small differences in rates among the various FI schedules. The effect of tyrosine hydroxylase inhibition was related to the pre-drug rate of responding (Schoenfeld and Seiden 1969). It is

also important to point out that the rate of responding decreased over time in rats that were lever-pressing on the FR 10 schedule. At the beginning of the session, the rate of lever pressing in the treated rats started close to saline-treated rats, but the rate of lever pressing among the AMT-treated rats rapidly decreased after about 15 minutes of the session had elapsed. DA and NE in brain were partially depleted by the AMT treatment at the beginning of the session. The rate of lever pressing decreased during the session, and it was shown that this rate decrease over time could be prevented with L-dopa, which replenished DA and NE. The decrease in responding with time and the restoration with L-dopa suggested that responding itself depleted DA or NE or both.

Lever pressing also had a direct effect on brain DA and NE in rats trained on a variable-interval (VI) schedule of reinforcement. Two groups were trained on the VI schedule, but one group was given tyrosine hydroxylase inhibitor while they were in their home cage, and the other group was given the drug while they were in the operant chamber. There was a significantly greater depletion of DA and NE depletion in the group in the operant chamber. This observation strongly suggested that lever pressing in the face of tyrosine hydroxylase inhibition could further deplete DA and NE. Another experiment confirmed that lever pressing influences NE metabolism in brain (Lewy and Seiden 1972). Tritiated NE was introduced into the lateral ventricle of rats pressing a lever on a VI schedule and the rats were sacrificed at the end of the session. Both the total activity of NE in brain and the specific activity of NE in brain were reduced as a result of lever pressing, suggesting that the behavior or some environmental aspect of the operant procedure was fed back to the brain and increased the release and synthesis of NE.

By using a multiple schedule, it was shown that amphetamine (AMPH) increases low response rates and decreases high response rates. This phenomenon is known as rate dependency (Kelleher and Morse 1968; Dews and Wenger 1977; Dews 1958). On the basis of the data that have been presented, which demonstrate that behavior can alter turnover of DA and NE and that drug effects depend on ongoing behavior, we could begin to conceptualize the mutual interactions among drugs, brain chemistry, and behavior (figure 1). Drugs influence brain chemistry, which can influence behavior. Behavior can alter the environment, and a specific alteration in the environment can reinforce behavior. In addition, behavior and the environment can also alter brain chemistry. Therefore, brain chemistry, behavior, and the environment are highly interactive. Drugs effects on behavior are not

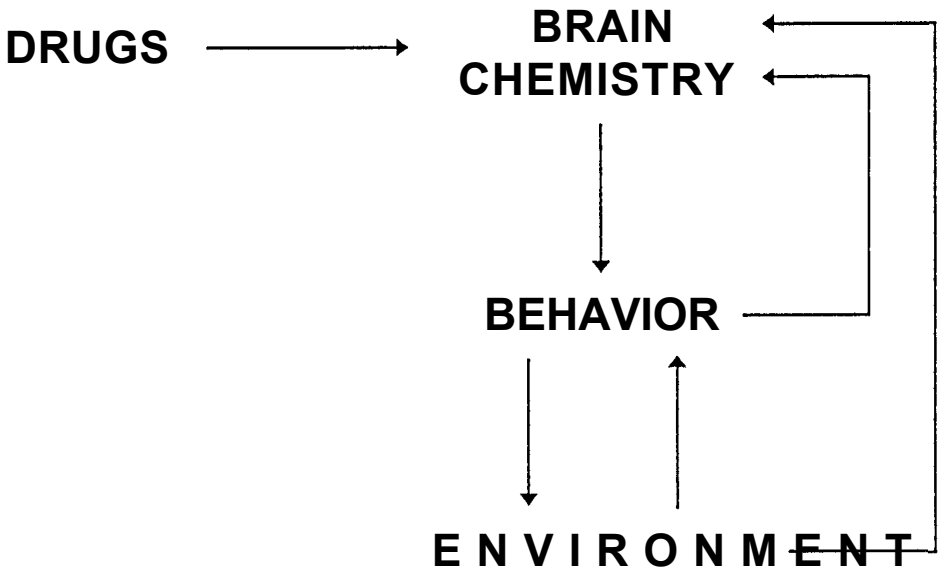

FIGURE 1. *Interactions among brain chemistry behavior, and environment.*

predictable entirely by the way in which drugs alter brain chemistry, because other mechanisms operate on brain chemistry, and these must be taken into account before one can determine the resultant behavior. A number of variables may account for the effects of operant behavior on brain chemistry: the consummatory response, deprivation, the nature of the operant, the amount of locomotion, the effects of the contingent relationship between the response and the reinforcement, and the history of the organism.

Feeding behavior affects the release of DA in several regions of the brain. The ratio between the DA metabolite dihydroxyphenylacetic acid (DOPAC) and DA was measured as a function of food deprivation; access to food and food consumption followed. There are regions of the brain where the levels of NE and DA do not change. In one study, food deprivation did not increase the turnover of DA but feeding for an hour engendered an increase in rate of turnover of DA in the hypothalamus and in some mesolimbic areas. Alterations in DA turnover as a function of feeding reveals specificity for certain areas of the brain. In the caudate-putamen and some mesolimbic areas, no changes occurred; changes in DA metabolism resulting from continuous access to food after food deprivation revealed that the nucleus accumbens, the amygdala, and

the hypothalamus showed an increase in DA turnover. Ingestion of food in a normal pattern, nutrition, and sensory signals engendered by food intake may account for the changes; these factors play a role in the food-deprived feeding rat. For this reason, tube-fed rats were used as controls in some experiments. These studies showed that all three factors-food, nutrition, and stimulation of the gut-increased DA turnover in the amygdala. In the hypothalamus and accumbens, only food and oral intake increased DA turnover (Heffner et al. 1980).

In another experiment NE turnover was measured as a function of water consumption. Rats were presented with 0.02 mL of water as a reinforcer on an operant schedule. The frequency of presentation and quantity of water were varied, and, in some experiments, the water was presented without a lever-pressing contingency. The results of these experiments show that the number of water presentations is an important determinant of the NE turnover rate. The number of presentations was proportional to the rate of turnover even when the total amount of water presented was held constant. The total amount of water was not related to the turnover rate in a linear fashion (Heff ner et al. 1984; Heff ner et al. 1981; Luttinger and Seiden 1981; Emmett-Oglesby et al. 1975; Albert et al. 1977).

In the caudate-putamen the turnover was directly related to the response rate. An experiment used lever pressing on an FR 5 schedule of water reinforcement. Rates of turnover differed depending on the area, but no direct relationship to the response rate existed in the areas of the brain examined (Heffner et al. 1984).

In the last experiment, we demonstrated that the effects of AMPH and lever pressing on DA turnover were additive and may account for the rate-decreasing effects engendered by AMPH because of the mutual interactions of AMPH and lever pressing on DA turnover. AMPH caused an increased rate of DA turnover in rats that were not pressing; saline-treated animals that were lever-pressing on a VI 30-s schedule also showed an increase in the rate of DA turnover. If AMPH and operant behavior were combined, the rate of DA turnover doubled. In this case, the response went down because rats that were lever-pressing on a VI 30-s schedule have an intermediate response rate. The drug effect on DA added to the behavioral effect and resulted in an altered behavioral effect (Seiden and Heff ner 1984). These results are consistent with the idea that the chemistry of the brain is determined by a multiplicity of factors and that these factors can result in the final state that influences behavior.

The data presented in this paper are quite consistent with the basic ideas expressed by Claude Bernard some 125 years ago. Bernard maintained that

stimulation of the organism that alters physiology will lead to further alterations in physiology or behavior or both, which will tend to correct the original alteration. Viewed in these terms, the rat, pressing the lever and depleting its CAs faster than they can be replenished, stops pressing the lever; this alteration in behavior then slows down the rate of amine utilization. Two important factors then are reciprocally interacting with each other: the brain chemistry (which is a necessary antecedent for the occurrence of the behavior) and the change in that biochemistry (which is a consequence of the behavior). By considering the dynamic interaction of the two with regard to Cannon's homeostasis principle, one should be able to predict behavior accurately over time, provided one understands the effects of the environment and behavior itself on brain chemistry as well as the role of that chemistry in behavior.

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Historical Influences Affecting the Behavioral Actions of Abused Drugs

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INTRODUCTION

Many attempts to describe factors contributing to the etiology of drug abuse have concentrated solely on the inherent characteristics of the drug and on the neurobiological substrates at which those drugs potentially exert their complex effects. An overriding emphasis on the pharmacology of the drug and on its interaction with multiple neuronal systems, while of unquestionable importance, potentially neglects other variables that can contribute significantly to the effects of a drug and, perhaps, to the likelihood that it will be abused. Experiments summarized in this chapter focus on what appears to be a behaviorally determined malleability in the effects of drugs of abuse. These studies indicate that the behavioral actions of abused drugs can be determined by conditions that have occurred in the past, even in the absence of overt features of behavior that normally predict drug activity. Importantly, the altered effects of these drugs persist long after those initiating conditions have terminated, leaving residual traces of previous experience that become apparent only after an abused drug is administered. These findings suggest that prior behavioral experience may actually predispose an individual to a particular drug effect by altering the initial drug experience, which may then influence the likelihood of subsequent drug use. This outcome could represent an initial step toward eventual drug abuse. Conversely, it is possible that other behavioral experiences could block particular drug effects, serving as “immunizations” against subsequent drug use. Together, these outcomes could account for some of the individual differences in drug effects and in the behavioral disposition to abuse drugs. Conditions that render an individual more or less vulnerable to the effects of abused drugs warrant careful evaluation. The experimental analysis of those conditions, with a corresponding emphasis on arriving at a better understanding of the involvement of correlated neurobiological processes, should yield considerable information about

individual vulnerability to the effects of abused drugs. This information should then permit more effective behavioral and pharmacologic strategies to be developed, which could be of use in treating and preventing drug abuse.

BEHAVIORAL HISTORY

The behavioral effects of many drugs often can be predicted on the basis of the characteristics of behavior occurring at the time the drug is administered. An example of this principle is frequently referred to as rate dependency, in which the effects of psychomotor stimulants are related inversely to the rate of responding in the absence of the drug (Kelleher and Morse 1968). The dependence of a drug effect on the existing rate or pattern of behavior is an instance of control by relatively proximal variables that exert more immediate influences on the effects that a drug has on behavior. There are, however, several examples in which the effects of a drug appear to be related to conditions that have occurred in the past but are not present in the immediate environment. For example, early experiments conducted by Steinberg and colleagues using exploratory behavior of rats found that an amylobarbitone-amphetamine mixture produced large increases in exploratory behavior in rats that had no experience in the maze, whereas with experienced subjects, these drugs had little effect (Steinberg et al. 1961). Subsequent experiments by these investigators evaluating both behavioral and pharmacologic history led to the general conclusion that “behavior under the influence of psychoactive drugs depends on striking a flexible balance between the drugs themselves, the precise circumstances under which they are administered, and the state of the recipient at the time of administration. The state of the recipient, in turn, depends on its previous experiences” (Rushton et al. 1963, p. 888).

One aspect of these studies involving exploratory or locomotor behavior warrants attention. In every instance in which experience was shown to alter the effects of a drug or drug combination in these studies, the experience itself also directly changed the behavior under study. For example, in the Steinberg et al. (1961) study, after repeated exposure to the maze, the experienced rats showed activity levels almost two times higher than those of the inexperienced rats. In view of the important contribution of baseline levels of activity in determining the effects of drugs (i.e., rate dependency), these findings must be interpreted somewhat cautiously. Any experience that modifies behavior before a drug is administered *and* leaves an enduring measurable effect on overt behavior would demonstrate an “experiential” effect. It would be the case, for

example, if animals trained under one schedule of reinforcement were provided with experience under a different schedule and then returned to the original procedure at which time the response rates differed from those obtained initially. If the behavioral effects of a drug differ before and after the intervening schedule history, it is impossible to separate the historical influences from those that directly affect behavior (Urbain et al. 1977). The isolation of a "pure" effect of experience on behavior should, therefore, be observed in the absence of a lasting overt influence on the behavior being studied, given that no other intervention such as the administration of a drug has occurred. Otherwise, it would be difficult to differentiate between effects that change behavior directly and then modify subsequent drug action and affects that do not appear to modify overt behavior but that, nevertheless, produce a change in the typical effects of a drug.

More recent experiments using squirrel monkeys, rats, and pigeons have supported and extended this conclusion about the influence of behavioral history on drug activity. For example, in one experiment with squirrel monkeys, d-amphetamine decreased food-maintained lever pressing that was punished by the delivery of electric shock (Barrett 1977). This outcome is typical for this drug under these conditions and has been reported by several investigators and in several species (e.g., Geller and Seifter 1960; Hanson et al. 1967; Spealman 1979). However, when monkeys trained initially under the punishment procedure were exposed subsequently to a shock-postponement schedule and then returned to the punishment schedule, *d*-amphetamine produced large increases in punished responding. These increases were not dependent on changes in response rate since these behavioral measures were comparable before and after the interpolated training under the avoidance schedule. In addition, the altered rate-increasing effects of amphetamine persisted unless other interventions occurred (Barrett 1985). Effects of different drugs, such as chlorpromazine, were not altered by the sequence of conditions that modified the effects of *d*-amphetamine (Bacotti and McKearney 1979), suggesting that only the effects of certain compounds may be changed by these conditions. It may be that only drugs of abuse, for reasons yet unknown, share this malleability to engender different effects that depend on nonpharmacologic as well as pharmacologic influences.

Additional experiments have shown that previous experience can reverse the effects of other abused drugs such as cocaine, morphine, and chlordiazepoxide (Barrett and Stanley 1983; Barrett and Witkin 1986; Barrett et al. 1989) as well as influence the effects of methadone and the development of tolerance to

methadone (Egli and Thompson 1989; Nader and Thompson 1987, 1989). One very striking feature of many of these studies is that drug effects were different after exposure to a particular condition even though no changes in ongoing behavior were manifested after that condition and even though those conditions were no longer part of the current environment. The administration of a drug can reveal characteristics of behavior and its control by *previous* environmental conditions that are otherwise not evident in ongoing behavior.

Despite the several experiments showing the importance of behavioral history in altering the effects of drugs, there are few studies directly addressing the specific variables that might contribute to those effects. It is known that a history of avoidance is necessary to reverse the effects of *d*-amphetamine on punished behavior, because monkeys in a yoked control condition that received response-independent shock did not show any changes in the effects of *d*-amphetamine (Barrett and Witkin 1986). Thus, it is not shock exposure per se that reverses the effects of d-amphetamine but a specific avoidance history. A history of exposure to certain schedules can modify drug actions, but it is not clear what aspect of that schedule history is important (Barrett 1986).

Several possible approaches might clarify how behavioral variables alter the effects of a drug. For example, in many of the experiments in which drug effects were modified by behavioral experience, the same event was used at different times to control behavior in different ways. The effects of *d*-amphetamine on responding suppressed by the *presentation of* shock were reversed by interpolated exposure to a shock *postponement* schedule (Barrett 1977). Thus, the same shock stimulus was used at different times either to maintain or suppress responding. As a result of these complex behavioral histories, stimuli may be established that have multiple behavioral functions, which are then revealed by the administration of a drug. How important is it that the stimulus be the same in order to obtain these effects? Is only an avoidance *history* important, regardless of the nature of the event, or must the punishment and avoidance stimuli be identical? These questions could be addressed by using different types of response consequences to control behavior. Studies of this type may also provide fundamental information about the functional consequences of establishing complex environmental stimuli (Sidman 1986) that may be of general significance to our understanding of how complex histories and stimulus control contribute to the behavioral effects of drugs.

It is also not clear at present whether changes in the effects of a drug by behavioral history also modify that drug's ability to function as a reinforcer. A

recent study that demonstrated individual vulnerability in response to the effects of amphetamine (AMPH) seems particularly appropriate to this issue. Piazza et al. (1989) reported that individual rats could be identified for "addiction liability" on the basis of their individual response to either environmental or pharmacologic challenges. Rats that displayed high levels of locomotor activity in a novel environment responded with even higher activity levels after AMPH was administered than did rats that showed lower activity levels in the absence of the drug. Moreover, the high-activity rats rapidly acquired intravenous AMPH self-administration, whereas rats from the other group did not. These findings suggest that there are individual differences in the effects of AMPH and in the predisposition to self-administer this drug and that such differences may be related to observable behavior. Although the study suggests that differences between rats in the behavioral and reinforcing effects of AMPH are genetically determined, it also raises the possibility, in view of the results that have been summarized, that differences in these effects may also be induced by behavioral manipulations.

NEUROBIOLOGICAL CORRELATES OF BEHAVIORAL HISTORY

Although the molecular properties of the drug do not seem solely responsible for its behavioral effects and abuse potential, it is clear that these compounds interact with neuroanatomically and neuropharmacologically distinct substrates in the central nervous system (CNS). How these substrates are modified by factors such as past behavioral history is not currently understood, but it is likely to be a fertile avenue for future research. In many respects, the factors that contribute to the qualitative and quantitative changes in drug effects may be related to more general principles involved in learning. Historical influences suggest that plasticity of the CNS continues well beyond the initial developmental period during which such changes normally occur. Techniques are now available for monitoring neurochemical correlates of behavioral processes. The combined approach to many of these issues, including the development of molecular biological procedures, should yield considerable insight into the transient and more enduring changes in CNS activity that are related to behavioral interventions (Barrett 1991; Barrett and Nader 1990; Dworkin and Smith 1989).

PHARMACOLOGIC HISTORY

Experiments demonstrating that the effects of abused drugs can be modified by a particular behavioral history are paralleled by experiments demonstrating that

pharmacologic history can also modify a drug's behavioral and discriminative stimulus effects. For example, the effects of pentobarbital on punished responding of squirrel monkeys maintained under a stimulus-shock schedule depended on whether those monkeys were previously administered d-amphetamine or morphine (Glowa and Barrett 1983). In monkeys that had received morphine, pentobarbital did not increase punished responding, whereas in those that had received AMPH, pentobarbital produced increases in punished behavior. These effects occurred long after pharmacologic factors related to the administration of either morphine or AMPH could have played a role in the effects of pentobarbital.

A similar result was obtained in studies examining the discriminative stimulus effects of novel antianxiety drugs. Using a drug discrimination procedure in which the mixed dopamine (DA) antagonist and serotonin (5-HT) agonist spiroxatrine was established as a discriminative stimulus, it was found that either the DA or the 5-HT elements of the compound stimulus could be salient, depending on the animal's drug history (Barrett and Olmstead 1989). If the progression of drug substitution testing was initially biased toward DA, the animals responded to buspirone (which is similar to spiroxatrine in its mixed 5-HT_{1A} and DA activity) but did not generalize to more specific 5-HT_{1A} agonists such as 8-OH-DPAT, which shares buspirone's 5-HT_{1A} actions. If the progression of drug substitution testing was initially biased toward 5-HT_{1A} compounds, then compounds such as 8-OH-DPAT and several other 5-HT_{1A} ligands occasioned spiroxatrine-key responding. Thus, the relative neurochemical balance of these drugs, as measured by their discriminative stimulus effects, could be altered by the pharmacologic history.

These results indicate that pharmacologic histories, much like behavioral histories, may either enhance or diminish a particular effect of a drug. The results with the drug discrimination procedure could be especially important for future analysis. It is commonly accepted that the discriminative stimulus effects of drugs are related to their subjective effects and that such effects may correlate with abuse potential. In the experiments with spiroxatrine as a discriminative stimulus, either the DA or the 5-HT component of spiroxatrine could be made dominant by varying the pharmacologic history. If this history can "direct" the discriminative stimulus effects of drugs with multiple pharmacologic actions by enhancing the relative balance of controlling stimulus influences, similar factors may operate in modifying the behavioral actions of drugs. Perhaps one mechanism that accounts for the modification of effects of a complex drug such as cocaine is that some components of the drug's

neuropharmacologic actions become more prominent while others are muted. If these factors are uniquely correlated with abuse, then they may begin a cascade of events culminating in enhanced abuse potential.

SELF-ADMINISTRATION IN PHARMACOLOGIC HISTORY

Results similar to those that have been described have been obtained using drug self-administration studies. For example, Schlichting et al. (1970) found that monkeys with a codeine self-administration history showed lower and more variable levels of d-amphetamine self-administration than monkeys with prior histories of either pentobarbital or cocaine self-administration. Young et al. (1981) reported that the ability of dextrorphan to maintain responding was much higher in animals with a history of ketamine self-administration than in animals with no such history or with a history of codeine self-administration. Similarly, it has been demonstrated recently that the self-administration of the noncompetitive *N*-methyl-D-aspartate antagonist MK-801 can depend on drug reinforcement history (Beardsley et al. 1990). In monkeys with a history of cocaine self-administration, MK-801 was not self-administered. However, in monkeys with a history of phencyclidine self-administration, MK-801 maintained self-administration behavior. The abuse potential of a particular drug, as measured by its ability to maintain drug self-administration behavior, may be critically related to the pharmacologic history or experience.

Recent work by Falk and colleagues also has shown that different drug histories can affect schedule-induced drug intake and preferences (Falk and Tang 1989*a, b;* Tang and Falk 1986, 1988). Thus, the ability of certain drugs to maintain responding can depend on a subject's self-administration history and the type of drugs with which the subject has had experience. It is significant that many of these same variables appear to play a role in determining the subjective effects of a variety of drugs in humans, as well as in the likelihood of drug abuse (Haertzen et al. 1983).

DRUG-BEHAVIOR INTERACTION HISTORY

Experimental studies also have demonstrated that the subsequent effects of a drug can be modified by the behavioral consequences that occurred at the time the drug was administered. A clear example of the importance of a drug-behavior interaction history was reported by Smith and McKearney (1977). Key pecking by pigeons was maintained under a procedure in which food was produced only if at least 30 s elapsed between pecks. Increases in responding

produced by an initial injection of *d*-amphetamine decreased the frequency of food presentation because the time between responses after drug typically was less than the 30-s schedule requirement. However, subsequent drug administrations, spaced days apart, resulted in progressively smaller increases in responding, eventually resulting in the failure of *d*-amphetamine to have any effect. When the contingency was removed during drug administration, these drugs continued to produce increases in responding. In many respects, these results resemble tolerancelike actions but actually derive from the dynamic interactions between behavior and the environment that are induced by the drug.

Another example of drug-behavior interaction history is provided by an experiment in which morphine, which does not typically increase punished behavior, did so after a drug effect occurred under a particular set of experimental conditions (Brady and Barrett 1986). The effects of morphine were studied under a multiple fixed-interval schedule in which responding in each of two components terminated a stimulus correlated with electric shock delivery. Morphine was first studied when responding of monkeys during one component was suppressed by arranging that a shock also followed every 30th response during the interval; this procedure reduced responding during that interval, but responding remained at a high rate during the alternate unpunished component. Morphine unexpectedly increased punished responding, an effect not previously reported for this drug; these increases also occurred when the punishment schedule was studied alone under a single-component schedule condition. Extensive analyses of the conditions responsible for this atypical effect resulted in the conclusion that the context in which behavior occurs when a drug is administered can interact with the effects that drug will have on behavior. Behavioral and pharmacologic histories can both be important, alone and in combination. What is remarkable about these results is that the conditions under which a particular effect is induced by the environment become less important once the effect has been obtained. The modified actions of the drug are then sustained in the absence of the inducing condition.

SUMMARY AND CONCLUSIONS

The studies summarized briefly in this chapter provide several examples of experiments in which a behavioral or pharmacologic history produced profound changes in the effects of an abused drug. In many instances these changes were qualitative, that is, the direction of the effects that drug usually has on behavior was modified dramatically. AMPH and cocaine-both of which may

normally decrease a particular behavior—will, after a suitable history, produce large increases in the behavior under study. If the behavioral effects of a drug are related to the abuse potential of that drug, then any condition that alters the behavioral effects of that drug could be an important component underlying vulnerability to drug abuse.

A noteworthy aspect of the studies that have been described is the lasting influence that prior experience can have on behavior and the behavioral effects of drugs in the absence of overt changes in behavior itself. The traces of past experience may not be apparent in ongoing behavior until a drug is administered. This feature makes drugs an important tool for studying behavioral processes and raises several related questions pertaining to the mechanisms responsible for these effects (Barrett 1986). Although at present there is little information available to answer the several questions raised by these experiments, newer techniques available in the neurosciences promise to help reveal the neurobiological correlates of these changes that should be of general importance in understanding both behavioral and pharmacologic processes.

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Importance of Behavioral Controls in the Analysis of Ongoing Events

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INTRODUCTION

One of the most compelling factors that have increased interest in the neurobiological mechanisms of substance abuse has been concern about the deleterious effects of the direct and indirect actions of behaviorally active compounds. For example, cocaine was considered a relatively benign compound until recent findings indicated the drug severely disrupts social and occupational functioning and is associated with a high degree of morbidity and mortality (Fischman 1987). Additional concerns resulting in increased efforts to understand and treat drug abuse include the deleterious effects of these drugs on the immune system and the increased risk of acquired immunodeficiency syndrome associated with the intravenous (IV) use of these compounds.

A second factor that may account for the growing interest in delineating the neurobiology of substance abuse is the rapid rise in technology over the past several years in pharmacology and the neurosciences. New or refined procedures have been developed for investigating the processes that occur in the central nervous system directly related to the behavior of an organism (Dworkin and Smith 1987, 1989). It is now reasonable to believe that a neurobiological approach to substance abuse not only will lead to a greater understanding of the factors responsible for drug abuse but will also provide some reasonable indications and directions for the improved treatment of this problem (Koob and Bloom 1988).

Most of the techniques and procedures for directly investigating the neurobiologic components of substance abuse are discussed in this monograph, ranging from those that determine the effects of pharmacologic agents with known mechanisms of action on measures of drug abuse to those that provide direct assessments of neuronal activity associated with drug

administration. Between these extremes are procedures that (1) provide correlational analysis of receptor binding with behavioral effects (Ritz et al. 1987; Spealman et al. 1989; Smith et al. 1984*b); (2)* determine the effects of specific neurotoxin lesions on the reinforcing and other behavioral effects of abused drugs (Dworkin and Smith 1988; Koob and Goeders 1989); (3) provide direct measures of the content, release, and utilization of neurotransmitters correlated with ongoing behavior (Miyauchi et al. 1988, 1989; Smith et al. 1981, 1984*a);* and (4) provide measures of functional neuronal activity (Pettit and Justice 1989; Porrino et al. 1984).

These neurobiological procedures must be coupled with appropriate and well designed behavioral procedures in order to provide an unambiguous analysis of the behavioral significance of any observed neurobiological changes. This paper deals with some of the issues related to the selection of appropriate behavioral baselines for neurobiological investigations.

Research in experimental analysis of behavior and behavioral pharmacology has provided several insights into some of the important variables that should be considered in any research effort to determine the behavioral effects of abused drugs. These variables include nonpharmacologic as well as pharmacologic factors (Barrett 1987). These same variables may also determine the behavioral effects of neurobiological manipulations. The nonpharmacologic variables that should be considered in any behavioral-neurobiological investigation include (1) the control rate of responding or activity, (2) the type of reinforcer or punisher, (3) the environmental context, and (4) the behavioral history of the organism. The list of pharmacologic factors is also of considerable importance: (1) the class and dose of the drug, (2) the route of administration, and (3) the pharmacologic history of the organism.

Analyzing the neurobiological aspects of drug abuse is further complicated by the interactions that can take place between the nonpharmacologic and the pharmacologic factors. For example, the effects of *d*-amphetamine on behavior are influenced by the previously mentioned factors. Amphetamine typically results in an inverted-U-shaped dose-effect curve on schedule-controlled behavior. Low doses of the drug have little or no effect on responding, and moderate doses increase operant responding, whereas larger doses decrease response rates; moreover, the effects of amphetamine are also related to the control rate of responding (Dews and Wenger 1977). Low to moderate rates of responding are increased by doses of the drug that have no effect on, or

decrease high rates of, responding in the same animal even in the same experimental session. Thus, both the dose and the control rate of responding can be important determinants of the behavioral effects of amphetamine. In addition, the behavioral effects of amphetamine are influenced by both the behavioral history of the organism and the current environmental context (Barrett and Witkin 1986). Two studies have shown that the typical effects of amphetamine on punished behavior can be reversed by behavioral variables in the absence of any apparent changes in the baseline performance. Amphetamine does not typically increase low rates of punished responding (Houser 1978). Significant increases in punished responding, however, were observed when *d*-amphetamine was given to monkeys with a history of responding maintained by electric shock (Barrett 1977) or when responding was maintained in alternating shock avoidance and shock punishment components (McKearney and Barrett 1975). These effects and their determinants influence the behavioral effects of other drugs of abuse as well as amphetamines (Barrett and Witkin 1986).

In addition to their rate-dependent effects, abused substances have both reinforcing and discriminative-stimulus functions. Their reinforcing effects can be directly measured by self-administration studies, and the stimulus functions are typically determined by means of drug discrimination procedures. These behavioral effects are of importance to any neurobiological investigation of drug abuse. Although the same or similar neurobiological techniques can be used to evaluate these effects, it is unlikely that similar behavioral designs can be used to measure all three effects. This chapter focuses on a few behavioral procedures that are used to evaluate the neurobiological components of the reinforcing effects of drugs and presents some of the behavioral controls that are necessary for these investigations. In particular, the influences of behavioral context and response contingency are presented.

BEHAVIORAL CONTEXT OF NEUROTOXIN LESIONS

Besides altering the effects of drugs on schedule-controlled behavior, environmental context can also influence the effects of 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens on morphine self-administration (Dworkin et al. 1988). Neurotoxin-lesion procedures are used to assess the involvement of specific neurotransmitter systems in the neurobiological components of drug reinforcement. A number of studies have reported that the selective destruction of dopaminergic pathways from the ventral tegmental area to the nucleus accumbens results in a decrease in the reinforcing efficacy of

stimulants (Roberts et al. 1980; Roberts and Koob 1982). Several different behavioral schedules have been used to evaluate the effects of dopaminergic lesions on cocaine self-administration, including fixed-ratio (FR) and progressive-ratio schedules and a concurrent chained schedule of food, water, and drug self-administration (Koob and Goeders 1989). All of these studies reported a decrease in cocaine self-administration, suggesting that this lesion causes a selective reduction in the reinforcing effects of cocaine and thus eliminating two other potential interpretations of the behavioral effects of this lesion, i.e., nonselective response decrements and changes in rate-dependent effects of the drug.

Similar attempts to elucidate the role of dopaminergic innervations of the nucleus accumbens in the reinforcing effects of opiates have not resulted in consistent effects. Lesions of the nucleus accumbens induced by 6-OHDA have resulted in increased morphine self-administration in opiate-dependent rats (Smith et al. 1985). It has been suggested that the increased rate of self-administration resulted from a decrease in the reinforcing efficacy of the drug. A similar lesion did not alter food, water, or morphine self-administration in opiate-dependent rats that were studied on a concurrent chained schedule (Dworkin et al. 1988). Furthermore, a more extensive dopamine lesion of the nucleus accumbens resulted in a 70 percent decrease in cocaine self-administration and only a 24 percent decrease in heroin self-administration in rats trained to self-administer both compounds on alternate days (Pettit et al. 1984). Because several reports indicate that nucleus accumbens dopaminergic neurons are involved in the behavioral effects of opiates, the results reported in the latter two studies may have been a function of the environmental context; i.e., in both of these studies, responding was maintained by other reinforcers in addition to the opiate. Moreover, the two lesion studies on morphine self-administration (Dworkin et al. 1988; Smith et al. 1985) were conducted in the same laboratory using similar procedures; the obvious difference was the food and water contingency. Thus, the results of these two studies clearly demonstrate that environmental context can influence the effect of this dopaminergic lesion on morphine self-administration in opiate-dependent rats.

RESPONSE CONTINGENCY

The term *reinforcementas* used in behavioral research has a fairly well accepted definition: an increase in the probability or frequency of some observable behavior when the presentation of an environmental event is contingent on that behavior (Skinner 1938). Behavior can be changed by the

presentation or removal of the event; the term *positive reinforcer* is used to describe the event in the first case, and *negative reinforcer* in the second. This definition stresses the contingent relationship between some measurable behavior and the delivery of the environmental event. The precise relationships between behavior and the environmental event are collectively referred to as schedules of reinforcement. A traditional view of reinforcement asserted that there were three mutually exclusive categories of contingent events: reinforcers, punishers, and neutral stimuli. An additional implication was that every environmental event is an incontrovertible member of one of these three categories, and thus it was assumed that the effects of all environments were trans-situational. After 30 years of research in experimental analysis of behavior and behavioral pharmacology, it is common knowledge among researchers that environmental events such as administration of food and electric shock can have both reinforcing and punishing effects (Morse and Kelleher 1977). Furthermore, abused drugs such as cocaine can function as both positive and negative reinforcers (Spealman 1979). The hedonic relevance of environmental events depends on several factors, including the behavioral and pharmacologic history of the organism and the current condition or environmental context in which the organism is placed. Most of the research in these areas has focused on the contingent delivery of environmental events. If the defining feature of reinforcement is the contingent delivery of these events, however, then what is the hedonic relevance of noncontingent presentations of putative reinforcers or punishers? The remainder of this chapter presents some of the behavioral and neurobiological differences observed between the contingent and noncontingent delivery of environmental events.

NEUROBIOLOGY OF DRUG REINFORCEMENT

The yoked-box procedure (figure 1) is the behavioral paradigm that has been used in several studies investigating the neurobiology of reinforcement. This procedure involves the use of triads to assess relative differences related to the contingent versus noncontingent presentation of environmental events. All subjects are randomly assigned to one of three conditions. Responding by one of the subjects results in the contingent delivery of a reinforcer and the simultaneous delivery of the same environmental event to a second member of the triad. The third subject is typically used as a control for nonspecific factors (i.e., handling, food deprivation, exposure to the experimental chamber) and is not exposed to the environmental event under investigation. As shown at the bottom of figure 1, if the contingent delivery of the environmental event results in a specific behavioral or neurobiological effect in the subject that is receiving

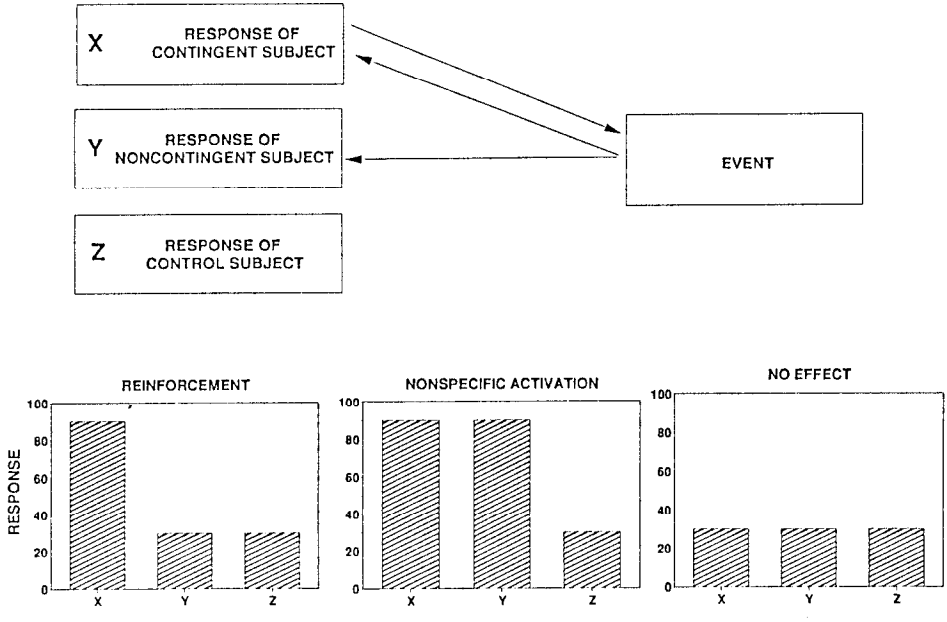

FIGURE 1. *Diagram of the yoked-box procedure.*

contingent deliveries of the reinforcer, then the effect is considered to be a function of reinforcement. If similar changes are observed in both subjects that are exposed to the environmental event under investigation, then the effect is considered to be a nonspecific activation produced by the delivery of the event. The third situation, in which all three subjects show the same effect, indicates that the event has no significant behavioral or neurobiological relevance under these conditions.

The yoked-box procedure for evaluating the neurobiology of reinforcement has several advantages. Many behaviorally relevant variables can be controlled simultaneously, and the potential influences of historical or contextual variables can be eliminated. The procedure is also relatively easy to establish. Disadvantages include various sources of bias and random error, such as individual differences in the effectiveness of the event-which would result in

misleading data-and potential fluctuations in attention, which might favor the contingent condition (Church 1964; Gardner and Gardner 1988). Selecting environmental events with a high degree of saliency (i.e., presentation of food to meal-deprived subjects, electric foot shock, or intravenous drug delivery) will most likely eliminate most of the disadvantages inherent in this behavioral design.

The yoked-box procedure was used by Smith and Dworkin (1986) to investigate the neurobiological correlates of the reinforcing effects of morphine. A triad of littermates, male F-344 rats, was used for these studies. Two of the rats in each triad were made physically dependent on morphine and then randomly assigned to one of two conditions. One rat was allowed to self-administer the drug, while the other received noncontingent injections yoked to the delivery schedule of the self-administering rat. A third subject was never exposed to morphine and received yoked saline infusions. It was reasoned that any differences observed between the rats receiving yoked-morphine and those receiving yoked-vehicle injections would be the result of the pharmacologic actions of the drug, whereas any additional differences between the self-administration condition and the yoked-morphine condition would be related to the reinforcing effects of the drug. A small FR schedule (FR 10) was used for the self-administration condition to minimize the influence of responding on the neurochemical measures. Neurotransmitter turnover rates (Smith et al. 1981, 1984*a)* and receptor-binding studies (Smith et al. *19846)* indicated that the contingent administration of the drug produced more extensive and larger neurobiological changes than did the noncontingent administration. As a result of these studies, it was suggested that two potential neuronal circuits are involved in the reinforcing effects of opiates. Moreover, the proposed systems are consistent with systems identified by 2-deoxyglucose (2-DG) autoradiography in rats exposed to contingent versus noncontingent electrical stimulation of the ventral tegmental area (Porrino et al. 1984).

In our attempts to evaluate the neurobiological aspects of the reinforcing effects of cocaine using the yoked-box procedure, we have observed a more profound and obvious difference in contingent versus noncontingent administration of cocaine. Littermate triads of male F-344 rats were surgically prepared with intravenous (IV) catheters. The rats were placed in three individual operant conditioning chambers in single sound-attenuating chambers and allowed to recover from the surgery. When food and water intake returned to baseline values, each rat was assigned to one of three conditions. One was trained to self-administer cocaine under an FR 1 schedule that was increased to FR 2

when stable rates of cocaine intake were observed. The other two littermates received noncontingent injections of either cocaine or saline yoked to the intake of the self-administering subject. The number of food pellets available each day for the yoked subjects was also yoked to the food consumed by the rat that was exposed to the contingent administration of cocaine. All subjects were continuously housed in the operant chambers under a reversed cycle of 12 hr light and 12 hr dark. Each drug and vehicle infusion (0.2 mL) was delivered over approximately a 5-s period and was paired with a 20-s light and tone presentation. Rats were exposed to one of two doses (0.33 or 0.67 mg per infusion) under one of three different access conditions: 2-hr drug availability, 6-hr drug availability, or termination of access after the 80th injection during a 24-hr period (80 infusions per day). For the latter condition, the drug was available from 5 a.m. until the 80th infusion was delivered. The rats were fed twice a day, with a maximum of 20 1-mg food pellets. The data obtained from the 0.33-mg-per-infusion, 80-infusion-per-day condition, and the 2-hr, 0.67-mg-per-infusion condition are shown in figure 2.

Three of the rats (14 percent) exposed to 0.33 mg per infusion for 80 infusions per day died within 30 days of contingent drug administration, whereas 12 of the rats (55 percent) that received yoked infusions of cocaine died before the 30th session. None of the rats exposed to yoked-vehicle injections died. The major behavioral difference observed between rats exposed to contingent administration of cocaine and those exposed to noncontingent administration of cocaine was the lack of, or slower development of, tolerance to the anorectic effects of cocaine. Figure 2, which shows the number of infusions and the number of food pellets consumed for three representative triads, also shows the development of tolerance to the anorectic effects of the drug. If tolerance to the effect of the drug on food intake did develop in the rats that were exposed to noncontingent infusions of cocaine, it took several more days to occur in them versus the self-administering littermate. These findings indicate that an enhanced morbidity and mortality are associated with noncontingent administration of cocaine compared with contingent administration of the drug and emphasize the importance of contingent relationships as defining features of reinforcement mechanisms.

Eight additional triads were prepared with indwelling IV catheters for the 2-DG studies. Cocaine infusions (0.67 mg per infusion) were available for a 2-hr period each day Monday through Friday. The animals were placed into operant conditioning chambers for the cocaine exposure period; when not in the operant chambers, they were placed in a home cage and given bihourly infusions of

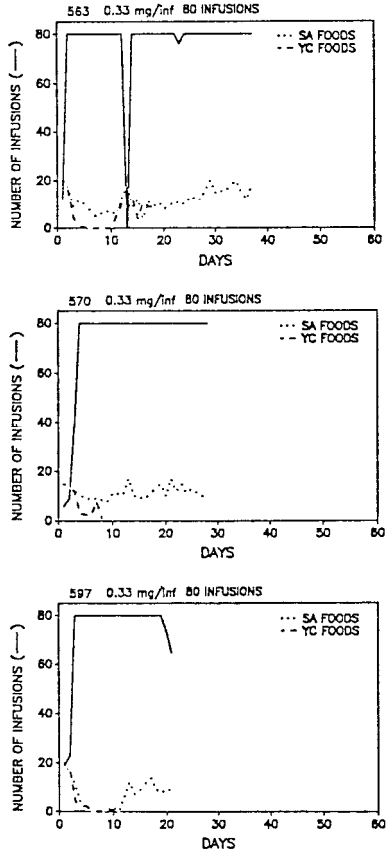

FIGURE 2. *Number of infusions (solid line) and food consumption for the rat receiving contingent (SA, dotted line) and noncontingent (YC, dashed line) over consecutive sessions. Panels shown contain data from three representative trials in which the yoked animal died.*

saline. The self-administering rats were trained under an FR 2 schedule. Three of the yoked-cocaine littermates died within 14 days of exposure to cocaine. Four of the triads were pulse labeled with 2-DG during the 15 days of drug exposure. All rats exposed to cocaine engaged in excessive conditioned stereotypy by the second day of exposure. The contingent administration of cocaine resulted in an increase in local cerebral glucose utilization in several regions: prefrontal cortex, ventral pallidum, globus pallidus, and nucleus

accumbens. It has been suggested that these regions are involved in mediating the reinforcing effects of cocaine. Furthermore, decreases in glucose utilization were observed in the lateral septum, lateral hypothalamus, and amygdala. The effects in these regions may indicate the potential aversive effects of noncontingent cocaine (figure 3).

NEUROCHEMICAL CORRELATES OF PUNISHMENT

The yoked-box procedure has also been used by Dworkin and colleagues to determine the neurobiological components of punishment (Dworkin and Smith 1989; Miyauchi et al. 1988, 1989; Izenwasser et al. 1989). Littermate triads were studied by means of an operant procedure that maintained similar patterns and rates of responding in punished and unpunished situations (Dworkin et al. 1989). A third rat received noncontingent food and electric shock presentations. Specifically, a random-ratio schedule of food reinforcement and electric foot-shock punishment was used to maintain low rates of responding by the punished subject, and a yoked-interval schedule maintained similar rates and patterns of responding by the unpunished subject. Pentobarbital (Dworkin et al. 1989) and chlordiazepoxide (Izenwasser et al. 1989) produced selective increases in punished responding and only dose-related decreases in unpunished responding, whereas cocaine increased only unpunished responding (Dworkin et al. 1989). A third subject that received noncontingent food and shock presentations yoked to the punished subject was added for the neurochemical studies. Initial studies indicated that punishment resulted in a selective increase in serotonin turnover in the frontal cortex and increased GABA utilization in the cingulate cortex and dentate gyrus. The turnover of serotonin and dopamine was shown to decrease in the hypothalamus and frontal cortex, respectively. Punishment-specific decreases in benzodiazepine receptor binding in the cerebellum were observed in an additional group of rats (Izenwasser et al. 1989). A replication of the neurotransmitter turnover studies has recently been completed using of 12 additional triads and more sensitive neurochemical procedures. The data shown in figure 4 indicate some of the selective punishment effects observed in that study.

CONCLUSION

The studies reviewed in this paper strongly indicate that behavioral variables can profoundly influence the neurobiological effects of manipulations of central nervous systems. The observation that environmental context can alter the behavioral effects of neurotoxin lesions suggests that behavioral factors that

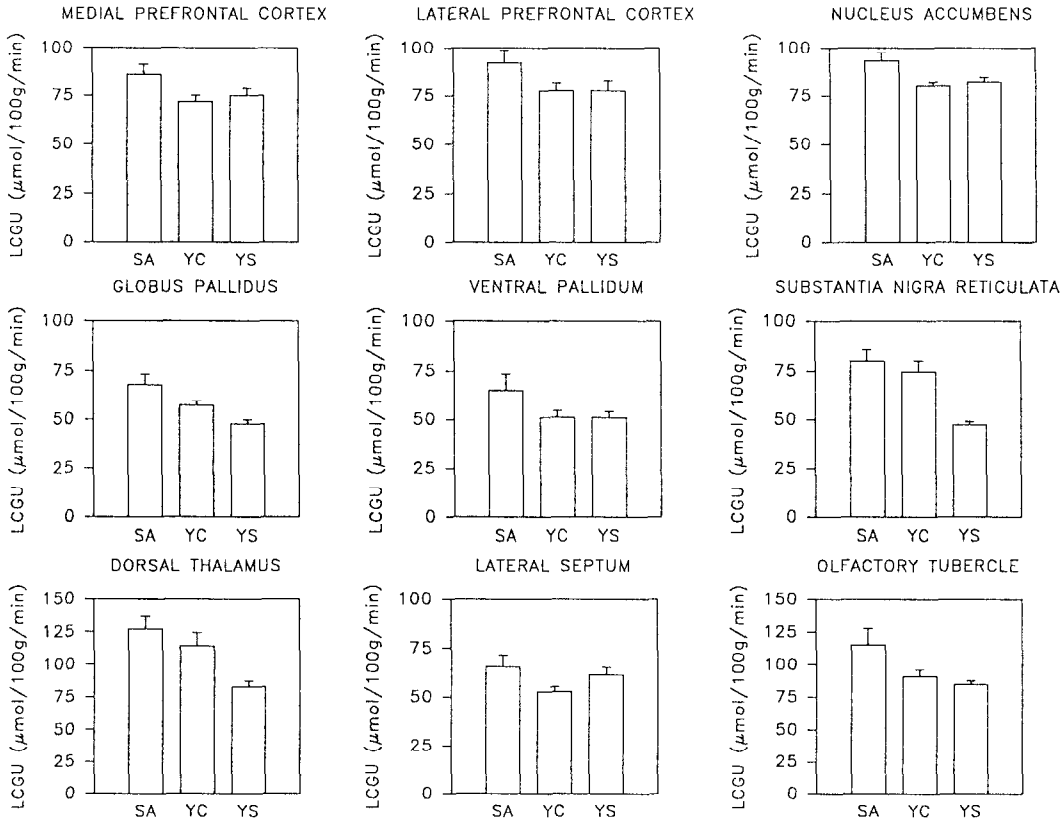

FIGURE 3. *Local cerebral glucose utilization of nine brain regions in groups of rats exposed to contingent (self-administering, SA bars) infusions of cocaine or noncontingent infusions of cocaine (yoked cocaine, YC bars) and saline (yoked saline, YS bars). Vertical bars indicate ±1 SE_M.*

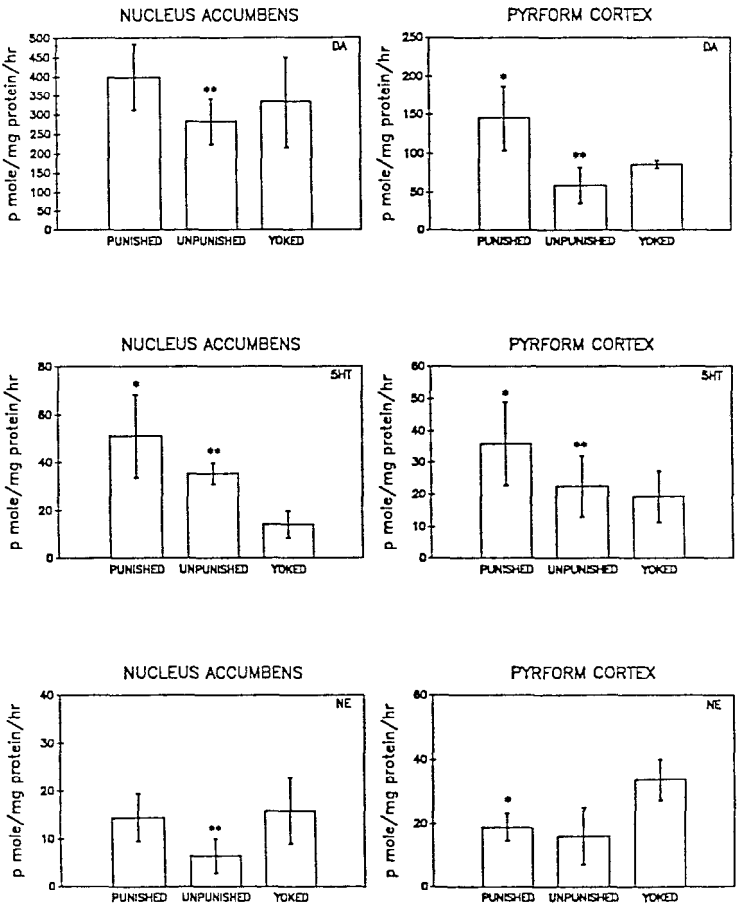

FIGURE 4. *Neurotransmitter utilization rates for dopamine (DA, top panels), serotonin (5 HT, middle panels) and norepinephrine (NE, bottom panels) for rats exposed to the different conditions of the punishment procedure. Vertical bars indicate ±1 SE_M: Significant differences ($p < .05$) between the punished condition and the yoke condition (*) and between the punished and unpunished conditions (**) were determined using Dunnett's* **t** *test for multiple comparison.*

have been demonstrated to alter the behavioral effects of pharmacologic agents may also alter the effects of other neurobiological manipulations. The importance of behavioral factors is further indicated by the general findings that the contingent administration of several environmental events results in significant differences in numerous neurobiological measures compared with the noncontingent delivery of the same environmental event. The effects of these behavioral factors must be evaluated and controlled if a complete neurobiological analysis of behavior is to emerge. Thus, a complete understanding of the neurobiological aspects of behavior requires a sophisticated behavioral as well as neurobiological approach. The behavioral procedures reviewed here are certainly not the only procedures that can or should be used, but they have provided results that stress the importance of behavioral variables in investigations of the neurobiology of behavioral processes. At best, only a limited neurobiological account of behavior will come from investigations that do not include an analysis of behavioral factors; at worst, without such an account our conclusions will be wrong.

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Cocaine Self-Administration: Pharmacology and Behavior

William L. Woolverton

INTRODUCTION

As the title implies, the purpose of this chapter is to consider cocaine self-administration from the point of view of two categories of independent variables that are determinants of the behavioral effects of drugs: pharmacologic and behavioral variables. By definition, pharmacologic variables are those related to the drug, such as neurotransmitters and receptors, events that are usually initiated inside the organism. Behavioral variables are those relating to the behavior and environment, generally events initiated outside the organism. I emphasize cocaine self-administration for two related reasons: because interest in cocaine, among both the public and the scientific community, is waxing at this time and because my primary research interest for the past 15 years has been cocaine.

The basic message-that drug abuse is an interaction among an organism, a drug, and the environment-is not new. It is, however, a message that bears reiterating lest it be forgotten in the euphoria generated by our newfound ability, well characterized in this meeting, to measure neurotransmitter changes and the like taking place inside living organisms. The new technologies clearly offer the opportunity for developing a more comprehensive understanding of the role of the brain in drug abuse. At the same time, it is essential to emphasize that behavior, including drug-seeking behavior, is powerfully determined by variables that originate outside the organism as well. Any comprehensive understanding of drug abuse necessarily includes a complete understanding of each of these categories and, ultimately, of their interaction. What follows, then, is a brief review of a series of laboratory self-administration experiments, some published and some not, that address the roles of behavioral and pharmacologic variables in cocaine self-administration.

PHARMACOLOGY

The first series of studies involves evaluating the reinforcing effects of a series of compounds in an intravenous (IV) self-administration paradigm that has proved to be highly predictive of abuse potential (Johanson and Balster 1978). The assumption of these studies is that if a compound shares a pharmacologic action with cocaine, and both the compound and cocaine function as positive reinforcers under the same conditions, there is then reason to suspect that the common pharmacologic action plays a role in the reinforcing effect of cocaine. Obviously, this result does not demonstrate that the common mechanism determines the reinforcing effects of both compounds, but it does imply that the mechanism merits further investigation. Because cocaine has several neuronal actions-among which are blockade of the reuptake of the monoamines dopamine (DA), norepinephrine (NE), and serotonin (5-HT) and local anesthetic effects-we evaluated the reinforcing effects of drugs that are more or less selective in each of these actions. In the present context, the question is which, or which combination, of these actions is involved in the reinforcing effect of cocaine. A second assumption is that, because the brain is the organ that controls behavior, we are talking about central nervous system (CNS) pharmacology.

The preparation used in these studies has been described in detail elsewhere (Woolverton et al. 1984). Briefly, rhesus monkeys were prepared with chronic IV catheters and allowed to self-administer a baseline drug (cocaine or *d*-amphetamine) for 2 to 3 hr per day by pressing a lever under a fixed-ratio 10 (FR 10) schedule of reinforcement. When behavior maintained by the baseline drug was stable, the drug vehicle (usually saline) was substituted for the baseline drug until responding declined to low levels (usually less than 10 sessions). Subsequently, responding maintained by the baseline drug was reestablished, and when it was again stable, a dose of the test drug was made available for the same number of sessions as had been required for responding to decline to low levels when the drug vehicle was available. In this way, rates of self-administration of several doses of the test drug were determined with a return to baseline conditions between doses. A dose of a test drug was considered to function as a positive reinforcer if the mean rate of self-administration over the last three sessions of availability was higher than the rate maintained by the vehicle and the ranges did not overlap.

The compound GBR 12909 is a highly selective DA reuptake blocker (Heikkila and Manzino 1984) that has high affinity for cocaine binding sites (Madras et al.

1989). When GBR 12909 was made available for self-administration, responding was maintained above vehicle levels in all three of the monkeys tested to date (figure 1) (Bergman et al. 1989; Kleven et al. 1988). In the monkey in which a complete dose-response function was determined (8618), the dose-response function was a typical inverted U, with doses that were too low to maintain behavior, a dose that maintained the peak rate of responding, and higher doses at which rates of self-administration decreased. The potency of GBR 12909 in this preparation was approximately equal to that of cocaine. In contrast, neither the NE reuptake blocker nisoxetine (Woolverton 1987) nor the 5-HT reuptake blocker fluoxetine (figure 2) functioned as a positive reinforcer. I should emphasize that the study with fluoxetine is now under way in my laboratory, and the data are not complete. Nevertheless, there was no evidence of fluoxetine self-administration at the doses that have been tested. Although the rate of responding for saline was unusually high in monkey 8623, I have included the data to make an important point: that responding maintained by

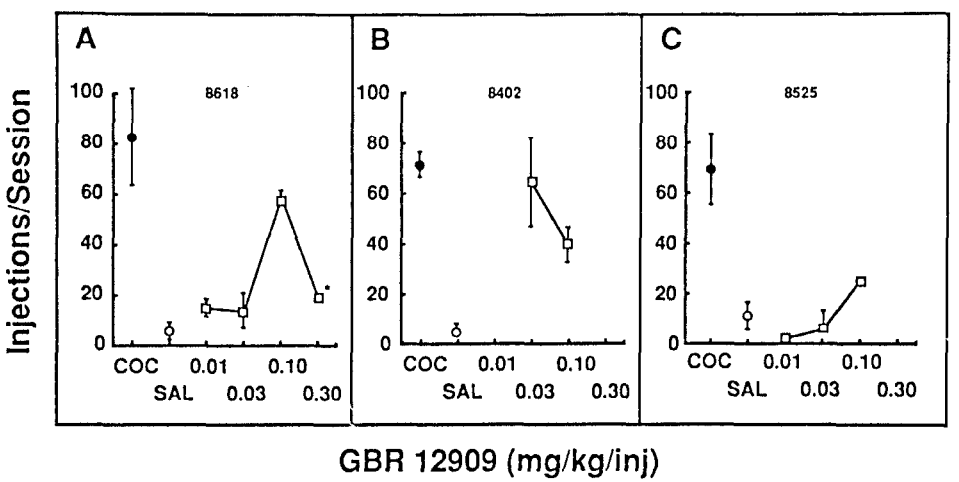

FIGURE 1. *Intravenous self-administration of the dopamine reuptake blocker GBR 72909 by rhesus monkeys. The number of injections was limited to 20 at this dose because the monkey became preconvulsive. The number of injections per 2-hr session is on the ordinate, and the dose of GBR 12909 is on the abscissa. Each point represents the mean of the last three sessions of availability of a dose, and the vertical lines represent the range.*

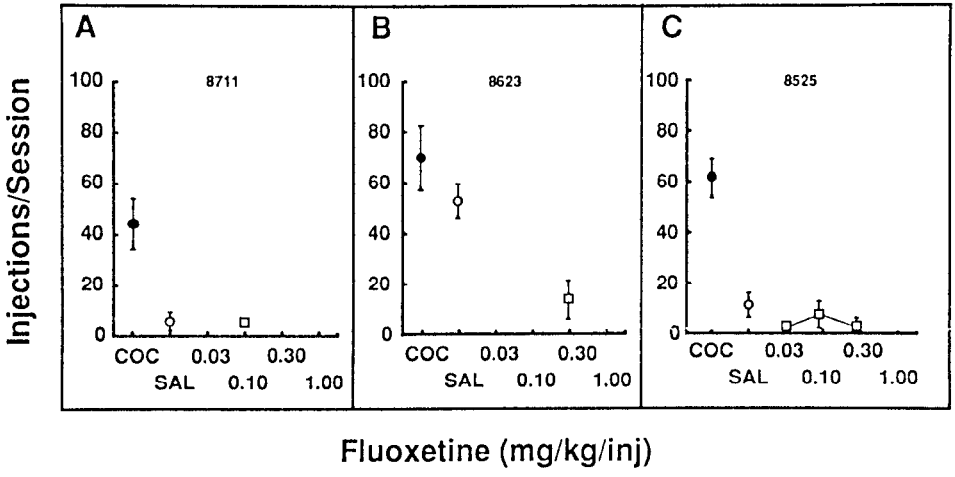

FIGURE 2. *Intravenous self-administration of the 5-HT reuptake blocker fluoxetine by rhesus monkeys. The number of injections was limited to 20 at this dose because the monkey became preconvulsive. The number of injections per 2-hr session is on the ordinate and dose of fluoxetine is on the abscissa. Each point represents the mean of the last three sessions of availability of a dose, and the vertical lines represent the range.*

fluoxetine occurred at a lower rate than did responding for saline is evidence that the doses of fluoxetine we have tested were behaviorally active.

In a study designed to examine the role of local anesthetic actions in the reinforcing effects of cocaine, we evaluated the reinforcing effects of a series of local anesthetics: procaine, lidocaine, tetracaine, and procainamide (Woolverton and Balster 1979). In fact, it is well known that some local anesthetics can function as positive reinforcers (Ford and Balster 1977; Hammerbeck and Mitchell 1978; Johanson 1980). This effect, however, seems to be limited to local anesthetics that are ester-linked compounds (Woolverton and Balster 1979). The amide-linked local anesthetics have not been found to function as positive reinforcers. Because not all local anesthetics function as positive reinforcers under these conditions, it seems unlikely that local anesthetic actions are responsible for the reinforcing effects of cocaine. Rather, the data suggest that cocaine and local anesthetics that can function as positive

reinforcers share some other action in common (blockage of DA reuptake?) that mediates their reinforcing effects.

If CNS DA is involved in the reinforcing effects of cocaine, then it is reasonable to ask which DA receptor subtype is involved. Woolverton and colleagues (1984) tested a number of direct DA agonists in this self-administration paradigm. All the compounds that stimulate D_2 receptors (apomorphine, propylbutyldopamine, bromocriptine, and piribedil) functioned as positive reinforcers in at least half the monkeys. The compound most selective for D_2 receptors (piribedil) was the most consistent positive reinforcer; four of four monkeys tested self-administered piribedil. In fact, we have used piribedil since then as a baseline drug in antagonist studies (Woolverton 1986). On the other hand, the D_1 agonist SKF 38393 was not self-administered by any monkey up to high doses, i.e., to doses that have been found to decrease the rate of lever pressing maintained by food in rhesus monkeys (Kamien and Woolverton 1989).

Taken together, these data support the not altogether surprising conclusion that pharmacology is important in drug self-administration. Across a constant set of behavioral conditions, drugs that act primarily as agonists of DA systems in the brain, either indirectly or directly, consistently functioned as positive reinforcers, whereas drugs that act primarily to block uptake of other monoamines or are local anesthetics did not. These findings support the belief, which has been held for some time (Wise and Bozarth 1981), that DA-reuptake blockage is the operative mechanism in cocaine pharmacology, mediating its reinforcing effect, and that an action of DA at D_2 receptors plays a necessary role in this effect. Pharmacology, however, is not sufficient to explain or characterize drug-maintained behavior.

BEHAVIOR

Drug-maintained behavior is not limited to the final drug self-administration response. It may be, particularly in humans, a long and elaborate sequence of complex behaviors that evolves over time and is ultimately maintained by the self-administration of a drug. Furthermore, alternatives to drug self-administration are usually available. The next series of studies involves evaluating the reinforcing effects of cocaine under several different behavioral conditions. The assumption (perhaps it is a question) of these studies is that the pharmacology of cocaine alluded to previously is constant across behavioral conditions. Ultimately, this assumption can and should be tested directly. Despite CNS pharmacology, however, rates and patterns of drug-maintained

behavior (indeed, whether a drug functions as a positive reinforcer at all) vary widely with the environmental circumstances under which a drug is made available.

The first environmental variable to emphasize, and the one for which there are the most data, is the schedule of reinforcement. It is well established that cocaine can function as a positive reinforcer under a wide variety of schedules of reinforcement (Spealman and Goldberg 1978; Johanson and Fischman 1989). In a study evaluating potential antagonists of the reinforcing effects of cocaine, we trained monkeys under a multiple schedule of food or cocaine availability in which food was available in the first and third component of the multiple schedule and cocaine was available in the middle component. The schedule of reinforcement was an FR 30, and there was a 2-min timeout (TO) after delivery of each reinforcer. As can be seen in figure 3, high rates and patterns of responding typical of FR schedules were maintained by cocaine injections. In contrast, when the same dose of cocaine was made available under a fixed-interval (FI) schedule of reinforcement, responding occurred at low rates early in the interval and gradually accelerated to high rates at the end of it (figure 4) (Johanson 1982); that is, simply manipulating the conditions of cocaine availability dramatically alters the rate and pattern of drug-maintained behavior. It seems unlikely that schedule of reinforcement has changed the CNS pharmacology of cocaine.

An experiment by Spealman (1979) makes this point in an even more dramatic way. In that study, squirrel monkeys were prepared with IV catheters and trained to press one lever (right lever) under a variable-interval (VI) 3-min schedule of reinforcement for injections of cocaine. After VI behavior was stable (approximately 2 wk), a second lever was added to the chamber. Responding on the second (left) lever terminated cocaine availability under an FI 3-min schedule of reinforcement, i.e., the chamber went dark and responding had no consequence for 1 min. Responding was maintained on both levers under these conditions, with rates and patterns typical of each schedule of reinforcement (figure 5); that is, behavior was simultaneously maintained by cocaine injection and by the termination of cocaine availability. When conditions were modified so that responding on the left lever produced cocaine injections under the VI schedule and responding on the right lever was maintained under the FI schedule of cocaine termination, rates and patterns of responding changed appropriately to the new schedule conditions. When saline was substituted for cocaine, responding declined on both levers, indicating that cocaine injections were necessary to maintain behavior on both levers. When the amount of

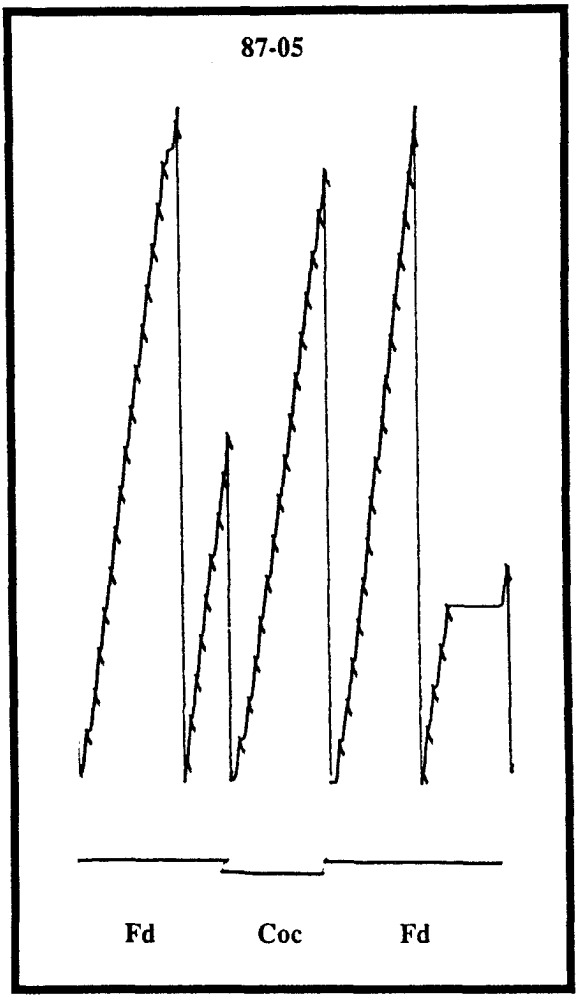

FIGURE 3. *Cumulative response records for a rhesus monkey responding under a multiple schedule of food (Fd) or cocaine (Coc) availability. Food was available in the first and third components, noted by the upward deflection of the lower pen. Cocaine (0.025 mg/kg per injection) was available in the middle component. In all three components, the schedule of reinforcement was FR 30, with a 2-min time out (TO) after delivery of each reinforcer. In addition, there was a 15-min TO between components of the multiple schedule. The recorder did not run during TO periods.*

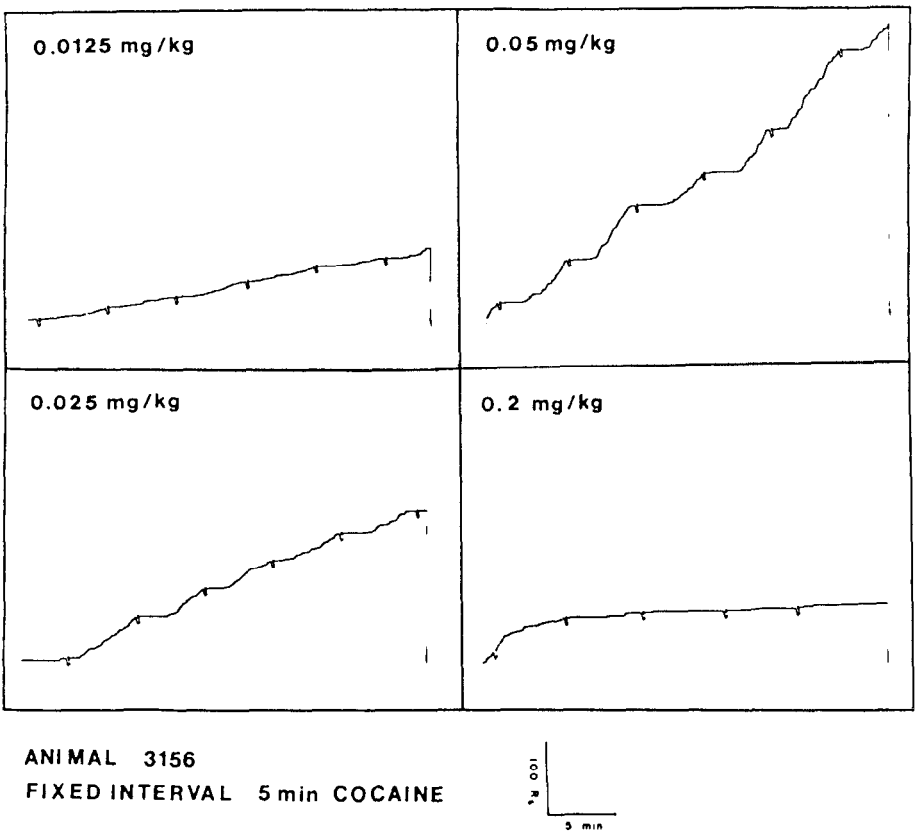

FIGURE 4. *Cumulative response records for a rhesus monkey responding under a fixed-interval 5-minute schedule of cocaine availability. Doses of cocaine are indicated in the upper left of each panel. Each record is the first 30 min of a 3-hr session. (From Johanson 1982.)*

cocaine that the monkey normally self-administered in a session was infused noncontingently, immediately before the session, responding was not seen on either lever, indicating that cocaine was not nonspecifically increasing lever pressing, i.e., to maintain behavior on both levers, cocaine had to be contingent on responding. When responding on the FI lever no longer terminated cocaine availability, responding on that lever decreased, whereas responding on the VI lever to receive cocaine continued to be maintained. This is convincing

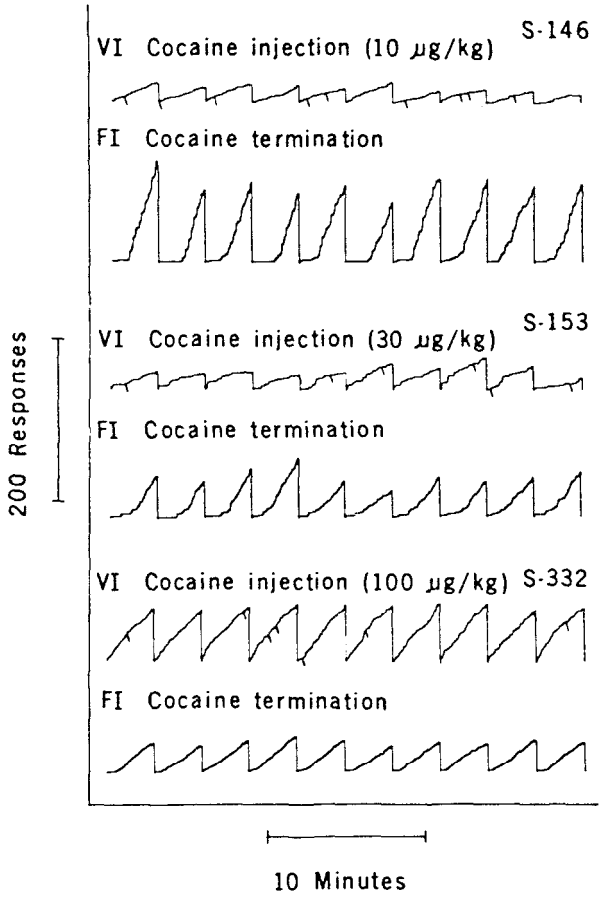

FIGURE 5. *Cumulative response records for squirrel monkeys responding under schedules of cocaine injection and termination of cocaine availability. Responses on the right lever (upper record in each panel) produced intravenous injections of cocaine under* a *variable-interval (VI)* schedule. *Responses on the* left lever *(lower record in each panel) terminated the schedule of cocaine injection for 1 min under a fixed-interval (FI) schedule. Pens were reset* at *the beginning of time out periods, during which recorders did not* run. Doses of cocaine are indicated in the *upper* left of each *panel. Each record is the first* 30 *min of a 3-hr session. (From Spealman 1979, copyright 1979, American Association for the Advancement of Science.)*

evidence that cocaine was functioning simultaneously as a positive reinforcer and as a negative reinforcer in the same monkey. Again, the question to ask is, How can we explain cocaine functioning simultaneously in the same organism as both a positive and a negative reinforcer on the basis of the pharmacology of cocaine?

The third study was conducted in my laboratory by Michael Nader (Nader et al. 1989). In this study, monkeys were trained in a discrete-trials choice paradigm and allowed to choose between an injection of cocaine and food pellets. The procedure has been described in detail elsewhere (Woolverton and Johanson 1984). Briefly, when a session begins, white lights are illuminated above the left lever, and red or green lights are illuminated above the right lever. Red and green are each associated with one of the reinforcers, cocaine or food. Responding on the left lever changes the light color above the right lever from red to green and from green to red. After a minimum of three color switches, a monkey can choose a reinforcer by pressing the right lever when the light associated with that reinforcer is illuminated. After a choice is completed, all the lights in the chamber go off and there is a 10-min TO between trials. After that TO, a new trial is initiated as before. Sessions consisted of 15 trials and were conducted each day.

When the number of food pellets available as the alternative to cocaine was held constant and the dose of cocaine was increased, the frequency of cocaine choice increased (figure 6). On the other hand, when the dose of cocaine was held constant and the number of food pellets available as the alternative to cocaine was increased, the frequency of drug choice decreased. Put simply, the reinforcing effect of cocaine varied with dose and with the magnitude of an alternative reinforcer that was simultaneously available.

SUMMARY

I would like to stress several major points. The first is that CNS pharmacology is important in drug self-administration. In the case of cocaine, the necessary effect seems to be blockade of reuptake of DA rather than blockade of reuptake of NE or 5-HT or local anesthetic effects. An action of that DA on the D_2 subtype of DA receptors appears to play an important role.

The second point is that increased DA in synapses in the CNS is not sufficient to explain drug-maintained behavior. The schedule of reinforcement critically determines rates and patterns of drug-maintained behavior. Barrett (this

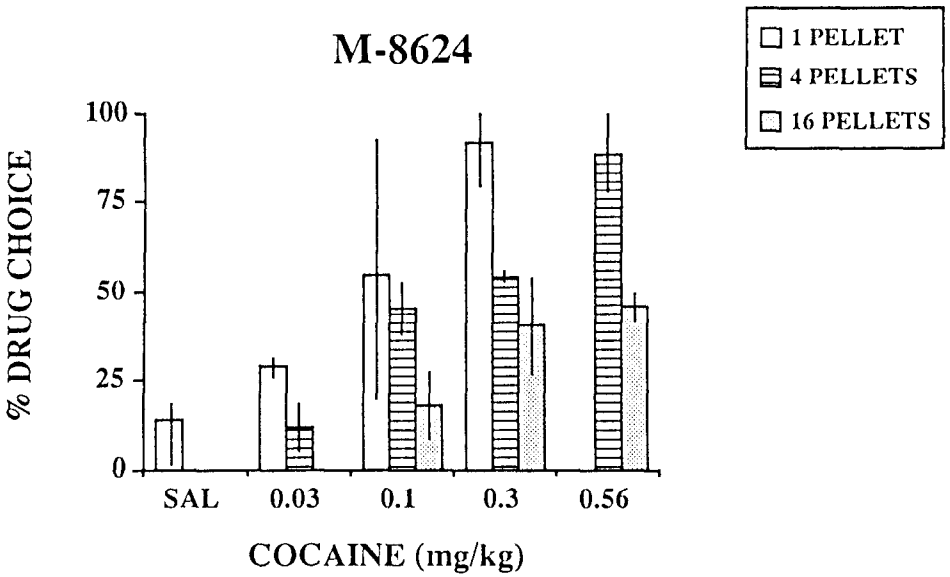

FIGURE 6. *Frequency of cocaine choice in monkeys given a choice between cocaine and food. Dose of cocaine is on the abscissa. Different bars represent different numbers of food pellets available as the alternative to cocaine. Vertical lines are the range of at /east two evaluations of each choice condition.*

volume) has presented convincing evidence that behavioral history, i.e., the sequential exposure to alternative behavioral conditions, can dramatically alter drug effects. What I hope to have made clear is that the simultaneous opportunity to engage in other behaviors can dramatically alter the reinforcing effects of drugs. Indeed, under appropriate conditions, a drug can function as both a positive and a negative reinforcer simultaneously in the same animal. The drug-choice studies I have described emphasize the dynamic nature of the interaction between behavioral and pharmacologic variables. The frequency of choosing to self-administer cocaine varied with the magnitude of the dose of cocaine (a pharmacologic variable) and the magnitude of an alternative positive reinforcer that was available (a behavioral variable). Increasing the magnitude of the alternative reinforcer decreased cocaine choice, but increasing the magnitude of the cocaine dose reestablished drug preference. In a sense, this is not a surprising result. The rhetoric of the times, however, would have it that

drug self-administration, particularly cocaine self-administration, is somehow a different class of behavior that is not subject to the usual laws governing the behavior of organisms. As our understanding of drug self-administration evolves, however, that position will become increasingly untenable.

These experimental findings challenge simple neurobiological assumptions about the determinants of drug self-administration. Clearly, a reinforcing effect, like any other behavioral effect of a drug, is not simply an immutable effect of a pharmacologic property of the drug. Given the pivotal role of reinforcing effects in drug abuse, this realization is critical to a comprehensive understanding of drug abuse. Any complete neurobiological model of drug abuse will have to account for these effects. Apparently, increasing the concentration of DA in synapses in the brain is not sufficient to account for drug-maintained behavior. As has been noted by Fibiger, neuroleptics increase the concentration of DA in synapses in the CNS, but there is little reason for concern about neuroleptic abuse. Alternatively, the conditions of drug availability may alter the neurobiology of cocaine. This is, indeed, a possibility and one that will ultimately need to be evaluated. Given the enormous number of environmental conditions that are known to modify drug effects, this is, at least for me, a sobering thought. The up side, however, is that there is enough to do to keep us all busy for a long time to come.

Finally, B.F. Skinner (1989, p. 18) eloquently generalizes the point that I have attempted to make:

> There are two unavoidable gaps in any behavioral account. One between the stimulating action of the environment and the response of the organism and one between the consequences and the resulting change in behavior. Only brain science can fill those gaps. In doing so it completes the account. It does not give a different account of the same thing. Human behavior will eventually be explained (as it can only be explained) by the cooperative action of ethology, brain science and behavior analysis.

A complete understanding of the behavior of drug abuse will require just such a collaborative approach.

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Multivariate and Nonlinear Approaches to Characterizing Drug Effects on the Locomotor and Investigatory Behavior of Rats

Mark A. Geyer and Martin P. Paulus

INTRODUCTION

The measurement of motor activity in rodents is one of the most fundamental behavioral tests used in the study of drugs of abuse. In many cases, the behavior is referred to as unconditioned or spontaneous motor activity because it has not been explicitly conditioned by the experimenter. The related term *genera/ motor activity* is commonly used when monitoring systems are employed that do not distinguish between different aspects of locomotor behavior; while the term *locomotor activity* is typically applied when ambulatory movements are monitored more specifically. Various instrumentally and operationally defined measures of motor activity have been used to assess the behavioral effects of drugs or other manipulations, either as strict measures of locomotor activity or as measures of more global and context-dependent constructs such as arousal, curiosity, emotionality, and exploration. In psychopharmacology, activity measures are often used as bioassays of drug effects or to establish macroscopic characteristics for drug classes. For example, psychoactive drugs are defined as stimulants or depressants largely on the basis of their effects on gross measures of the motor activity of rodents.

MEASUREMENT CONSIDERATIONS

Motor Behavior Versus Locomotor Behavior

The definitions of terms that have been mentioned rest on the assumption that motor activity is a category of behavior. However, so-called spontaneous or general motor activity is not a unitary class of behavior. Rather, depending on the nature of the recording technique, many different behavioral actions may be

included within the measured behavior. Each of these behavioral actions could be defined as a response that can be measured and studied in its own right (Skinner 1933). Such an approach recognizes that macroscopic behavior consists of assemblies of microscopic responses. Hence, any attempt to indiscriminately detect any movement of the animal and combine all these responses into one category called motor activity is an oversimplification **of the** behavioral observations. Although such approaches have demonstrated some utility insofar as they are able to detect the global effects of drugs, these coarse measures of motor activity provide little or no information about the behavior of the animal or the nature of a drug-induced change in behavior.

Such indications have led most researchers to rely on measures of locomotor behavior rather than motor behavior. Locomotor activity can be defined operationally as movement from place to place and is virtually always one of the behavioral responses that provides a major contribution to any measure of general motor activity. It is, however, a much more specific measure because the monitored behavior is limited to units that specifically reflect the animal's movements of some minimal distance or from one place to another. Such units, which are often called crossovers or crossings, require ambulation by the animal. By design, such measures are insensitive to movements related to sniffing, grooming, eating, drinking, tremor, or breathing. As the field has advanced, many investigators have begun to measure locomotor responses concurrently with other behaviors, such as rearings, object contacts, hole pokes, or patterns of behavior (e.g., circling) to enhance their ability to characterize drug effects and interpret their results.

Automated Monitoring Systems

Only a limited number of different measurement techniques have been applied to detecting locomotor activity in rodents. The primary approaches have relied on photobeams, wheels, touch plates, field detectors based on ultrasonic or capacitance circuits, and mechanical transducers such as jiggle cages, tilt cages, and force platforms. More recently, video imaging techniques were introduced to monitor locomotor activity. Most of the devices, which were reviewed by Reiter and MacPhail (1979), incorporate mechanical transducers, infrared motion detectors, or field detectors and are limited by the difficulty of differentiating locomotor from nonlocomotor movements (e.g., grooming, sniffing); dependence on the size, weight, or temperature of the animal; and complications associated with standardization and calibration. Different measurement techniques that are purported to measure horizontal locomotion

may yield different results depending on the particular sensitivities of the recording device. For instance, Ljungberg (1978) combined two standard activity-monitoring devices, a commercial capacitance-based activity meter and a photobeam box, for assessing simultaneously the effects of several catecholaminergic drugs in rats. He found that the two devices reflected behavioral changes differently and that their results were not correlated.

To date, the most successful and reliable measures of locomotor activity have come from devices that use infrared photobeams. Photobeams are relatively trouble free in operation and can be calibrated and standardized easily. Infrared beams can function independent of the ambient illumination and provide no feedback to the animal even during the nocturnal portion of the animal's sleep-wake cycle. Photobeams can also be used to detect rearings by placing an array of beams at an appropriate height above the animal; the same technology can be used to monitor hole pokes as an explicit measure of exploratory behavior. An important consideration is that repeated interruptions of the same photobeam should not be counted in a measure of locomotor behavior, because they may reflect movements on very small scales. Although the total number of photobeam interruptions has been used widely as a measure of activity, a measure based on successive interruptions of separate beams is more specific to locomotor movements and is therefore generally preferable. There is strong support for the recommendation of Reiter and MacPhail (1979) that all automated activity devices should include a measure that is selectively sensitive to locomotor movements to distinguish them from fine movements associated with grooming, sniffing, tremor, etc. Such an approach will make possible comparisons with other reports in the literature and thereby increase our understanding of at least this major component of motor activity.

Length of Test Sessions

In much of the early work using the traditional open field systems and visual observations of behavior, test sessions were commonly as brief as 3 min to 5 min. Even in the recent literature, some procedures limit the test session to only 10 min. Such short test sessions may be suitable for assessing drug effects on emotionality or exploration, but they are seldom adequate for characterizing treatment effects on the levels or patterns of locomotor activity. Short test sessions maximize the influences of factors such as handling, the familiarity of the animal with the test chamber, and the contribution of behaviors related to attempts to escape. Most such factors are as likely as locomotor

activity to be influenced by the drug or other experimental manipulation. The guidelines for motor activity testing established by the U.S. Environmental Protection Agency (EPA 1986, p. 17, 891) provide that "the test session shall be long enough for motor activity to approach asymptotic levels by the last 20 percent of the test session for most treatments and animals' activity counts shall be collected in equal time periods of no greater than 10 minutes duration." In practice, these considerations suggest making test sessions at least 30 min long and preferably 60 min or more.

Nature of the Test Chamber

Several factors must be considered in selecting the size and shape of the test chamber (Geyer 1990). If locomotion is the object of study, the chamber must be large enough to elicit this behavior. Further, there are advantages to a chamber that is large enough to permit a meaningful division between central and peripheral areas. For example, a study on the influences of a depletion of central norepinephrine on the hyperactivity induced by amphetamine revealed that although the depletion has no effect on the amount of locomotor hyperactivity, it resulted in a significant increase in the time spent in the center and a significant decrease in time spent in the corners of the chamber (Geyer et al. 1986*a).* Conversely, a neurotoxin-induced depletion of brain serotonin had the diametrically opposite effect on the locomotor response to amphetamine, again without altering the amount of activity (Gately et al. 1986).

The nature of the test chamber also has important consequences with regard to the sensitivity of the measures to both increases and decreases in the locomotor activity. Beyond general principles of measurement, the advisability of using an experimental design in which one can detect bidirectional changes-i.e., to avoid "ceiling" and "floor" effects-in the measured behavior is clear. Indeed, EPA (1986, p. 17, 891) includes the requirement in its guidelines for measuring motor activity that "the device used must be capable of detecting both increases and decreases in activity, i.e., baseline activity as measured by the device must not be so low as to preclude decreases nor so high as to preclude increases." *(Federal Register* 1986, p. 17,891).

One reasonable criterion that has been suggested and could be applied more widely is that the test be capable of detecting the activating effects of amphetamine and the inhibiting effects of chlorpromazine. To satisfy such requirements, the test chamber should be sufficiently novel for the animal so that an adequate level of exploratory locomotor activity is elicited. Potentially

misleading floor effects may also be avoided by testing animals during the waking part of their sleep-wake cycle. Unfortunately, the use of paradigms that have limited ability to detect drug-induced decreases in activity is one of the most common and unfortunate weaknesses in the behavioral literature on the effects of psychoactive drugs.

The animal's degree of familiarity with the test environment can have a profound influence on the observed effect of a drug. For example, the reductions in locomotion and investigatory hole poking produced by low doses of hallucinogens in rats tested in a novel test chamber are not seen if the rats are already familiar with the chamber (Geyer and Light 1979; Adams and Geyer 1985*a).* Such effects are comparable to the profound and complex influences of the animal's experiential history on the effects of drugs on scheduled behavior.

Importance of the Center of the Chamber (Thigmotaxis)

One of the most noticeable aspects of the locomotor behavior of a rat in an enclosed chamber is the tendency to remain close to the walls, referred to as thigmotaxis. In traditional open field devices, entries into the central portion-usually defined as being more than half a body length away from and therefore not in contact with any wall-often have been scored separately from movements around the periphery. Measures of center entries have proved to be very sensitive to the effects of drugs. For example, hallucinogens decrease entries into the center of a chamber even when they have no effect on the level of activity as measured by either peripheral movements or total number of photobeam interruptions (Adams and Geyer 1985*a).* Such findings can be interpreted as a hallucinogen-induced increase in agoraphobia (fear of open spaces), that is, a specific potentiation of the animals' normal avoidance of the presumably threatening central area. The demonstrable significance to the animal of the central portion of an activity chamber underscores the advantages of using a chamber that is large enough to elicit thigmotaxis and to enable the separate detection of central and peripheral movements to be detected separately.

ADVANTAGES OF MULTIVARIATE ASSESSMENTS

Value of Multiple Concurrent Measures

The fundamental conclusion of virtually all critical reviews of activity measures is that it is highly advantageous to assess multiple aspects of exploratory and

locomotor activity concurrently (Geyer 1990; Lat 1965; Reiter and MacPhail 1979; Robbins 1977). The multivariate assessment of unconditioned behavior allows the investigator to assess the validity of hypothetical constructs, make more confident comparisons with other results in the literature, examine the generality and specificity of an observed effect, identify the contribution of response competition, and detect artifacts. For example, many investigators have used locomotor activity measures **as** indicators **of** constructs such as arousal and exploration. The challenge has been to distinguish between activity related to an animal's internal level of arousal and activity elicited by external stimuli. Most theorists have concluded that the amount of exploratory behavior is directly related to the novelty and complexity of stimuli in the environment and, in reciprocity with the process of habituation, inversely proportional to the organism's prior experience with those stimuli (Berlyne 1960; Kumar 1968; McReynolds 1962; Montgomery 1955).

Because standard activity measures are influenced by many factors, behavioral scientists have begun to use hole boards to provide specific measures of investigatory responding. A hole board is simply a test chamber with holes into which burrowing animals such as rats frequently poke their noses. Thus, the holes serve as specific stimuli that elicit easily measured inspective responses. Many hole board chambers rely solely on holes in the floor, a design that maximizes the likelihood that inadvertent missteps by the animal could lead to erroneous counts being generated. Such artifactual responses could easily be related systematically to the drug treatment and therefore could be very misleading. However, holes in the walls can be used with equal success and somewhat more reliability (Flicker and Geyer 1982). Just as measures **of** locomotion in the absence of specific measures of investigatory behavior are difficult to interpret in terms of exploratory behavior, inferences based solely **on** measures of hole poking or head dipping are very questionable unless treatment effects on general levels of activity are assessed simultaneously. **For** example, although both amphetamine and apomorphine increase locomotor activity, amphetamine increases the frequency of hole pokes and apomorphine decreases it (Geyer et al. 1979; Ljungberg and Ungerstedt 1976). Such observations indicate that measures of hole poking should not be used without concurrent measures of locomotor activity.

An Example: The Behavioral Pattern Monitor

In the context of characterizing the behavioral effects of psychoactive drugs by themselves and especially in their interactions with other drugs or experimental

manipulations, the availability of multiple measures enables the behavioral profiles to be developed and used. The Behavioral Pattern Monitor (BPM) was designed to combine the features of activity and hole board chambers and to measure individual response frequencies and durations (Flicker and Geyer 1982; Geyer 1982). Each chamber consists of a large (30.5- by 60-cm) black Plexiglas box containing three floor holes and seven wall holes equipped with infrared beams and a wall touch plate to detect rearings. The chamber is crisscrossed with infrared beams, which are sampled by a microcomputer. The computer records the sequences of hole pokes and rearings and the current position with a temporal resolution of 0.1 s and a spatial resolution of 3.8 cm. Various descriptors are obtained from the raw data, including the total number of photobeam breaks and crossovers from one 15-cm square to another, and more qualitative descriptors such as entries into and time spent in the center and the corners. Because the record of all movements is permanent, it may be used for computer reconstructions of the pattern of movements on paper or on a video display or for calculating of descriptive statistics reflective of treatment-induced differences in these patterns. Further, objects or lights may be placed in specific holes to manipulate their novelty or complexity and elicit discrete inspective and novelty responses (Flicker and Geyer 1982).

Characterizing the Specificity of Drug Action

A major and fundamental issue in the characterizing of drug effects on behavior is specificity. In the present context, an example is provided by the category of drugs labeled stimulants. Many, but not all, of these drugs are also drugs of abuse. In sharp contrast to the advances in precision and specificity so apparent in most areas of neurosciences and pharmacology, much of the behavioral psychopharmacology literature continues to rely on measures of behavioral activity that cannot differentiate between drugs such as amphetamine and caffeine or between apomorphine and cocaine. That is, if one collects only a single measure of locomotor activity, drugs as different as amphetamine, apomorphine, scopolamine, caffeine, methylenedioxy-methamphetamine (MDMA), and nicotine are often indistinguishable. Hence, to assess the value of the multivariate behavioral assessment provided by the BPM system, the effects of various stimulant drugs have been compared in systematic dose-response studies (Geyer 1982; Geyer et al. 1986*b;* Gold et al. 1988). The goal was to determine whether developing profiles of locomotor and investigatory behaviors would enable distinctions to be made that cannot be made with standard measures of the amount of activity.

In the studies summarized here, naive male rats were tested only once during their initial exposure to the BPM chambers, Each experiment involving a stimulant drug included four or five groups of 8 to 12 animals each. Test sessions were conducted during the dark phase of the animals' light-dark cycle and lasted 60 min. Subcutaneous injections of saline or one of several doses of the test drug were given 10 min before the animal was introduced to the chamber. The following doses (in milligrams per kilogram) of each drug were tested: amphetamine, 0.25, 0.5, 1.0, and 2.0; scopolamine, 0.125, 0.25, 0.5, and 2.0; caffeine, 2.5, 5.0, 10.0, 15.0, and 20.0; apomorphine 0.1, 0.5, 1.0, and 2.0; nicotine, 0.0625, 0.125, 0.25, and 0.5; MDMA, 1.25, 2.5, 5.0, and 10.0; and lisuride, 0.005, 0.015, 0.03, and 0.06. Details of these experiments may be found in the original reports (Adams and Geyer 1985*b;* Geyer et al. 1986*b;* Gold et al. 1988). For each experiment, repeated-measure and mixed-design analyses of variance were performed for selected variables. Dunnett's tests were used to compare each treatment group with the control group. All statistical comparisons reported here were derived from comparisons of the particular dose group with the corresponding control group, although some figures depict only the results from the most typical control group.

All the drugs elicited dose-related increases in either total photobeam breaks or crossovers, which are defined as movements from one 15-cm square to another. The effects of a representative dose of each drug on crossovers during the first and second halves of the hour-long test session are shown in figure 1. Clearly, all the drugs can be classified as stimulants on the basis of their shared ability to increase such measures of locomotor activity. Even the simplest multivariate assessment, however, began to differentiate these drugs from one another. Many drugs have differential effects on entries into a central area that are unrelated to differences in overall levels of activity. Scopolamine, apomorphine, and MDMA shared a common effect in that animals tended not to enter the center of the chamber (figure 2).

These three drugs produce characteristic patterns of hyperactivity in which the animals rarely move away from the walls. On the other hand, the increase in center entries produced by caffeine is in direct proportion to the effects of the drug on total movements. Such observations demonstrate that the separate measurement of central versus peripheral movements is of considerable value both in detecting the behavioral effects of drugs and in differentiating the effects of seemingly similar drugs. The dopamine agonist lisuride, which is a structural relative of lysergic acid diethylamide (LSD) and has important effects on serotonergic systems (Adams and Geyer 1985*b),* also increased entries into the

center to about the same extent that it increased crossovers. Thus, its effects contrast with those of LSD, which markedly decreases center entries (Adams and Geyer 1985*a).* In contrast, amphetamine was the only drug that produced a preferential increase in center entries.

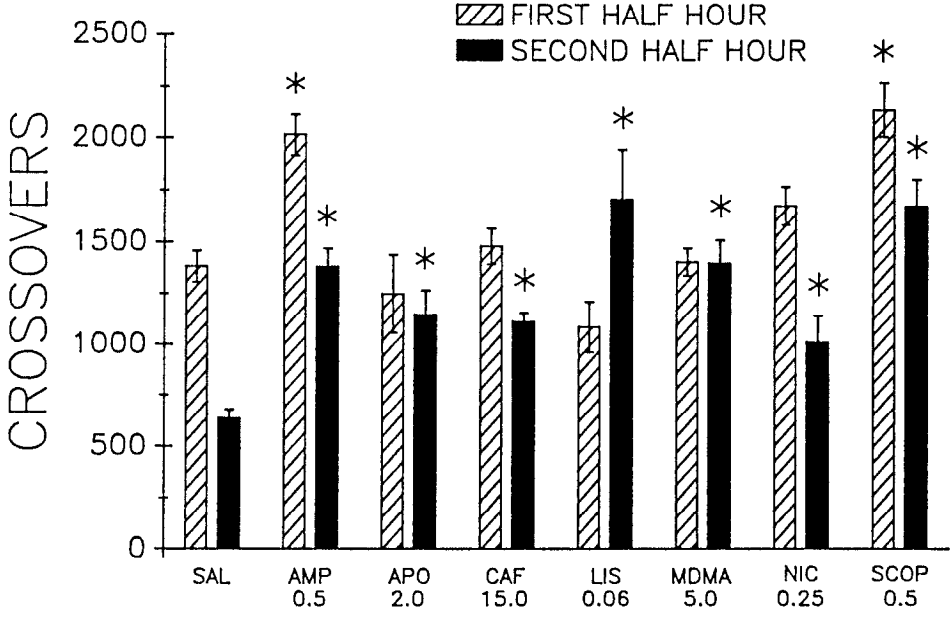


FIGURE 1. *Effects of stimulants on crossovers. The effects of the selected doses of the various stimulant drugs on crossovers are shown as group (N = 10-12) means ± SE_M for successive halves of the hour-long test sessions. At these doses (shown in milligrams per kilogram), each drug significantly increased locomotor activity during the last ha/f of the test session. The control values shown are the median values from the separate control groups used for each stimulant study. Statistical comparisons were based on each particular control group. SAL = saline; AMP = d-amphetamine; APO = apomorphine; CAF = caffeine; LIS = lisuride; MDMA = methylenedioxy-methamphetamine; N/C = nicotine; SCOP = scopolamine. * = significantly different from corresponding control,* $p < 0.05$.

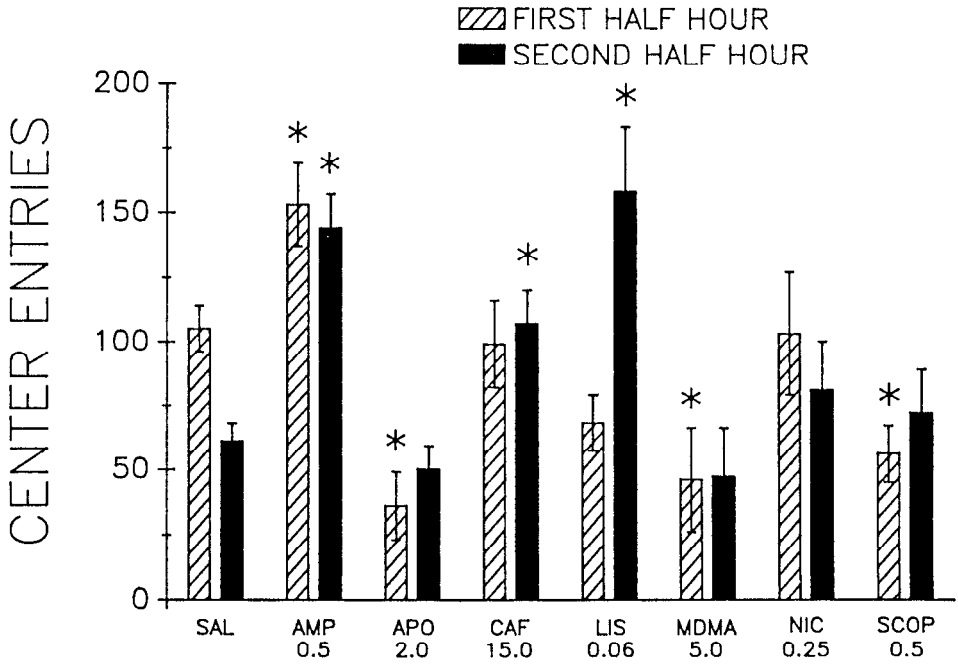


FIGURE 2. *Effects of stimulants on center entries. Group means for center entries are shown as in figure 1. The center region is illustrated in figure 6. * = significantly different from corresponding control,* $p < 0.05$.

The BPM also provides explicit measures of exploratory or investigatory behavior, namely hole pokes and rearings. In all cases, each drug had similar effects on both these behaviors, as shown for hole pokes in figure 3. Scopolamine markedly increased both hole pokes and rearings, whereas apomorphine virtually abolished both behaviors and MDMA reduced them. Lisuride also significantly decreased both hole pokes and rearings despite increasing crossovers and center entries. Although caffeine and nicotine produced similar increases in crossovers and at least tended to increase center entries, caffeine increased and nicotine decreased both hole pokes and rearings. Thus, although at the doses selected for comparison all these drugs produce roughly comparable increases in the amount of locomotor activity, they are readily discriminable by means of a rather simple form of multivariate or profile analysis. Just as these drugs may be differentiated biochemically on the

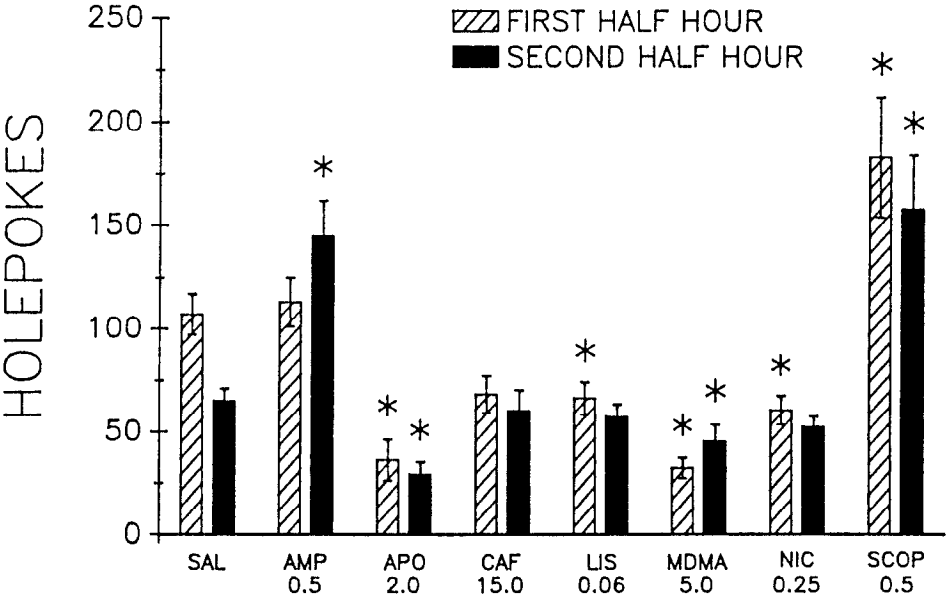


FIGURE 3. *Effects of stimulants on hole pokes. Group means for ho/e pokes are shown as in figure 1. Each chamber contains three floor holes and seven wall holes.* = significantly different from corresponding control,* $p < 0.05$.

basis of binding or other neurochemical effects, or pharmacologically by virtue of their differential sensitivities to receptor antagonists or synthesis inhibitors, so may they be differentiated at a behavioral level even by means of a single test paradigm.

UTILITY OF ANALYSES OF SPATIAL AND TEMPORAL PATTERNS OF ACTIVITY

Patterns of Locomotor Activity

A new and sensitive approach to the study of drug effects on locomotor and exploratory behavior is based on examining the patterns of locomotion by plotting the sequence of movements as the animal explores the chamber. The BPM system permanently stores all the movement patterns of the animal together with the duration of each investigatory response or pause in a

particular *x-y* position. One of the most instructive uses of these data with regard to understanding the structured manner in which a rat explores its environment has been the computer generation of reconstructed visual images of the sequences of hole pokes, rearings, and locomotor movements. As expected, virtually all untreated animals tend consistently to avoid the center region and to stay near a corner of the chamber. The structure of the movement patterns themselves was most easily identified by observing the video displays of the movements when animals were tested in a free-exploration paradigm in which they could move freely between a home cage and the larger BPM chamber. The animal's location in the home cage serves as an organizing focus; excursions to various parts of the larger chamber and back follow progressively more fixed routes over time. Typically, the outbound part of an excursion is more frequently interrupted by investigatory hole poking and rearing than the return. When tested in the more typical forced-exploration situation, the behavior is similar except that each animal selects a particular corner as its home area. Each rat, however, clearly develops its own particular spatial pattern of movements, and this pattern is predictable across time within a session and between sessions.

Drug Effects on Spatial Patterns of Locomotion

The consistency in the locomotor patterns of untreated rats has led to the study of drug-induced changes in these patterns per se. For example, the stimulant drugs that have been discussed above in the context of multivariate assessments have also been examined in terms of their influences on spatial patterns of locomotion (Geyer et al. 1986*b;* Gold et al. 1988). Even at doses that produce comparable increases in the amount of locomotor activity, some of the stimulant drugs are readily distinguishable by virtue of qualitative changes in the animals' locomotor patterns. Some drugs, such as low doses of amphetamine, disrupt the normal structure by producing highly varied patterns of directional changes (figure 4). At higher doses, amphetamine can produce perseverative patterns (Lat 1965; Schiorring 1979). Other stimulant drugs essentially replace the normal patterns of locomotion with new, even more highly structured patterns. For example, both apomorphine and scopolamine induce movement patterns that are very predictable and seemingly characteristic of each drug. Most animals treated with apomorphine run around the perimeter of the chamber consistently in one direction for most of the session (figure 5). Although scopolamine-treated animals also move around the perimeter of the chamber, they frequently change directions and pause enroute

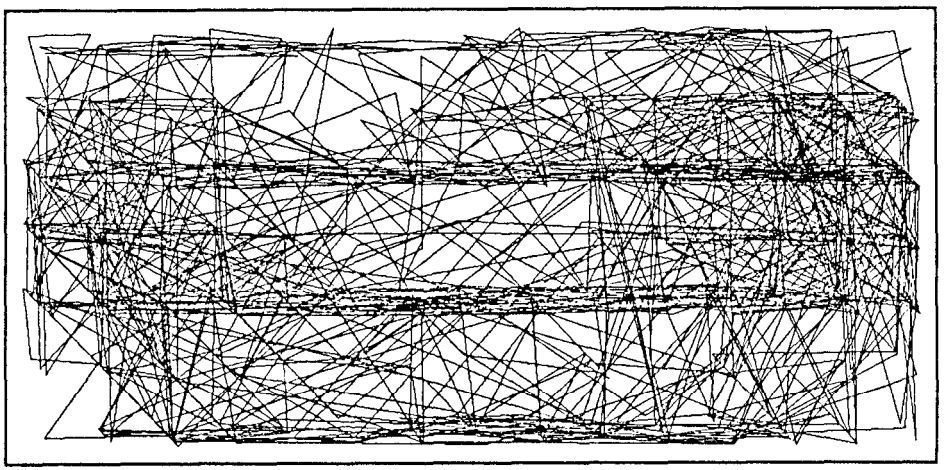


FIGURE 4. *The locomotor pattern induced by amphetamine. Shown here are the movement patterns exhibited by a representative animal given amphetamine and tested for 1 hr in the Behavioral Pattern Monitor. To avoid exact retracings of the same lines, ± 40 percent variability has been added to successive x - y positions.*

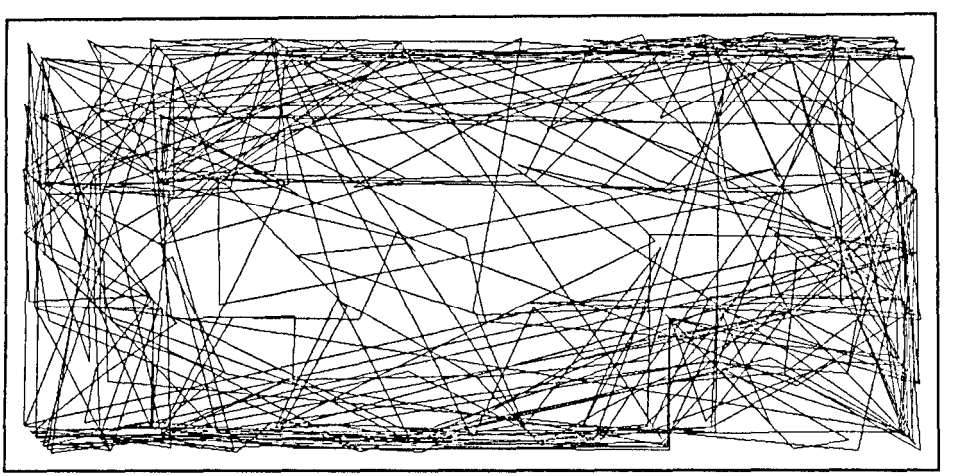


FIGURE 5. *The locomotor pattern induced by apomorphine. Shown here are the movement patterns exhibited by a representative animal given apomorphine and tested for 1 hr in the Behavioral Pattern Monitor. To avoid exact retracings of the same lines, ± 40 percent variability has been added to successive x - y positions.*

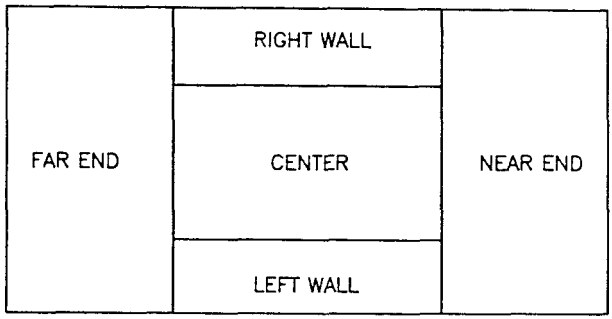
to investigate the holes and rear against the wall, responses only rarely seen with apomorphine-treated rats (Geyer 1982).

Intermediate combinations have also been noted. For example, doses of 5 to 10 mg/kg of the serotonin-releasing compound MDMA cause animals to move around the perimeter of the chamber, changing directions occasionally as with scopolamine, but without hole poking or rearing as they do with apomorphine. It also appears that stimulants such as caffeine and nicotine do not disrupt the normal structure of the animals' spatial patterns of locomotion. With these drug treatments, it is evident that each animal adopts a preference for a particular home area and establishes preferred excursion routes that are as predictable as those of controls. That is, the structure of their locomotor patterns is largely similar to those exhibited by untreated or saline-treated control animals. These drugs increase activity primarily by reducing the duration of each visit in the home corner. Accordingly, the strongest differences between caffeine or nicotine versus saline controls occur after 20 to 30 min, when the control animals begin to pause longer in their self-selected home corner.

Measures of Perseverative Patterns of Locomotion

Despite the recent advent of automated devices that **can** record such patterns, the statistical description and analysis of the resulting data has posed a difficult problem. We have had some success with a measure called the spatial coefficient of variation (CV) (Geyer 1982; Geyer et al. *19866).* In our first attempts to use the metric to statistically describe and evaluate the sequences of position changes, the data were reduced into transitions among five areas: the two ends, the center, and the two long walls (figure 6). Subsequent applications of this approach have involved calculating transitions among nine areas (Gold et al. *19866;* Geyer et al. 1988). In either case, transitions among the five or nine areas can be displayed in a matrix with 16 or 40 permissible cells (see figure 6). Relative transition frequencies are then calculated as percent of total, and the spatial CV is derived from this set of numbers. To the extent that an animal preferentially repeated certain transitions, the spatial CV increases. A more uniform or random distribution of spatial transitions produces a low spatial CV.

Figure 7 illustrates the differential effects of the various stimulant drugs on the CV statistic, a measure of the degree of structure or predictability in the spatial pattern of locomotion. As appropriate to the pattern differences described above, the effects of the drugs on the CV were independent of their effects on



	NEAR	LEFT	CENT	RIGHT	FAR
NEAR		78	19	105	
LEFT	132		14		49
CENT	26	5		10	15
RIGHT	44		4		127
FAR		112	19	60	

FIGURE 6. *The spatial coefficient of variation (CV) statistic. The diagram at the top represents the five regions into which the chamber is divided by the analysis program for assessing the CV and defining center entries. To calculate the CV, transitions of the rat from one region to another are cumulated as illustrated in the matrix This particular matrix comes from an animal treated with scopolamine, which makes animals run around the periphery of the chamber in both directions. Hence, the numbers in this matrix include several large numbers and many small numbers because this animal exhibited the same movement pattern consistent/y. According/y, the standard deviation or coefficient of variation-CV-of this set of numbers is relatively high, i. e., 0.890. An animal that distributed its movements more random/y or uniform/y throughout the chamber would produce a matrix filled with more similar numbers and therefore a low CV. Thus, the CV statistic increases to the extent that an animal repeats certain spatial movement patterns to the exclusion of others.*

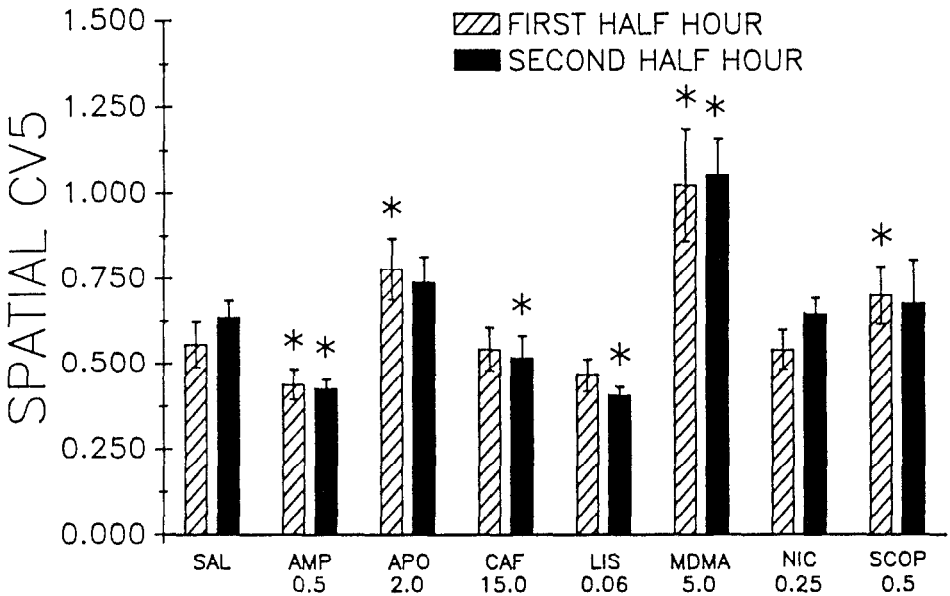


FIGURE 7. *Effects of stimulants on the spatial coefficient of variation. Group means for CV, or the coefficient of variation, are shown as in figure 1. The spatial CV is defined in figure 6. An increase in the spatial CV reflects a more repetitive pattern of movements.* * = *significant/y different from corresponding control,* $p < 0.05$.

the amount of activity. The drugs that were observed to produce repetitive or stereotyped patterns of locomotor hyperactivity—scopolamine, MDMA, and especially apomorphine—all significantly increased the CV. Conversely, amphetamine, which increased the frequency of directional changes and produced an unpredictable pattern of locomotion at the doses tested, significantly decreased the CV. Nicotine and caffeine had minimal effects on it. Although lisuride was observed to produce repetitive rotational movements, these predictable patterns were limited to the early part of the test session and tended to form oval patterns centered on the diagonal of the chamber (Adams and Geyer 1985*b).* Because this particular pattern did not conform to the arbitrary Cartesian coordinate system used in calculating of the CV, the statistical measure was inconsistent with the observed behavior. Such an example reflects the constant need to compare the results of abstracted descriptive statistics with the object being measured-in this case, locomotor

behavior. Discussed below are some newer measures that do not suffer from the low-resolution weaknesses of the CV measure and therefore more accurately describe the behavioral changes induced by lisuride. Despite such occasional limitations of the spatial CV measures, the results depicted in figure 7 reveal that they provide additional information that is not evident in any other measure and that is independent of drug-induced changes in amount of activity.

Although descriptive statistics such as the spatial CV have proved useful, much more work in this area is warranted. The combination of multivariate assessments and pattern analyses provided by systems such as the BPM promises to enhance the study of drug effects on locomotor activity in much the same way the switch from whole-brain to regional analyses of brain chemistry revolutionized our understanding of drug effects on neurotransmitters. The advent of new technologies such as video tracking systems and the associated computer-based pattern analyses should have considerable impact in this area. Further, the application of methods used for analyzing nonlinear dynamical systems is certain to supply a variety of additional measures that have enormous potential to quantify and characterize the effects of drugs on such a complex, dynamic, and metastable behavioral system as spontaneous locomotor activity (e.g., Geyer et al. 1986*b;* Paulus et al. 1990).

SCALING APPROACHES IN BEHAVIORAL PHARMACOLOGY

Problem

The data acquisition systems that are currently being used in behavioral experiments are far more sophisticated than the concepts that are being used to deal with the amount and variety of information. The preceding discussions emphasize that more elaborate techniques are necessary for dealing with the information obtained from such experiments.

Approach

In the study of physical systems that are near phase transitions (Stanley 1971), an important concept emerged that is now widely used to categorize different

systems in so-called universality classes. The basic idea is that an important quantity that describes the system (such as its free energy) has the following property:

$$F(\lambda t) = \lambda^{\alpha} F(t)$$

In words, replacing the argument of this quantity by an argument that is scaled by a factor of λ, the quantity can be obtained by multiplying the quantity by the factor λ to some power a. Functions that obey this relationship are said to be *scaling.* Central to the treatment of statistical mechanical systems near phase transitions (i.e., complex systems that exhibit large fluctuations) are equations that yield a function rather than a number as their solution. Transferring these results to behavioral systems leads to the basic assumption that a single measure will not describe the results adequately. Rather, a function that relates the quantity of interest to its variation depending on intervening variables may provide a more complete description. In other words, insights about the system may be gained by observing a quantity of interest (e.g., locomotor activity) on different levels of observational perspective. Scaling concepts have been extensively used for studying fractal objects in mathematics and physical experiments. A fractal may be described simply as “a shape made of parts similar to the whole in some way” (Mandelbrot 1977). A concrete example of a scaling relationship is that the mass of an object scales with the measuring resolution with which the object is studied. For a regular “geometric” fractal, this scaling relationship holds as a strict equality, whereas for a “statistical” fractal, it may be described as follows:

$$[\text{mass}] \text{ — } \text{lengthscale}^{\text{exponent}}$$

In addition, the range of this scaling ansatz may be specified.

System

When the influence of drugs on unconditioned locomotion is studied, counts of activity based on photobeam breaks provide a quantity that may be a candidate for a scaling approach. The information provided by photobeams depends on the particular apparatus and is not related in a simple fashion to explicit behavioral responses. The locomotor activity of rats in the BPM may be conceptualized as movements that occur on different scales. The movements of an animal may involve small local movements characterized by consecutive photobeam breaks within the same region of the chamber. Such patterns of

beam breaks may reflect small head movements, movements that occur during grooming or investigatory behaviors such as rearing or hole poking, or repetitive focal perseverative behaviors such as biting or gnawing. In addition, the animal will exhibit some long, distance-covering movements, which appear as straight paths without interruption by local movements and often reflect the frequently observed rotating pattern of locomotion.

A normally behaving animal will exhibit both local and distance-covering movements, as well as intermediates that are combinations of these movements, during the test session. Although this chapter focuses on the spatial domain, a similar distribution of the durations of events provides a description of the frequencies of movements in the temporal domain. Descriptors based on response durations may indicate changes in rate of activity. Thus, to describe the behavior exhibited in the BPM, a plane can be constructed that consists of two axes; the first can be used to describe changes in the spatial composition of local and distance-covering movements, and the second displays the overall level of activity. Effects of psychoactive substances in the BPM can therefore be discriminated further to include qualitative aspects of locomotor behavior indicated by changes in the composition of movements.

The locomotor activity observed in this chamber shown in figure 8 has already been expressed as a function of the distance between photobeam breaks on several discrete levels (Geyer et al. 1986*b).* It was suggested that the total count of all events serves as a measure of small movement activity, the recordings of crossovers serves as measures of ambulations, and movements between regions such as those in figure 6 serve as indicators of larger movements within the BPM. This idea can be extended using the scaling approach to introduce the following basic relationship,

$$< L\,(l) > \sim l^{-d}$$

where L is the average length of consecutive response sequences, i.e., positions of the rat in the BPM chamber, measured with resolution *l.* The value *d* is often identified with the fractal dimension (Mandelbrot 1977) of an object. We will refer to it here as the spatial scaling exponent and have deliberately avoided calling it *dimension,* because new developments in the application of ergodic theory to experimental data sets have resulted in a variety of dimension measures that may be obtained by embedding the data into higher dimensional spaces (for an overview, see Farmer et al. 1983). The average distance of consecutive responses can be measured by introducing a metric on a space

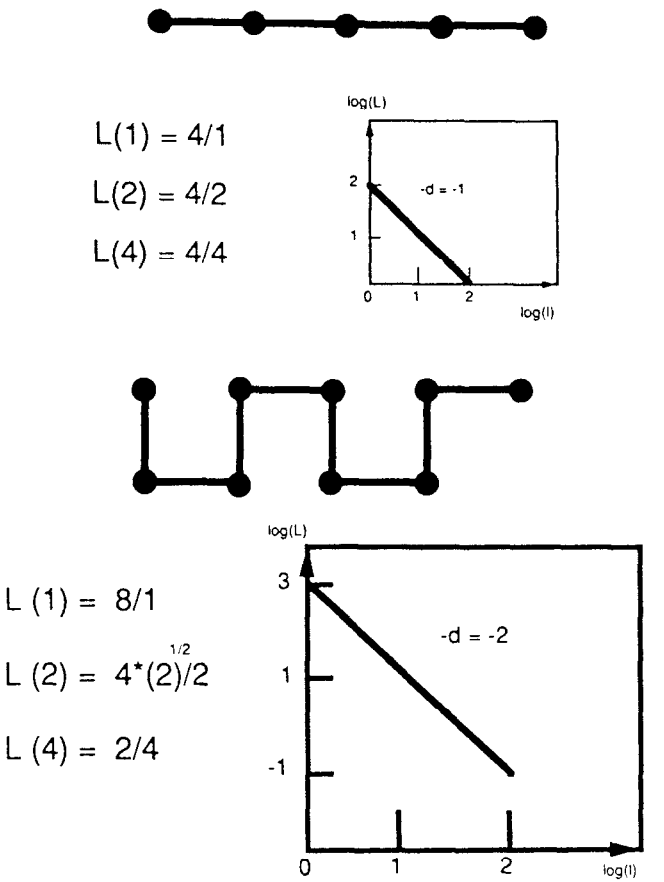


FIGURE 8. *Calculation of the spatial scaling exponent* d. *On the left, two different patterns of four consecutive movements within the Behavioral Pattern Monitor are displayed and connected by lines. By computing the average distance between these responses using different inter-response distances, the spatial scaling exponent describes the change in length with the change in the inter-response distance. The lower part displays an example with consecutive responses whose distance measures are more strongly affected by increasing the inter-response measuring unit leading to shorter distances compared to the upper part with the same measuring resolution. The scaling exponent is obtained by finding the slope between the measuring resolution and the average distance computed.*

constructed from the recorded data sets. The city block metric was chosen as a suitable distance metric because it seems to be particularly sensitive to the correspondence between changes in the photobeams and changes in the (x-y) position of the animal in the BPM. It is computed as

$$d(X,Y) = \sum |x_i - y_i|$$

The average distance of response sequences, *L,* starting at the *m*th position in the data set, reads

$$L_m(l) = \frac{\sum_j d(x_{m+i\times l}, x_{m+(i-1)\times l}) \times C}{l}$$

where C is a normalization factor ensuring that end effects are not influencing the distance calculation. The scaling exponent dis computed by fitting the slope of the line of log*(L)* vs. log*(l)* with a least-square procedure. The physical interpretation of the exponent *d* can be obtained by considering an example of a path observed in the BPM chamber, which is schematically given in figure 8. In the first case, successive observations result in locations of the rat in the box that are connected by straight lines. The function *L(l)* results in a linear dependence on *l,* thus giving the simplest result, namely, that the scaling exponent for a straight line is 1. In the second case, consider a path that is very irregular and covers only a moderate distance after many successive observations. In this case, the average distance measured is smaller with a low-resolution instrument than with a high-resolution instrument, resulting in a stronger decay of the average distance than with the straight path and therefore in a higher *d* exponent. The spatial scaling exponent *d* consequently characterizes how path lengths depend on the resolution of the instrument and allows one to quantify different qualitative geometrical features of the locomotor behavior.

So far, the response was defined to be a movement within a given measurement resolution without considering how long it takes to observe one response. A second scaling approach can be formulated by considering a measuring instrument that is recording the position of the animal in the box with different time resolutions. Thus, the following relationship may be assumed:

$$N(t, \Delta t) \sim t^{\alpha}$$

which states that the number of observations within a given time resolution depends on the time and a characteristic time scaling exponent, alpha or α.

More simply stated, the number of responses having a certain length $(t, \Delta_i t)$ in the BPM chamber decays with a power law behavior. The interpretation of α may be facilitated by an example. An animal treated with a stimulant drug like amphetamine exhibits many fast locomotor responses because of the increased amount of activity. Conversely, slow responses are correspondingly more infrequent than saline control animals and faster responses dominate the behavior. Such an effect results in a rapid decay of the number of responses observed with increasing response time; thus a larger time scaling exponent α is obtained. A rat that shows less locomotor activity in the BPM than controls exhibits relatively more slow responses, thereby slowing the decay of the number of the responses across increasing response times and yielding a smaller α.

The Goal

The eventual goals of this line of investigation are (1) to develop metrics that can facilitate comparisons among the effects of substances and other manipulations and then (2) to identify a small number of parameters that describe the macroscopic properties of the complex system. The main concept of synergetics (Haken 1983) is that interacting systems self-organize and exhibit global properties that are not dependent on the microscopic details of the system. These properties are described by order parameters, which consist of macroscopic collective variables and characterize the self-organized behavior. The characterization of the effects of substances on different behavioral paradigms is undoubtedly influenced by many factors, some of which were presented in papers at this meeting ("Importance of Behavioral Controls in the Analysis of Ongoing Events" and "Cocaine Self-Administration: Pharmacology and Behavior"). Substances may influence behavior through the interaction of several neurotransmitters and neuropeptides in different locations in the brain, as pointed out by Koob ("Neurobiological Approaches to Brain-Behavior Interaction: Summary and Overview"). Therefore, with unconditioned locomotion as the dependent variable, one has to assume that the effects of different substances reflect combinations of many effects on different neuronal systems. To capture the differential effects of psychoactive substances, the spatial scaling exponent dand the temporal scaling exponent α were taken to be order parameters that describe the macroscopic organization of locomotor behavior in the BPM. The first objective was to identify categories of substance effects in the *d versus* α plane.

Implementation

The implementation and application of scaling approaches to this behavioral data set follow a scheme that is broadly applicable to a wide variety of data sets. There are, however, important subtleties that may have strong influences on the identification of the order parameter or parameters. The implementation consists of a four-stage process.

1. A set of behavioral observations is considered for evaluating the effect of the independent variable in the experiment. In the case of locomotor activity experiments, these observations depend strongly on the specifics of the apparatus used; e.g., for the BPM, the sets recorded consist of a location in the BPM chamber, whether rearing or hole poking has occurred, and the time since the last response.

2. The second stage, an important step in determining the order parameters, involves a transformation of the data set in a suitable space. The choice of the space depends strongly on the theoretical construct used to assess changes induced by drugs. In our case, to calculate the spatial scaling exponent *d,* we transformed the data set into Euclidean space equipped with the city block metric.

3. A measure is defined, i.e., a function that converts elements of the previously defined space into a number *(M:S* $\rightarrow$ *R).* We chose the average length of the paths traveled in the BPM chamber and the number of responses with a certain response time as the measures of interest to describe the effects of the drugs.

4. It is determined whether a scaling relationship exists between the measure and a variable that influences the measure. If a scaling relationship is observed, then this variable may serve as an order parameter, because it combines the information of the measurement on different levels of observation. For the paths of the rats in the BPM, the average length served as the measure and the length scale was used as an order parameter leading to the calculation of *d.* Similarly, the number of observed responses having a response time between *t* and *(t* + Δ *t)* served as another measure that showed a scaling relationship with the response time as an order parameter resulting in the calculation of α.

Results

Figure 9 displays the results of the evaluation of amphetamine-treated animals in the *d-versus* -α plane. The results are expressed in terms of deviations in units of standard deviations from the saline controls, i.e., a *z*-transform

$$x_d^{\wedge} = \frac{< x_s > - < x_d >}{\sigma}$$

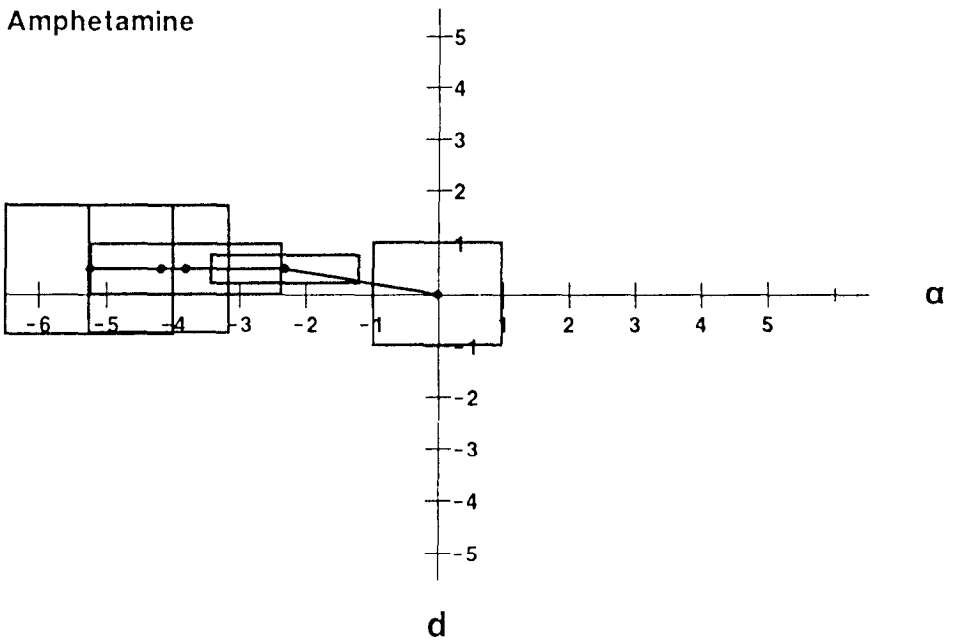


FIGURE 9. *Effects of amphetamine on scaling exponents. The results from amphetamine-treated animals are shown in z-transformed coordinates in the* d-versus-α *plane. The origin corresponds to the saline control group and the boxes indicate ± 1 standard deviation from the mean in the* d *and* α *directions. Increasing doses are connected with lines to signify the dose-response curve. Amphetamine results in a strong activating effect, indicated by a decrease in a with no strong changes in* d.

was carried out to obtain these data points. Consecutive doses of drug are connected with lines to indicate the direction of the dose-response curve. At the origin and corresponding to the location of the saline control group, a square with a side length of 2 units indicates the 66 percent confidence interval (±1 standard deviation) of the transformed data. The points in the plane correspond to the transformed group mean and the boxes around the points are the transformed standard deviations for *d* and α_1, respectively.

The animals treated with *d*-amphetamine did not exhibit significant differences in the spatial scaling exponent *d* over the 60-min test session *(F*[4, 37] = 0.61). The temporal scaling exponent a yielding information about the distribution of different "pausing times" during the test session gives the expected result of an increase in "fast" time bins because of the increased activity of the amphetamine-treated animals (α:: *F* [4, 37] = 26.99). This hyperactivity leads to an increased negative slope of the logarithmic decay of number of observed times versus the response time. The effect of amphetamine on this measure corresponds to a rotation of the distribution. Contributions from slower time slices are redistributed into faster bins. The higher doses of amphetamine (1.0 and 2.0 mg/kg) were significantly different not only from the saline controls but also from the lowest dose amphetamine group (0.25 mg/kg). In the *d*-α plane, the dose-response curve is pointed horizontally (no change in *d)* to the left toward more negative α values.

The rats treated with apomorphine showed more complicated group differences *(d: F*[4, 421 = 6.10) (figure 10). The lowest dose (0.1 mg/kg) resulted in an increased spatial scaling exponent, *d,* and higher doses (0.5 and 1.0 mg/kg) reduced this scaling exponent to a level that was somewhat lower (for 2.0 mg/kg) than that of the saline control group. Thus, the paths of the low-dose apomorphine animals can be characterized as more jagged, covering less distance with increasing number of consecutive responses. The higher dose animals exhibit straightened paths that are characterized by a lower scaling exponent resulting in longer distances observed with increasing response length. The changes in the temporal scaling exponent α, which differed significantly among the different dose groups *(α: F*[4, 42] = 29.97) documented an increased decay of time occurrences for the higher doses of apomorphine (0.5 to 2.0 mg/kg) as a result of the increased activity that was displayed by the animals. As with amphetamine, the distribution changes correspond to a rotation of the response time distribution. This response is portrayed in the *d-a* plane indicating the biphasic relationship with increasing dose, the lower dose

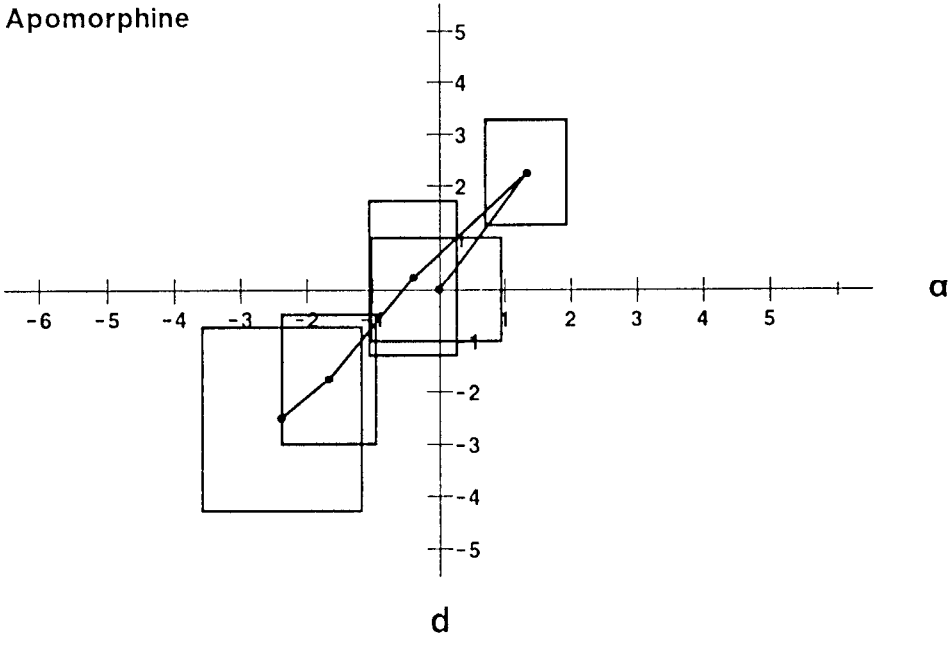


FIGURE 10. *Effects of apomorphine on scaling exponents. The* z*-transformed results for apomorphine are displayed as in figure 9. The increasing dose results in an initial small increase of* α *and* d *followed by a decrease in* d *and* α*..*

being displaced to the upper right, whereas increasing doses shift the groups to the lower left.

The dose-response curve of lisuride is displayed in figure 11. Significant changes among the different dose groups *(F*[4,38] = 21.93) reflect the straightened paths characteristic of animals treated with higher doses (30 and 60 µg/kg) and paths that are unchanged with respect to the saline control group for the lower doses (5.0 and 15.0 µg/kg). The temporal scaling exponent *a* showed significant differences *(F*[4,38] = 28.36), indicating that for the three lower doses a reduction of locomotor activity occurred, which agrees with other measures taken from these same animals and reported by Adams and Geyer (1985*b).* The distribution of response times is also indicative of a stimulant effect producing more populated fast time bins for the highest dose (60 µg/kg). The effect of lisuride can therefore be characterized as a low-dose slowing and

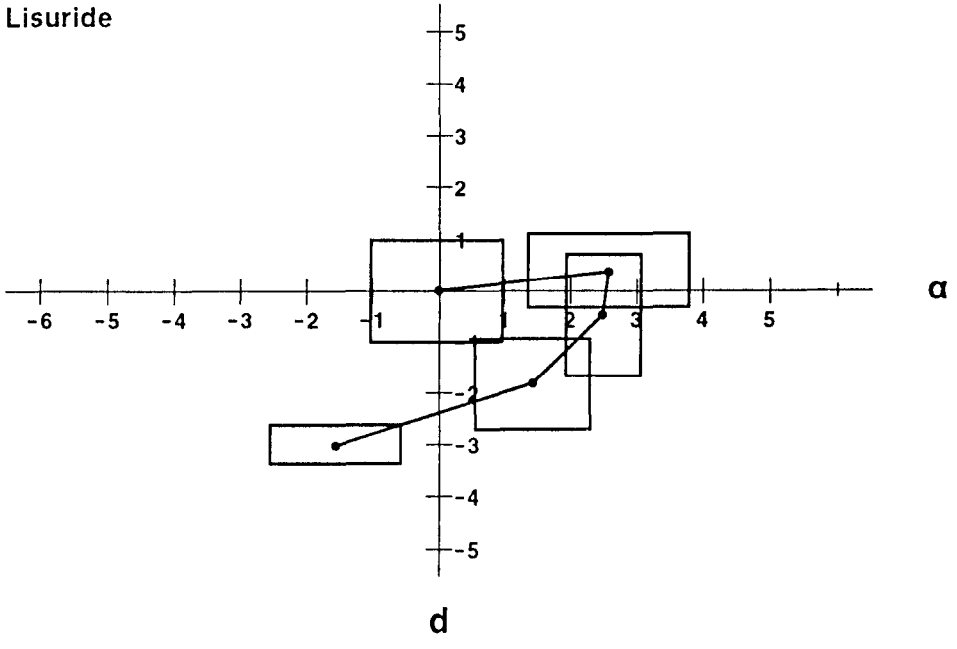


FIGURE 11. *Effects of lisuride on scaling exponents. The z-transformed results for lisuride are shown as in figure 9. Lisuride results in a similar dose-response curve in the* d *direction but shows less stimulant properties when compared to apomorphine; see text.*

a high-dose stimulating effect. In the *d*-α plane, the biphasic relationship of the lisuride dose to both the temporal and spatial scaling exponents results in a slightly different curve than the apomorphine curve. Lisuride suppresses the activity for most doses tested, and a reduction in *d* is observed even with lower levels of activity. The structure of the paths in the BPM chamber is therefore relatively independent of the amount of activity observed; in this particular case, long distance-covering moves predominate the behavior even with a reduction in the overall activity. Furthermore, this pattern of results is more representative of the observed behavior of the animals than was the spatial CV statistic shown in figure 7. Although the CV was insensitive to the long distance-covering movements because they were not oriented in a suitable Cartesian frame, the *d* measure captured this effect of the drug more adequately.

Finally, the significant changes *(F*[4,34] = 4.60) between the different doses of MDMA are characterized by a progressive decrease in *d* with increasing dose. Therefore, animals treated with MDMA travel farther in the BPM chamber and follow straight paths. That both results corroborate previous findings (Gold et al. 1988), indicates a strong circling behavior, particularly for higher doses. The stimulant effect is substantiated by the distribution of times characterized by *a,* which is significantly changed *(F*[3,34] = 9.50) for the two highest doses versus the saline control and versus the 1.25- and 2.5-mg/kg groups. This result points to a temporal restructuring of the responses toward faster movements with less time being spent at each successive position in the BPM chamber. Across all the drugs compared here, the sensitivity of *a* has proven to be much greater than either crossovers or total beam breaks as a measure of changes in the amount of activity. Such results indicate that *a* is an important indicator that enables us to distinguish even small changes in activity.

Several patterns seem to emerge from this evaluation. First, amphetamine proved to be the most potent stimulant of the drugs compared; it displaced the *a* exponent farthest from the origin. Next were MDMA, apomorphine, and lisuride, in that order. Second, apomorphine and lisuride show a low-dose increase in *a* with a small increase in *d,* suggesting that in both cases the paths become more clustered as the temporal behavior slows down. Third, MDMA and lisuride lead to the strongest decrease in *d,* followed by apomorphine, indicating a stimulating action that leads to both a speeding of responses and a decreased clustering of consecutive responses. Last, it can be summarized that the change in a is not uniformly associated with a similar change in *d* for the different substances evaluated here; thus is emphasized the distinctness of the information that can be obtained from these measures.

Outlook

The application of scaling concepts to behavioral data points toward several interesting directions. First, an enormous reduction and compression of information may be obtained by finding a suitable variable that shows scaling of the quantities of interest, e.g., measures of locomotor activity. Second, distinct subgroups of behavioral reactions could be identified based on the basis of different changes of the temporal and spatial scaling exponents. These subgroups can be distinguished from one another by a measure of distance within the plane defined by both exponents *d* and α. Thus, a quantitative descriptor of similarity may be defined, either as a vector quantity or as a measure describing the distance between different drug reactions and the

directions of the different effects. Finally, to predict drug effects, to distinguish between different phases of drug effects, or to study rapidly occurring fluctuations induced by drugs, a quantitative model could be based on the order parameter concept. In such a model, a set of independent variables-which may be based on any of the behavioral, biochemical, or neurophysiological measures described in this volume (e.g., "Psychomotor Stimulant Effects on Single Neurons in Awake, Behaving Rats," "Microdialysis in the Study of Psychostimulants and the Neural Substrate for Reinforcement: Focus on Dopamine and Serotonin," and "Neurophysiological Approaches to Receptor Pharmacodynamics")—define a function describing the quantitative change of the order parameter, e.g.,

$$d(x_1, x_2)$$

The change in x_1 and x_2 may correspond to effects on different receptor types, which eventually should result in the development of suitable combinations of substances affecting the different receptor systems in order to obtain a macroscopic change in behavior compatible with the change in the order parameters.

CONCLUSIONS

This review has discussed some of the important considerations in assessing the effects of drugs or other treatments on the locomotor activity of rodents. The need to clearly define the behavior being studied has led to most researchers, and this review, focusing on measures of locomotor activity versus more inclusive measures of general motor activity. For a variety of reasons, the most reliable measures of rodent activity are those that are selective for locomotor movements from one place to another. Additional considerations, including the importance of using a test system that enables the detection of either increases or decreases in activity, indicate that the test chambers to be used should be of sufficient novelty or complexity for the control subjects so that they exhibit an adequate level of activity. For most practical purposes, therefore, the minimal system suitable for most investigators is one in which the test chamber is (1) large enough to engender in the animals locomotion that is qualitatively different from their locomotion in the residential caging and (2) equipped for automated measures selective to locomotor movements. The further ability to use the system in the nocturnal portion of the animal's sleep-wake cycle without providing feedback to the animal is advantageous from the point of view of flexibility and sensitivity. Such criteria are readily satisfied by systems based on

infrared photobeams, which are clearly the most common and generally satisfactory detectors. The additional advantage of photobeam systems is that they can easily provide a secondary measure of general or fine motor activity, which, while having limited behavioral meaning, can serve as an assay of drug effects and indicate the need for further studies.

A number of more complex issues are relevant to more thorough and behaviorally meaningful characterizations of drug effects on locomotor activity. The foremost concern involves the desirability of multiple concurrent measures, preferably of explicit investigatory responses as well as locomotor movements. Maximizing the sensitivity of measures of locomotor activity requires some recognition of the qualitative features and structure of the spatiotemporal patterns of locomotion exhibited by rodents in the given test environment. From a behavioral point of view, the nature of the test chamber has profound effects on the kinds of questions the investigator may address. The example described here, the BPM system, demonstrates some of the ways that detailed analyses of patterns of locomotion may increase the sensitivity and interpretability of one's measures. The behavioral profiles provided by such multivariate assessment techniques greatly enhance the specificity with which drug effects can be characterized. With the advent of new detector systems such as video imaging methods and the further development of new metrics, such as those evolving from the study of nonlinear dynamic systems, future state-of-the-art studies of locomotor activity are likely to provide ever more informative and powerful approaches to understanding the behavioral effects of drugs.

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Neurobiological Approaches to Brain-Behavior Interaction: Summary and Overview

George F. Koob

The technical review developed in such a way that one could easily rearrange the presentations to reflect three major themes. First, there were dependent variables from neurobiology ranging from positron emission tomography (PET), single photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI) techniques to in vivo voltammetry and dependent variables from behavior involving measures of brain reward, drug self-administration, and motor activity. Independent variables that critically influence the dependent variables included behavioral history that can affect subsequent behavior and behavioral history that can affect neurochemical measures. Finally, the important role of contingency control was discussed.

The presentation of PET, SPECT, and MRI techniques by William Jagust focused on introducing these techniques and their relative resolution and practicality. It was clear that the PET technique currently provides higher resolution and more versatility but is more expensive than SPECT. PET also has the potential advantage that tracers for specific neurotransmitter receptors may be measured. SPECT is inexpensive but has low sensitivity and poor temporal resolution and is used largely as a measure of blood flow. MRI produces a much greater contrast sensitivity between white and gray matter yet is limited by having no isotopes and thus no tracers by which to measure the activity of specific receptors.

Using PET, Jagust showed that patients with Alzheimer's disease had decreases in glucose metabolism in the temporal and parietal cortex with little change in subcortical areas. In a preliminary study on drug abusers, labeled spiroperidol binding was decreased in the caudate nucleus of a nondetoxified cocaine user.

Harriet de Wit then showed the power of the PET technique when combined with innovative experimental design and correlational analysis. Using a within-subjects design, de Wit was able to administer drug and take behavioral measures in a natural setting and then repeat many of these behavioral measures after drug in the unnatural setting of the PET measurement. Studies following ethanol ingestion showed increases in measures of positive mood, elation, friendliness, etc., at blood alcohol levels of 40 to 60 mg percent. Identical amounts of ethanol produced a decrease in global metabolic rate as measured by PET in all regions examined, including all the cortices, basal ganglia, and cerebellum. No major correlations were evident between individual differences in mood changes and differences in regional metabolism. Similar results were obtained with diazepam except that the behavioral measures reflected more decreases in anger, anxiety, and arousal. The experimental designs used by de Wit were very powerful; these designs combined with better PET resolution and new methods of PET analysis (see “Importance of Behavioral Controls in the Analysis of Ongoing Events”) should provide an exciting means by which to examine the neural basis of human mood states after drugs.

Linda Porrino discussed the use of the 2-deoxyglucose (2-DG) technique for evaluating brain activity changes in animals associated with various independent variables such as electrical brain-stimulation reward, drug self-administration, and brain pathophysiology. In the context of this thorough exploration of the 2-DG technique, Porrino emphasized highly critical issues regarding what was being measured and how it was analyzed.

First, it appears clear that the 2-DG technique measures brain synaptic activity and changes in the afferent input to neurons but not changes in cell body activity. The 2-DG measures do not correlate well with electrophysiologic measures of cell firing, but in support of the hypothesis regarding synaptic activity, blockade of ion gradients with ouabain blocks glucose metabolism. (See “Metabolic Mapping Methods for Identifying the Neural Substrates of the Effects of Abused Substances.“)

The advantage of the 2-DG technique is that it provides a visualization of activity of the entire central nervous system at the resolution of autoradiography in conscious, freely moving animals. The disadvantages include inability to distinguish direct from indirect effects and excitation from inhibition or neurochemical specificity. In addition, the 2-DG method currently has poor time resolution.

Perhaps the most critical issue, however, centers on the problems of data analysis. Porrino argued very convincingly that one must show in 2-DG studies that the reference measure (referent) is not changing with the independent variable. She showed that the relative optical density measure used by others can give misleading results and may explain much of the controversy in the literature. Using measures of local cerebral glucose utilization (nontransformed rates of glucose utilization), Porrino showed that regions of the ventral striatum such as the nucleus accumbens (NAC) and olfactory tubercle as well as their efferent projections had increased 2-DG activity following intracranial self-stimulation and following administration of drugs that activated the dopamine (DA) system, such as cocaine and amphetamine.

Mark West demonstrated the utility and versatility of chronic unit recording in the freely moving rat for identifying the actions of drugs at the cellular/systems level of analysis. Rats were prepared with multiple microelectrodes using microwires with which up to 10 electrodes could be recorded at the same time. This technique can measure spontaneous tonic firing rates as well as phasic firing patterns that can be correlated with behavior. Specific regional cerebral stimulation or specific neurotransmitter activation using a four-barreled micropipette can be combined with electrical recordings in awake animals. The recordings appear to be stable over days and weeks, indeed, up to 60 days.

Using this technique, West showed that there was a topographical representation of striatal responses to body stimulation and that other striatal cells respond to active movement such as a rat moving on a treadmill. The cells in which firing correlated with treadmill locomotion showed enhanced responsiveness following administration of amphetamine but decreased excitation following a dopamine receptor antagonist. Sixty percent of the cells that fired during locomotion were excited by administration of DA. Recordings of neural activity from the NAC showed increased firing during the licking of a spout for chocolate milk but not during licking of a dry spout, suggesting that increases in spontaneous activity were more related to rewarding stimuli. These results emphasize the power of this technique in correlating unit activity with behavioral responses to drugs at the system level. Such a technique provides a critical bridge between global measures such as the PET and 2-DG techniques and single-unit responding in anesthetized animals (see “Neurophysiological Approaches to Receptor Pharmacodynamics”).

Francis White provided an indepth analysis of the power of neurophysiology to explain receptor pharmacodynamics by exploration of the interaction of the D_1

and D_2 DA receptor subtypes. Using an anesthetized preparation, sophisticated electrophysiological recordings allowed specific neuronal systems and the effects of drugs on these systems to be identified as well as the exploration of the mechanism and its relevance to behavior. The power of such a technique is the ability to use classic electrophysiological techniques such as orthodromic and antidromic stimulation for identifying projection neurons and afferent inputs. A potential significant weakness of such an approach is the anesthesia. Although not necessarily a problem with recording from DA neurons, anesthesia apparently diminishes stimulatory effects particularly in acetylcholine neurons (comment of H. Chris Fibiger). Consistent with this observation, the studies discussed by White of recordings from DA neurons in anesthetized rats show a predominance of DA inhibition whereas in awake freely moving rats there was a predominance of dopamine excitation (see "Psychomotor Stimulant Effects on Single Neurons in Awake, Behaving Rats").

In single-unit recordings from the NAC accumbens in anesthetized rats, White showed that intact neurons responded to both D_1 and D_2 agonists with an inhibition of firing. However, acute depletion of DA from the NAC with alphamethylparatyrosine (AMPT) produced an abolishment of the decrease in firing produced by the D_2 agonist LY 141865 (quinpirole). This responsiveness of the DA receptor could be reinstated with a combination of the D_2 agonist and the D_1 agonist SKF 38393, suggesting that D_1 receptors play a critical enabling role in the activity of D_2 agonists. Similar results were obtained at the behavioral level, where the stereotyped behavior produced by DA D_2 agonists was also eliminated by pretreatment with AMPT but could be reinstated by simultaneous administration of the D_1 agonist SKF 38393 with the D_2 agonist. The converse requirement of D_2 activation for D_1 effects did not appear to be true. These results suggest that the behavioral interactions produced by activation of brain DA receptors result from an enabling action of the D_1 receptor subtype; these results also show the power of the electrophysiological approach in predicting pharmacologic interactions at the behavioral level.

Bartley Hoebel's presentation explored the power of the technique of brain microdialysis in the context of measuring DA and dihydroxyphenylacetic acid (DOPAC) from the NAC in freely moving rats. In this technique a chronic cannula is implanted in the brain of the rat through which a microdialysis probe can be inserted at any time. Through this probe, brain neurotransmitters that form an equilibrium with the extracellular space can be measured.

The advantage of this technique is that the liberation of neurotransmitters from synaptic terminals can be correlated with ongoing behavior. Specific neurotransmitters can be measured that presumably reflect the activity of synaptic terminals and, because of the size of the dialysate, membrane enzymes are excluded. Other potential uses for this technique are that it can be used for drug or transmitter stimulation of specific neuronal receptors by injecting through the dialysis probe. Disadvantages include a long time resolution (5 to 20 min), lack of sensitivity, and a limitation in what transmitters can be measured as well as possible interactions with other simultaneously released neurotransmitters. Many of these disadvantages will probably be resolved with refinement of the technique as well as application of more sophisticated assays and assay techniques.

Using microdialysis, Hoebel has extensively explored the conditions under which DA release in the NAC can be modified. Most drugs of abuse such as cocaine, phencyclidine, and nicotine provoked a release of DA when infused directly into the NAC in freely moving, awake animals. More drug-reinforcing stimuli such as food and rewarding brain stimulation also caused an increase in nucleus accumbens DA release. In an elegant experiment, Hoebel showed that saccharin infused on the tongue of a naive rat increased NAC dopamine release but actually decreased DA release in a rat for which saccharin had been the conditioned stimulus in a conditioned taste aversion experiment. These results demonstrated the power and sensitivity of this technique for brain-behavior interactions and add impetus to hypotheses suggesting a role for DA in the NAC in reward/incentive motivation.

Behavior-dependent variables are equally important in brain-behavior interactions and also have limitations specific to each technique. They also require important control measures not unlike those for neurochemical-dependent variables. In addition, behavioral measures can lend themselves to equally sophisticated analyses, allowing a greater potential for correlation to discrete electrophysiological and neurochemical events.

The hypothesis that electrical brain self-stimulation provides a direct measure of the activity of brain reward systems is the premise that guides Conan Kornetsky's pursuit of the use of brain-stimulation reward (BSR) as a behavior-dependent variable. Kornetsky has developed a powerful threshold measure of BSR that has the major advantage of minimizing the response rate confounds of other conventional BSR techniques. The technique involves a discrete trials procedure in which the rat receives a free BSR stimulus train with

the option of responding by rotating a wheel manipulandum to obtain a second stimulation train. Using this procedure Kornetsky can readily obtain reliable threshold measures that can be subject to probit analysis for statistical testing.

When this procedure was used, most drugs that have abuse potential produced a lowering of reward threshold. These include drugs such as amphetamine, cocaine, nicotine, opiates, and phencyclidine. DA receptor antagonists such as haloperidol and pimozide raised BSR threshold. The selectivity of these effects has been demonstrated by Kornetsky, who showed that those same drugs and doses of drugs failed to alter or change in a different direction the threshold for BSR detection. Here the rat performs exactly the same behavioral task, but the BSR is presented at an intensity lower than threshold and the rat is asked to respond to the discriminability of that stimulus.

Finally, BSR has the potential to link drug studies directly to brain function. Two deoxyglucose measures taken after BSR show that ventral forebrain structures such as the NAC, olfactory tubercle, and prefrontal cortex show a significant activation (see “Metabolic Mapping Methods for Identifying the Neural Substrates of the Effects of Abused Substances”). Drugs of abuse combined with BSR can potentiate some of these effects, but preliminary results suggest that some of these drug effects may be common to drugs of abuse and others may be different for each class of drug.

Drug self-administration as a dependent variable provides one of the most direct measures of the reinforcing properties of a drug. William Woolverton described how the behavioral contingencies under which self-administration was maintained could emphasize either the pharmacology or the history of reinforcement. A substitution procedure was used in primates to assess the different potential of drugs to maintain intravenous self-administration. Animals established a baseline of cocaine self-administration, were subjected to an extinction probe, and then after several baseline days were subjected to a new drug probe at a given dose. With this approach, drugs such as the DA agonists, apomorphine, bromocriptine, and piribedil were readily self-administered, whereas fluoxetine, a serotonin reuptake inhibitor, was not. Thus, the pharmacology of the drugs is clearly important for self-administration.

However, the schedule of reinforcement that maintains self-administration can significantly alter the pattern of responding. Indeed, in earlier work Spealman showed that cocaine could act as both a positive and negative reinforcer. This point was demonstrated in monkeys working on a concurrent schedule in which

a variable-interval schedule produced cocaine infusions and a fixed-interval schedule terminated the schedule of cocaine infusion. In addition, choice procedures can be used to manipulate the relative choice of responding for a drug. In monkeys trained in a discrete trials procedure for food and cocaine, increasing the dose of cocaine increased the frequency of cocaine choices; however, increasing the number of food pellets delivered decreased cocaine choices. Such a choice procedure may be very useful for brain-behavior interaction studies, particularly to control for nonspecificity associated with responding.

Spontaneous locomotor activity has been classically used as a means of measuring the arousal level of animals and the stimulant and sedative properties of drugs. However, simple measures of motor activity have significant limitations in discriminating differential drug effects and even dose-effect functions. Mark Geyer and Martin Paulus described a new approach to locomotor activity measures that involves both quantitative and qualitative measures. A Behavioral Pattern Monitor (BPM) system developed by Geyer not only incorporates measures of crossovers and investigatory behaviors but also provides a description of the activity pattern of the animal. Qualitatively, this pattern can be described by video or actual line drawing. Quantitatively, a coefficient of variation can be calculated that basically describes the distribution or uniformity of the locomotor transitions. A high coefficient of variation indicates a stereotyped pattern of activity. These measures have allowed a clear discrimination to be made between stimulant drugs that were previously difficult to separate on motor activity measures. Paulus described a novel approach to measuring the complex patterns of locomotor activity described by the BPM. Borrowing from physics and nonlinear dynamics, Paulus used a scaling transformation to describe the complexity of the locomotor path structure. For example, the measure *d,* a spatial scaling component, increased with the complexity of the path structure but decreased with a preservative path structure. Thus, apomorphine, which produced stereotyped locomotion and thigmotaxis (a high coefficient of variation), produced a decrease in *d.* However, amphetamine produced nonstereotyped locomotion, a low coefficient of variation, and no major change in *d.* These sophisticated mathematical models provide an innovative way to simplify complex events. They may have great potential for linking locomotor activation to neurobiological systems and for identifying and characterizing the actions of stimulant drugs.

Three presentations focused on independent variables that transcend a given dependent variable or experimental design in experiments designed to

elucidate interactions between brain and behavior. These issues affect both the behavior to be measured and the neurochemistry; not only are they critical for interpretation of results but they also provide intriguing questions for brain-behavior interactions.

The role of an organism's history in altering the behavioral response to drugs was discussed by James Barrett. Barrett provided several examples in which the behavioral response to drugs changed dramatically depending on the previous training of the animal. For example, animals with no history of avoidance showed a decrease in punished responding following amphetamine treatment. However, animals with a history of avoidance showed an increase in punished responding following amphetamine treatment.

Similarly, morphine decreased avoidance responding in animals before an experience with shock presentation but increased responding on avoidance after a history of shock presentation. Thus, previous experience left a residual effect that altered subsequent drug responses. This type of behavioral analysis appears to be critical for studies of brain-behavior interactions. Unknown at this time is how such historical effects could alter the reinforcing properties of drugs or drug dependence. Finally, it should be noted that the residual behavioral effect produced by a different behavioral history presumably has a neurochemical correlate.

This thesis was clearly elaborated by Lewis Seiden, who showed that a variety of behavioral factors can influence catecholamine metabolism; these factors can differentially affect catecholamine metabolism in different brain regions. For example, food-deprived rats that were given food showed increases in DOPAC levels (reflecting increases in DA metabolism) in the hypothalamus, amygdala, and NAC. However, tube-fed animals given food showed no change in DOPAC in the NAC and posterior hypothalamus but still showed the increase in DOPAC in the amygdala. Thus, an increase in DA metabolism in the NAC was associated with the motor activity of feeding (see "Microdialysis in the Study of Psychostimulants and the Neural Substrate for Reinforcement: Focus on Dopamine and Serotonin"; "Psychomotor Stimulant Effects on Single Neurons in Awake, Behaving Rats"; and "Brain-Stimulation Reward: A Model for the Study of the Rewarding Effects of Abused Drugs"). Seiden emphasized that the brain-behavior interface with drugs reflected a dynamic interaction in which both behavior and the environment could alter brain chemistry with or without drug, supporting the hypothesis that the residual effects observed with different behavioral histories can reflect significant changes in brain neurobiology.

The importance of behavioral controls in the analysis of brain behavior interactions was explored by Steven Dworkin. In addition to pharmacologic factors and behavioral history, the need for subject control over contingencies was emphasized. To control for nonspecific effects associated with drug effects when examining brain-behavior interactions, a yoked-control procedure has been developed. Here, the neurobiological response of the contingent subject is thought to measure reinforcement mechanisms, whereas the neurobiological response of the noncontingent subject is thought to reflect nonspecific activation. The control subject receives no treatment and thus its neurobiological response presumably reflects no event.

Using this approach, Dworkin and colleagues showed significant differences in limbic brain regions associated with morphine self-administration between contingent and yoked subjects. Most notably the amino acids, GABA and glutamate, and the monoamines, DA, norepinephrine, and serotonin, were altered. Results from this work have been the driving force in developing hypotheses about the neurochemical basis for drug reinforcement and dependence.

This need for appropriate behavioral controls was extended by Dworkin to other behavioral situations including punishment. DA metabolism was decreased and serotonin metabolism was increased in the frontal cortex of the punished subjects versus yoked animals. A behavioral confirmation of the power of the contingent situation versus the noncontingent situation was manifested by the significant increase in toxicity associated with noncontingent administration of cocaine. Thus, control of events can significantly affect brain neurobiology and the experimental designs used for establishing contingent effects could be easily used with other neurochemical-dependent variables.

Finally, George F. Koob addressed the question of drug dependence and different measures of drug dependence. Most of the techniques described in the technical review focused on behavioral measures of the motivational effects of drugs and the interface of neurobiological measures in acute drug situations. Largely lacking were studies in dependent animals.

Previous work exploring the neurobiological substrates for opiate withdrawal have focused on “physical” signs such as wet dog shakes and ptosis and have identified a diffuse localization in the amygdala, thalamus, and mesencephalic-diencephalic function of sites sensitive for opiate antagonist precipitation of withdrawal. Using more motivational tasks for measuring

withdrawal such as response suppression and place aversion, Koob and colleagues have shown that the region of the nucleus accumbens is a very sensitive substrate for precipitating the aversive effects of opiate withdrawal with opiate antagonists. Because the NAC appears to be particularly sensitive to the effects of opiate antagonists in blocking the reinforcing effects of opiates, these results suggest that the same sites in the brain important for the acute motivational effects of drugs may be involved in the plasticity associated with the motivational aspects of drug dependence.

Several important conclusions developed from this technical review, particularly in regard to the dependent variables that are used to explore brain-behavior interactions. Attempts should probably be made to standardize behavioral tests from rats to primates to humans. Some of the experimental designs elaborated in the human work attest to this as a possibility. Technical guidelines for the proper use of voltammetry, microdialysis, neurophysiology, and 2-DG would be helpful and useful. Clearly, all techniques have some limitations, as amply elaborated by the presentations. Nevertheless, a standardization of control procedures and methodology would save unnecessary frustration and lost effort.

Another point evident from the review is that data analysis is continually in need of improvement and sophistication. New means of analyzing neurochemistry, motor activity, and glucose metabolism were presented. Such sophisticated analysis promises an ultimately finer resolution of brain-behavior interactions.

Although the focus on the brain monoamine systems demonstrates the breadth of knowledge available in this area, studies of brain-behavior interaction ultimately must demand a broader perspective. New techniques must be developed for measuring and manipulating neuropeptides and amino acids.

Finally, this technical review focused largely on dependent variables and our advances therein. However, the presentations on independent variables raised these questions: What hypothesis is one testing? and What knowledge will be gained from testing that hypothesis? Crosstalk between the disciplines is still somewhat limited, and the courageous efforts of participants to transcend discipline chauvinism and to interact across levels of analysis should continue and be strongly encouraged.

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