# DOPAMINE D1 AND D2 RECEPTOR INTERACTION MAY INITIATE AMPHETAMINE-INDUCED HYPO-ACTIVITY IN RATS

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Susan L. Roy

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Wesley White
Director of Thesis

Master's Committee: Wesley White, Chairperson

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DOPAMINE D1 AND D2 RECEPTOR INTERACTION

MAY INITIATE AMPHETAMINE-INDUCED HYPO-ACTIVITY IN RATS

Susan L. Roy, M.S.
Morehead State University, 2005

Director of Thesis: Wesley White

Abstract

Amphetamine (2.0 mg/kg) produces hypo-activity around hour 20 post-administration in rats (White, Feldon & White, 2004). The hypo-activity may be an aspect of acute withdrawal, because other indications of withdrawal are present at the same time, including REM sleep rebound, unwillingness to work for reward, and a Haloperidol cue state (Barr & Phillips, 1999; Barrett, White & Caul, 1992; Edgar & Seidel, 1997). The purpose of these studies was to investigate the dopaminergic mechanisms involved in producing amphetamine-induced hypo-activity. More specifically, the goal was to study the contribution of different dopamine receptor subtypes to the phenomenon.

In order to pursue this objective two experiments were conducted. In both experiments, male Wistar rats were individually housed in plastic cubicles, where they were on a 12-12 hr light-dark cycle and had free access to food and water. A camera mounted above each cubicle was connected to a computer by a multiplexer. Tracking software used camera images to quantify activity in terms of total distance moved per hour. Different groups of rats were given subcutaneous administrations of different doses of SKF 81297 (SKF), a dopamine D1 receptor agonist, or of

Quinpirole, a dopamine D2 receptor agonist. In Experiment 1 different groups of rats were given administrations of saline, 2.0 mg/kg amphetamine, 0.2 mg/kg SKF, 0.4 mg/kg SKF, 0.2 mg/kg Quinpirole, or 0.4 mg/kg Quinpirole. In Experiment 2 different groups of rats were given combined administrations of 0.0 SKF/ 0.4 Quinpirole, 0.2 SKF/ 0.4 Quinpirole, 0.4 SKF/ 0.4 Quinpirole or 0.8 SKF/ 0.4 Quinpirole. All treatments occurred at the start of the light period and were separated by at least a 48-hr interval. Activity was monitored for 33 hr after each treatment.

In Experiment 1 only amphetamine produced hypo-activity 20 hr post-administration. Selective stimulation of D1 or D2 receptors was not sufficient to produce such hypo-activity. In Experiment 2 the two higher dose combinations (0.4 SKF/ 0.4 Quinpirole and 0.8 SKF/ 0.4 Quinpirole) produced hypo-activity 20 hr post-administration. The lower dose combinations did not.

Combined stimulation of D1 and D2 receptors above a threshold level by amphetamine may be sufficient to initiate amphetamine-induced hypo-activity. This raises the possibility that the cascade of events by which amphetamine produces other indications of acute withdrawal is also initiated by the short term activation of D1 and D2 receptors. The methods used here may provide a convenient animal model with which to further study the determinants of amphetamine-induced hypo-activity and acute withdrawal.

Accepted by: Wesley White, Chairperson

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Cary S. Feria

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Though imperfect, I am proud of this work. I dedicate this, my first major work of science and writing, to these wonderful people to whom I am indebted, and who are the most important in my life. Without your support, understanding and encouragement, this would not have been possible.

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May Initiate Amphetamine-Induced Hypo-Activity in Rats

Research has shown that certain doses of amphetamine can cause hypo-activity 20 hr post-treatment in rats (White, Feldon & White, 2004). The objective of the current research was to evaluate whether certain dopaminergic mechanisms might have contributed to this amphetamine-induced hypo-activity.

In order to pursue this objective, different groups of rats were given different doses of SKF 81297, a D1-like receptor agonist, or of Quinpirole, a D2-like receptor agonist; or they received doses of the agonists combined. Effects on activity were evaluated by housing rats in individual stations where activity was continuously monitored. If a particular treatment had the ability to produce hypo-activity, then the specific mechanism affected might also be involved in amphetamine's ability to induce hypo-activity.

In the remainder of this introduction I will do the following. I will describe some of amphetamine's immediate effects in humans. Because I am focusing on activity, I will then describe amphetamine's immediate and longer-term effects on activity and on other measures in rats. Amphetamine has several time-dependent effects, so I will introduce a terminological distinction that clarifies what I am trying to account for. Amphetamine affects multiple neurotransmitters, so I will describe evidence that justifies the focus on dopamine (DA). I will then review dopaminergic mechanisms, with a focus on dopamine pathways and the structure and function of the dopamine receptor subtypes. Then, I will explain why activity is a good

behavioral measure. Finally, I will talk about specific goals and hypotheses and conclude with the significance of this research.

*Immediate effects in humans.* The immediate effects of amphetamine in humans are well known (reviewed in Julien, 2004; Segal & Kuczenski, 1994). During the first several hours post-administration, amphetamine produces signs of autonomic arousal including an increase in blood pressure, heart rate, respiration, metabolism, and body temperature, as well as dilatation of the pupils. Amphetamine also produces signs of cognitive, motivational, and emotional arousal including an increase in alertness, increased energy, and a feeling of well-being and euphoria (Julien, 2004).

*Immediate and longer-term effects in rats.* The immediate effects of amphetamine on the activity of rats are dose-dependent. A moderate dose of 1.0 mg/kg produces an increase in locomotor activity and rearing, as well as mild sniffing and head bobbing. A moderately high dose of 2.0 mg/kg produces an increase in locomotor activity intermixed with low gauge stereotypy. A high dose of 4.0 mg/kg produces a multi-phasic pattern: An initial increase in locomotion is replaced by intense stereotypy which is then followed by another increase in locomotion (reviewed in Feldman, Meyer & Quenzer, 1997).

Additional immediate effects of amphetamine in rats include autonomic changes. Drug discrimination, conditioned place preference, and drug self-administration studies indicate that amphetamine produces discriminable internal cues, some of which are rewarding (reviewed in Hoffman, 1989).

Amphetamine also has some longer term effects on activity. White et al. (2004) found that male Sprague-Dawley rats, when given 1.0 mg/kg amphetamine, were hypo-active around hours 19-21 post-treatment. White and White (in press) gave male Wistar rats a range of amphetamine doses (1.0 mg/kg, 2.0 mg/kg and 4.0 mg/kg). They found that the 1.0 mg/kg dose did not produce hypo-activity around hour 20 post-treatment but that the 2.0 and 4.0 mg/kg doses did.

In addition to immediate effects and effects 20 hr after administration, amphetamine and other psychomotor stimulants enhance activity and autonomic function for several hours beginning approximately 24 hr after administration (Tornatzky & Miczek, 1999; White et al., 2004; White & White, in press). The changes may indicate a recovery state.

*Induction and expression.* Amphetamine is followed by different effects at different times. Therefore, it is useful to introduce new terminology in order to clarify my interest. 'Induction' will be used to refer to the events that must occur in the short term to produce the hypo-activity near hour 20. 'Expression' will be used to refer to the events involved in the manifestation of the hypo-activity. I am interested in investigating what form of short-term dopaminergic stimulation is sufficient to produce hypo-activity 20 hr later, therefore, I am interested in the process of induction.

The purpose of this research was not to investigate how induction and expression might be linked. However, the opponent process theory (Solomon &

Corbit, 1974) and the distinction between within and between system adaptations (Koob & Bloom, 1988) explain how induction and expression could be linked.

According to opponent process theory, intense states are followed later by a rebound in the opposite direction (Solomon & Corbit, 1974). The opponent process theory of drug addiction describes how the withdrawal from a drug can promote drug dependence and addiction through negative reinforcement (Barr, Markou & Phillips, 2002). Thus, an individual will try to restore hedonic equilibrium by self medication through drug intake.

A within-system adaptation produces a rebound when "the primary cellular response responsible for the acute hedonic effects of the drug would itself adapt to oppose and neutralize the drug's effects; persistence of the opposing effects after the drug disappears would produce the motivational withdrawal response." A between-systems adaptation produces a rebound when "cellular and molecular systems different from those responsible for the acute hedonic effects of the drug, triggered by the changes in the primary drug response neurons, would contribute to or produce the motivational effects of withdrawal after drug removal (Koob & Bloom, 1988)." In other words, in a within-system adaptation, the same mechanisms that are involved in induction are also involved in expression. Alternatively, in a between-systems adaptation, different mechanisms are involved in induction and expression. How these processes might be applicable to this research will be discussed in more detail later.

*Justification for dopamine involvement.* Amphetamine is an indirect agonist of catecholamines, including dopamine, norepinephrine and serotonin. For example, amphetamine promotes the release of dopamine from the presynapse, and it blocks reuptake of dopamine and norepinephrine and serotonin. Amphetamine can also inhibit the storage of dopamine in vesicles and inhibit the destruction of dopamine by enzymes (Julien, 2004). The stimulation of one or more catecholaminergic receptors in the short-term by amphetamine would appear to be involved in induction.

I focused on dopamine because non-selective stimulation of dopaminergic receptors in the short-term appears to produce hypo-activity near hour 20. Apomorphine is a non-selective direct agonist of dopamine. White, Mattingly, Duke, Liu, Dunkman, Charles, and White (2002) gave male Wistar rats 1.0 mg/kg or 2.0 mg/kg apomorphine and found that the rats were hypo-active 19-21 hr post-treatment. This time course parallels the time course seen with amphetamine. The study suggests that amphetamine's ability to produce hypo-activity might be mediated via dopamine receptors.

Other research has suggested that dopamine might be involved in the expression of hypo-activity. Barrett, White, and Caul (1992), Persico, Schindler, Zaczek, Brannock, and Uhl (1995), and Tonge (1974) have all found molecular and neurochemical changes in dopamine 20 hr after amphetamine treatment that might be related.

Because research has established that dopamine is involved in amphetamine-induced hypo-activity, it becomes reasonable to investigate the specific dopaminergic mechanism/s that might underlie the phenomenon.

*Review of dopaminergic mechanisms.* Dopamine is a main neurotransmitter that has an important role in the control of motor activity, reward-related mechanisms, and emotional and cognitive processes. The dopaminergic system includes several pathways. The main dopaminergic pathways in the brain are the nigrostriatal, mesolimbic, and mesocortical. The nigrostriatal pathway projects from the substantia nigra to the striatum. The mesolimbic pathway projects from the vental tegmental area (VTA) to the nucleus accumbens, olfactory tubercle, septum, and amygdala. Finally, the mesocortical pathway projects from the VTA to the prefrontal cortex (Julien, 2004; Wishaw, Kolb & Wishaw, 2003).

Five dopamine receptor sub-types have been identified on the basis of structural, pharmacological, and functional characteristics. The D1 and D5 subtypes are categorized under the D1-like family and the D2, D3 and D4 subtypes are categorized under the D2-like family. In these studies I will use pharmacological agents to selectively stimulate the two main dopamine receptors, the D1 and D2 receptors. After I review the molecular structure, the localization and the functions of the D1 receptor, I will do the same for the D2 receptor.

The D1-like receptors are seven transmembrane domain metabotrophic receptors. Structurally the D1-like receptor has the N-terminus localized on the extracellular surface, a long C-terminus which projects into the cytosol, and a small

third cytoplasmic loop. D1-like receptors are found in the hypothalamus, thalamus, ofactory tubercle, substantia nigra pars reticulata, striatum and nucleus accumbens. D1-like receptors are found primarily at the post-synapse (Sibley, Monsuma & Shen, 1993).

D1-like receptor activation has been found to increase motor activity. D1 receptor stimulation with agonists elicits locomotion, grooming and rearing in rats (Molloy & Wadington, 1984). Direct infusion of D1 agonist into the dorsal striatum or nucleus accumbens elicits hyperactivity and the development of behavioral sensitization to the locomotor-activating effects of amphetamine. Furthermore, D1 agonist infusion into the nucleus accumbens produces a conditioned place preference and supports drug discrimination, whereas D1 anatagonists attenuate these effects (Waddington, 1986).

The D2-like receptor is also a seven transmembrane domain metabotrophic receptor. However, structurally the D2-like receptor differs from the D1-like receptor because its C-terminus is small and its third cytoplasmic loop is large. D2-like receptors are found in the ventral tegmental area, granule area of the hippocampal formation, substantia nigra pars compata, septal region, cingulate, nucleus accumbens, amygdala and prefrontal region. D2-like receptors are found at both the pre- and post-synapse (Sibley et al., 1993).

D2-like receptor stimulation has been found to increase locomotion, sniffing and snout contact (Eilam, Golani & Szechtman, 1989; Molloy & Waddington, 1985; Walters, Bergstrom, Carlson, Chase & Braun, 1987). D2 agonists do not produce a

conditioned place preference but do produce a discriminative stimulus effect (Nielsen & Scheel-Krueger, 1986). D2 receptor stimulation has been found to produce behavioral effects similar to those of non-selective dopamine agonists.

*Activity as the dependent variable.* The main dependent variable in this research was an activity measure. An activity measure has several advantages. First, the rat circadian activity pattern is well understood: Activity occurs in bouts that are separated by one to several hours, and most activity occurs during the dark period. Second, the effects of drugs on activity can be easily identified against this well known background. Finally, changes in activity can be used to make inferences about the drug-induced state that an animal is in.

*Goals and hypothesis.* The purpose of this research was to investigate which dopaminergic receptors produce amphetamine-induced hypo-activity. Specifically:

- In Experiment 1, activity was measured following one of several treatments. Treatments included administration of amphetamine, saline, and different doses of either dopamine D1 receptor agonist (SKF 81297) or dopamine D2 receptor agonist (Quinpirole).
- In Experiment 2, activity was measured following one of several treatments. Treatments included administration of different doses of D1 receptor agonist (SKF 81297) combined with D2 receptor agonist (Quinpirole).

The ability either of a selective agonist or of the agonists in combination to produce hypo-activity would indicate which specific dopaminergic receptors are involved in amphetamine-induced hypo-activity.

*Significance and implications.* The longer-term effects of amphetamine need to be studied for various reasons. Acute administration of psychostimulants is not thought to produce symptoms of hangover. However, the presence of hypo-activity 20 hr post-amphetamine administration suggests that there may be costs to even acute amphetamine use. Longer term motivational processes might be affected by amphetamine. In particular, amphetamine-induced hypo-activity may compete with psychological processes necessary for effective task performance. Amphetamine-induced hypo-activity may even be an indicator of an acute withdrawal syndrome. This research may help us identify novel side effects produced by amphetamine administration.

Additionally, this research may promote the development of an animal model of amphetamine-induced acute withdrawal. We eventually want to use such a model to understand the mechanisms underlying different symptoms of acute withdrawal. Information regarding the symptoms present during amphetamine-induced acute withdrawal in humans has not been obtained, primarily because human drug use occurs under very complex circumstances. Inducing these symptoms in humans under controlled circumstances would not be ethical. Hence, it is necessary to use an animal model approach. Understanding the psychological processes and identifying the

mechanisms that contribute to amphetamine-induced hypo-activity and acute withdrawal may help us identify potential treatments for amphetamine abuse.

## Experiment 1: Selective Dopamine Receptor Activation

Treatment with either amphetamine or apomorphine produces hypo-activity 20 hr later, suggesting that the hypo-activity may be dependent on short term stimulation of dopaminergic receptors. Apomorphine is a nonselective dopamine agonist: It activates both D1 and D2 receptors. Consequently, whether apomorphine (and by inference, amphetamine) produces hypo-activity by activating the D1 receptor subtype, the D2 receptor subtype, or both is uncertain. Experiment 1 investigated whether hypo-activity 20 hr after treatment could be elicited by stimulating either D1 or D2 receptor subtypes alone.

To justify my methods, I will briefly review the dopamine agonists that I used. First, I will review SKF 81297, a selective direct D1-like agonist. Then I will review Quinpirole, a selective direct D2-like agonist. Because I am measuring activity, I will describe how the drugs affect immediate activity and why they might result in hypo-activity in the longer-term. Finally, I will identify neural structures that may be involved in the agonist-induced behaviors.

*Hypotheses.*

- Selective dopamine D1 receptor activation with the agonist SKF 81297 would produce hypo-activity 20 hr post-treatment.

- Selective dopamine D2 receptor activation with the agonist Quinpirole would produce hypo-activity 20 hr post-treatment.

If either selective agonist (SKF 81297 or Quinpirole) produced hypo-activity 20 hr later, then amphetamine might do the same via a similar mechanism.

*D1-like agonist: SKF 81297.* SKF 81297 (SKF) is a full dopamine D1-like receptor agonist. The agonist is a benzazepine derivative. Andersen and Jansen (1990) showed, with a measure of adenylate cyclase stimulation, that SKF has a very high affinity for D1 receptors.

A number of studies have shown the immediate effects of SKF 81297 on activity in rats (Alleweireldt, Weber, Kirschner, Bullock & Neisewander, 2002; Arnt, Hyttel & Sanchez, 1992; Chausmer & Katz, 2002; Gendreau, Gariepy, Petitto & Lewis, 1997; Heijtz, Beraki, Scott, Aperia & Forssberg, 2002; Reavill, Bond, Overend & Hunter, 1993). Heijtz et al. (2002), utilizing a range of doses (0.3, 3.0 and 10 mg/kg), showed the biphasic effect SKF has on motor activity in the rat. SKF appeared to have an initial short inhibition on activity that was then followed by a longer-lasting increase in activity. The inhibitory period did not appear to involve an increase in stereotypy. SKF has also been found to increase grooming behavior in the rodent. In addition to the immediate motor effects of SKF, the D1 agonist also produces immediate autonomic and physiological arousal effects similar to those of cocaine and amphetamine (Chausmer & Katz, 2002). For example, Reavill et al. (1993) found that SKF could replace cocaine and amphetamine in drug

discriminiation studies. Rosenzweig-Lipson and Bergman (1993) found that SKF acted as a partial substitute for cocaine in rats.

D1 receptors are primarily localized post-synaptically, especially in the striatum, nucleus accumbens, olfactory tubercle and the cerebral cortex (Sibley et al., 1993). Additionally, these areas have been found to be stimulated with SKF administration (Reavill et al., 1993; Rosenzweig-Lipson et al., 1993). Heijtz et al. (2002) suggested that the stimulatory effects of SKF are mediated via the striatum, whereas the inhibitory effects are mediated via the medial prefrontal cortex.

SKF administration could produce longer term activity patterns similar to those seen following amphetamine administration. The areas in which D1 receptors are located and that SKF stimulates are involved in the mediation of motor activity. Consistent with a within system adaptation, the same D1 receptors stimulated by amphetamine in the short term could be involved in the longer term expression of hypo-activity. Therefore, SKF could produce hypo-activity 20 hr after administration.

*D2-like agonist: Quinpirole.* Quinpirole is a dopamine D2-like receptor agonist. Andersen and Jansen (1990) showed that Quinpirole has a high affinity for both D2 and D3 receptors, with a greater affinity for D3 receptors. However, Quinpirole is regularly used as a D2-like receptor agonist.

A number of studies have shown the immediate effects of Quinpirole on activity in rats (Eilam, Golani & Szechtman, 1989; Eilam & Szechtman, 1989; Van Hartesveldt, 1997). Eilam and Szechtman (1989) showed the biphasic locomotor

effects of Quinpirole by administering a range of doses to rats. The researchers found that moderate to high doses (0.5 – 8 mg/kg) produced an initial inhibition of activity followed by stimulation of activity that lasted for about 2 hr. Others such as Van Hartesveldt (1997) showed that Quinpirole was similar in locomotor-activating effect to apomorphine, a non-selective dopamine agonist. The study found that lower subcutaneous doses (0.0, 0.02 or 0.2 mg/kg) could also elicit a biphasic activity profile. However, Quinpirole administration did not produce stereotypy.

Quinpirole appears to produce activity via the striatum and the nucleus accumbens. Van Hartesveldt, Cottrell, Potter, and Meyer (1992) found that Quinpirole injected into the striatum but not into the nucleus accumbens produced the same locomotor effects as a systemic administration. Others such as Mogenson and Wu (1991) found that injection of Quinpirole into the nucleus accumbens reduced amphetamine-elicited locomotion. Mogenson and Wu (1991) and Furmidge, Tong, Petry, and Clark (1991) suggested that the initial inhibitory effects of Quinpirole may be mediated by DA receptors in the nucleus accumbens, whereas the secondary stimulatory effects may be due to the striatum. These areas are well known to be involved in locomotion.

Quinpirole administration could produce longer-term activity patterns similar to those seen following amphetamine administration. D2 receptor stimulation produces many of the same behavioral effects as non-selective dopamine agonists, though not as robustly. Consistent with a within system adaptation, the same D2 receptors stimulated by amphetamine in the short term could be involved in the

longer term expression of hypo-activity. Therefore, Quinpirole could produce hypo-activity 20 hr after administration.

## *Method*

### *Subjects*

The subjects were 64 male rats of the Wistar strain, purchased from Harlan (Indianapolis, IN). Prior to the start of the study all rats were housed in pairs in the departmental colony in a temperature of 20°C – 22°C and on a 12-hr light/ 12-hr dark cycle. Rats had free access to food (5001 Rodent Diet, Lab Diet) and water, and weighed between 300 and 400 g at the start of the experiment. Animals were treated in accordance with ethical guidelines established by the National Institutes of Mental Health and approved by Morehead State University IACUC.

### *Apparatus*

Sixteen stations were used (see Figure 1). The 16 stations were located in two well-isolated rooms (each approximately 1.8 m X 2.1 m X 2.6 m high). Within each room there were eight sound attenuating, wooden compartments (58 cm X 42 cm X 71 cm high, with a shelf 58 cm from the bottom). The interior of each compartment was white and each compartment had a fan (Sunon, sf11580A) mounted in the upper portion of one side wall for ventilation and to mask out sound. A light fixture (Lampi-Pico accent light, 4-W) was mounted in the middle of the back wall. The lights were used to produce the same 12-hr light/ 12-hr dark cycle in all stations.

In each compartment was a plastic cubicle (40 cm X 20 cm). The floor of the cubicle was a black metal pan, which contained a thin layer of micro-waved topsoil to

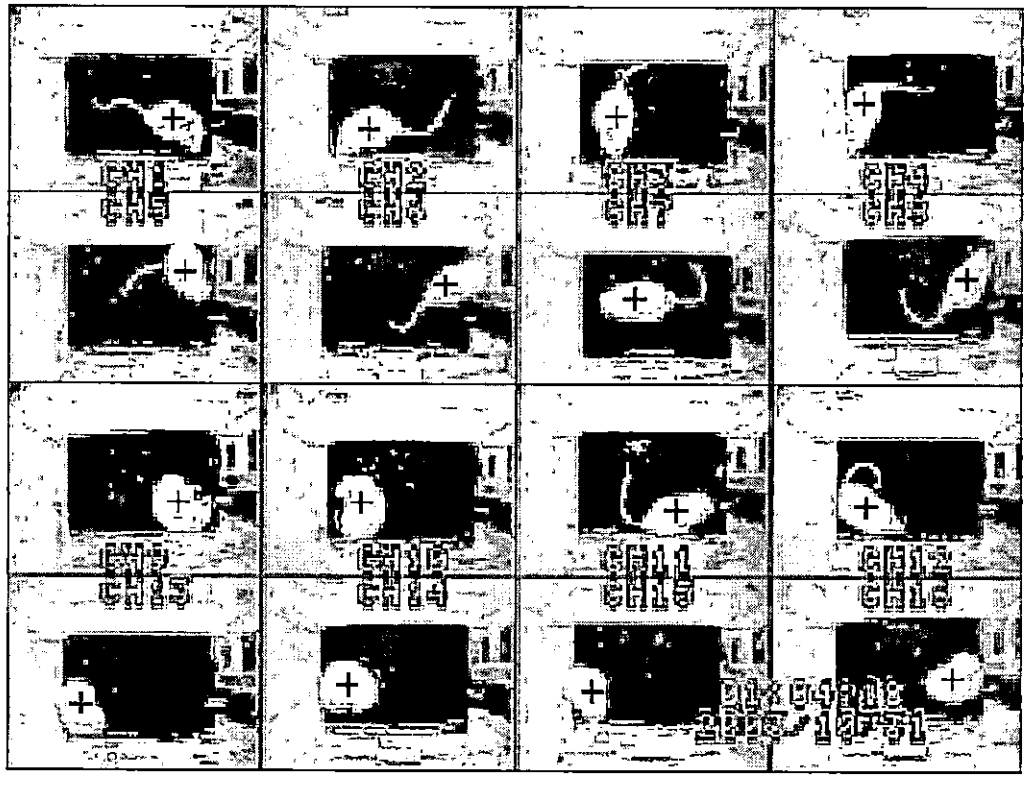

*Figure 1.* Picture of 16 stations used to house and record activity of rats. Plus symbols indicate software tracking the activity of rats.

provide absorbency and minimize reflections. The right wall had two vertical masonite panels, one of which held a drinking tube (8 cm from the front of the cubicle and 6 cm from the floor) attached to a 250 ml bottle of water. The other panel contained an opening (15 cm from the front and 6 cm from the floor) to a feeding bin, which held 200 g of powdered meal (Lab Diet, Rodent Diet 5001). In the ceiling of the compartment, and centered 50 cm above the floor of each cubicle, was a monochrome infrared camera (Super Circuits #DC 12-500R). The lens of the camera was surrounded with IR-emitting diodes to monitor locomotor activity in the dark. A paper disk encircled the lens so that direct light could not shine on the floor below.

The cameras were connected by cables to a monochrome multiplexer (Robot-Duplex Digital Video Multiplexer, DMV16Q), which combined images from the cameras in each of the 16 stations into one image for recording and quantification. The multiplexer was connected to a video recorder (JVC, SR-V10U), a monitor (14" Trinitron high resolution video monitor, ECM-1402H), and a computer. The computer (Dell, M782p) contained a piccolo frame grabber. An EthoVision Pro 3.0 Video Tracking, Motion Analysis and Behavior Recognition System collected and analyzed the data. Each animal was tracked within an area corresponding to the dimensions of the floor. The system was programmed to take two samples per second. The gray scaling method was used in object detection. Control equipment was located in an adjacent room.

*Drugs*

SKF 81297 (0.4 mg/ml and 0.2 mg/ml) (Sigma) was mixed in distilled water. Quinpirole (0.4 mg/ml and 0.2 mg/ml) (Sigma) was mixed in saline. Amphetamine (2.0 mg/ml) (Sigma) was mixed in saline. All drugs were injected subcutaneously in the back of the neck in a volume of 1.0 ml/kg. Saline was the control treatment.

*Procedure*

The experiment included 6 treatment groups: saline ($N$=10), amphetamine ($N$=12), 0.2 Quinpirole ($N$=12), 0.4 Quinpirole ($N$=12), 0.2 SKF ($N$=8), and 0.4 SKF ($N$=8). The first 4 groups were run in one series of studies, and the last 2 groups were run in a separate series. Throughout each study rats were continuously housed in one of the stations in a 12-hr light/ 12-hr dark cycle and allowed free access to food and water.

Figure 2 displays a schematic diagram of the basic procedure. Up to 2 habituation ("Hab") cycles were run. No treatments were given during habituation cycles. Rats then received a series of control treatments followed by a series of drug treatments. All treatments occurred at the start of the light cycle. During the first control treatment, each rat was removed in turn from its station, weighed and vigorously rubbed on the back of the neck. During subsequent control treatments ("Saline"), animals also received a subcutaneous injection of saline (0.5 ml) into the nape of the neck. Control treatments were separated by 48 hr. Control treatments were given until patterns of activity across groups were similar for 2 consecutive control-treatment cycles. While a rat was being treated, its apparatus was maintained.

| | | L/D cycle | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Cycle | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| 1 | Hab1 | I/H | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | M | | | | | | | | | | | | | |
| 3 | Hab2 | H/M | | | | | | | | | | | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | M | | | | | | | | | | | | | |
| 5 | Saline1 | T/M | | | | | | | | | | | | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | M | | | | | | | | | | | | | |
| 7 | Saline2 | T/M | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | M | | | | | | | | | | | | | |
| 9 | Drug1 | T/M | | | | | | | | | | | | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | M | | | | | | | | | | | | | |
| 11 | | | | | | | | | | | | | | | | | | | | | | | | | |
| 12 | Drug2 | T/M | | | | | | | | | | | | | | | | | | | | | | | |
| 13 | | | | | | | | | | | | O | | | | | | | | | | | | | |

I In apparatus
H Habituation
T Treatment
M Maintenance
O Out of apparatus
System recording
Animals handled

*Figure 2.* Diagram of procedure used in Experiment 1 showing habituation (Hab), control (Saline), and treatment (Drug) cycles. Lights-on/ lights-off indicated on top by light and dark boxes. Each row is a day.

Maintenance of the apparatus consisted of wiping down the pan, adding new top soil, and replenishing food and water. Treatment and maintenance took approximately 2 min per station.

Following control treatment cycles, 2 drug treatments cycles were run ("Drug"). Drug treatment cycles were similar to control treatment cycles except that different groups of rats received different drug treatments. Drug treatments included saline, 2.0 mg/kg amphetamine, 0.2 mg/kg Quinpirole, 0.4 mg/kg Quinpirole, 0.2 mg/kg SKF 81297, or 0.4 mg/kg SKF 81297. All drugs were injected subcutaneously into the back of the neck in a volume of 1.0 ml/kg. At least 72 hr elapsed between drug treatments to allow drug to be cleared from the body. Throughout the study, monitoring began at the same time in all stations -- at lights on and shortly before treatments began. Activity of animals was monitored continuously for 33 hr. Additional station and animal maintenance occurred between treatment cycles, when animals were not being monitored.

*Data Analysis*

Tracking software was used to quantify total distance moved for each rat into 10-min bins. Though monitoring began at lights on, for each rat activity was quantified beginning with the 10-min bin following treatment. Data were then combined into 33 1-hr bins for each rat. All hourly activity totals were expressed as a percentage of each rat's mean control value. To obtain this mean for each rat, hours 2-25 were averaged across the last 2 control cycles. Hourly percent control values were averaged for each rat across these 2 control cycles and across the 2 drug

treatment cycles. Activity was also averaged into 3-hr bins for each rat. Activity was then averaged across subjects in a treatment group.

Changes in immediate activity (hours 1-6 post-treatment) and longer-term activity (hours 7-33 post-treatment averaged into 3-hr bins) were analyzed with an analysis of variance (ANOVA). One-way between and within ANOVAs were performed on significant main effects. All significant results were followed-up with Fisher's PLSD post hoc analysis or paired *t*-tests.

*Results*

*Immediate results.* Figure 3 shows mean activity (as a percentage of control) for the first 6 hr post-treatment for all 6 groups during the control cycles. Activity appeared to be elevated for the first hour after treatment and then to decline to normal levels for hours 2-6 post-treatment. A six (drug treatment groups) by six (post-treatment hours) mixed two-way analysis of variance (ANOVA) was conducted. No significant effect of group or significant group by hour interaction was obtained, $F(5, 56) = 0.717$, $p > .05$ and $F(25, 280) = 0.80$, $p > .05$. A significant effect of hour was obtained, $F(5, 280) = 116.206$, $p < .0001$. Fisher's PLSD post hoc analysis indicated that overall activity was significantly higher during the first hour post-treatment than during all other hours ($p < .0001$). No other differences were found.

Figure 4 shows mean activity (as a percentage of control) for the first 6 hr post-treatment for all 6 groups during the treatment cycles. The amphetamine group appeared to be elevated in activity through hour 5. All other treatment groups showed a decline to normal activity levels after the first or second hour. Again, a six (drug

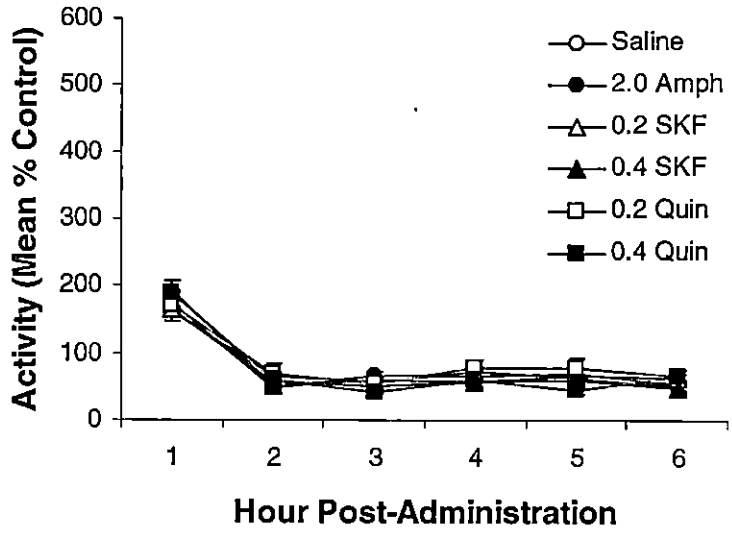


*Figure 3.* Mean activity during hr 1-6 post-administration for saline, amphetamine, 0.2 mg SKF, 0.4 mg SKF, 0.2 mg Quinpirole, and 0.4 mg Quinpirole groups during control cycles. ± *SEM* bars are shown.

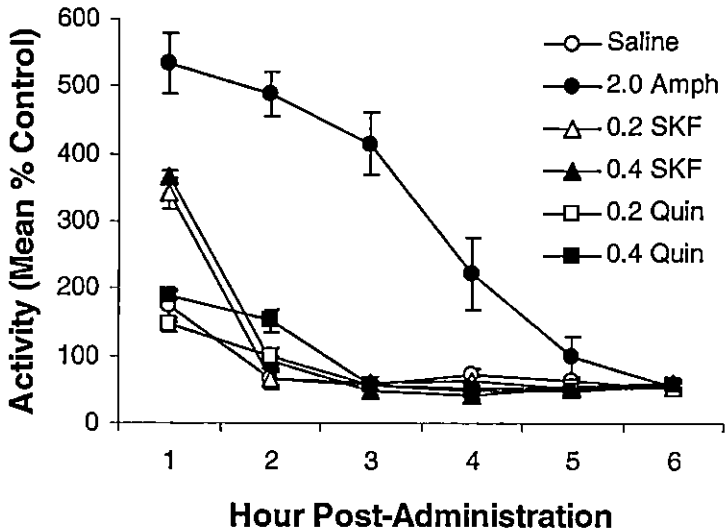


*Figure 4.* Mean activity for hr 1-6 post-administration for saline, amphetamine, 0.2 mg SKF, 0.4 mg SKF, 0.2 mg Quinpirole and 0.4 mg Quinpirole groups during drug cycles. ± *SEM* bars are shown.

treatment groups) by six (post-treatment hours) mixed two-way ANOVA was conducted. Significant effects of group and hour were obtained, as well as a significant interaction, $F(5, 56) = 98.373$, $p < .0001$, $F(5, 280) = 114.310$, $p < .0001$ and $F(25, 280) = 16.525$, $p < .0001$. Additional analyses were conducted to compare the activity of groups at each hour and the activity within each group across hours.

First, one-way ANOVAs were conducted between groups at each hour and additional Fisher's post hoc analyses were conducted to examine significant effects. Groups differed during the first hour post treatment, $F(5, 56) = 38.698$, $p < .0001$. The amphetamine group was greater in activity than all other groups, *ps* < .0001, and both the low and the high dose SKF groups were greater in activity than the Quinpirole and saline groups, *ps* < .0005. Groups also differed in the second hour post-treatment $F(5, 56) = 77.181$, $p < .0001$. The amphetamine group was again greater in activity than all other groups, *ps* < .0001. The high dose Quinpirole group (0.4 mg/kg) was significantly greater in activity than the low dose Quinpirole group, the SKF groups and the saline group, *ps* < .05. Groups also differed during hours 3 and 4, $F(5, 56) = 45.696$ and 7.845, *ps* = < .0001. During these hours the activity of only the amphetamine group was elevated, *ps* < .0001. By hour 6 post-treatment there were no group differences in activity, *ps* > .05.

Second, separate one-way within group ANOVAs were conducted for each treatment group. All groups showed a significant decrease in activity across hours, $F(5, 45) = 18.628$, $F(5, 55) = 28.125$, $F(5, 35) = 111.652$, $F(5, 35) = 547.702$, $F(5, 55) = 20.521$ and $F(5, 55) = 60.419$, all *ps* < .0001, for saline, amphetamine, 0.2 SKF,

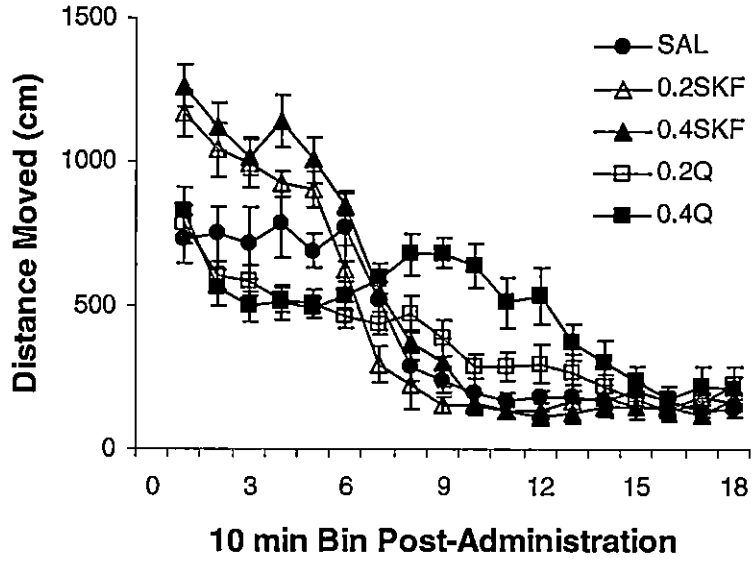


*Figure 5.* Mean distance moved in 10-min bins during first 3 hr post-administration for saline, 0.2 mg SKF, 0.4 mg SKF, 0.2 mg Quinpirole, and 0.4 mg Quinpirole groups during drug cycles. ± *SEM* bars are shown.

0.4 SKF, 0.2 Quinpirole and 0.4 Quinpirole groups, respectively. For the amphetamine group, activity declined from hours 1 to 3, 3 to 4 and 4 to 5, *ps* < .0001. For the 0.2 SKF group, activity declined from hours 1 to 2, $p < .0001$. For the 0.4 SKF group, activity declined from hours 1 to 2 and 2 to 3, *ps* < .0001. For the Quinpirole groups, activity declined from hours 1 to 2 and 2 to 3, *ps* < .005 and .0001. Finally, for the saline group, activity declined from hours 1 to 2, $p < .0001$.

Figure 5 shows activity (as centimeters moved) in 10-min bins for the first 3 hr post-treatment. The figure shows activity for the 0.2 mg/kg and 0.4 mg/kg SKF, 0.2 mg/kg and 0.4 mg/kg Quinpirole and saline groups. The function for the 0.4 Quinpirole group was biphasic, whereas the functions for the other groups were monotonically decreasing.

*Longer-term effects.* Figure 6 shows mean activity (as a percentage of control) averaged across 3-hr bins for hours 7-33 post-treatment for all 6 groups during the control cycles. Activity appeared to be lower during the light-period bins than during the dark-period bins and appeared to be particularly elevated during the last dark-period bin. A six (drug treatment groups) by nine (post-treatment hour bins) mixed two-way ANOVA was conducted. No significant effect of group or significant group by hour interaction was obtained, $F(5, 56) = 0.725$, $p > .05$ and $F(40, 448) = 1.367$, $p > .05$. A significant effect of hour was obtained, $F(8, 448) = 400.552$, $p < .0001$. Fisher's PLSD post hoc analysis indicated that overall activity was significantly higher during hours 22-24 than at other times and was significantly higher during the

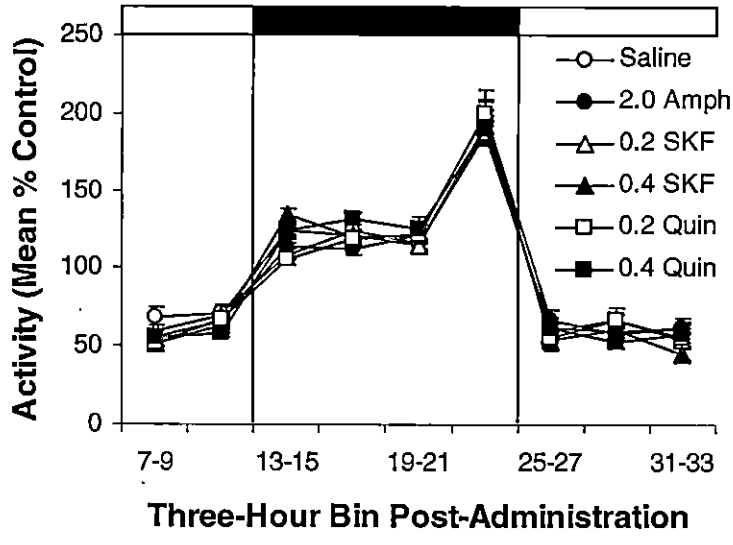


*Figure 6.* Mean activity during hr 7-33 post-administration averaged into 3-hr bins for saline, amphetamine, 0.2 mg SKF, 0.4 mg SKF, 0.2 mg Quinpirole and 0.4 mg Quinpirole groups during control cycles. Light/ dark bars across top indicate lights-on/ lights-off. ± *SEM* bars are shown.

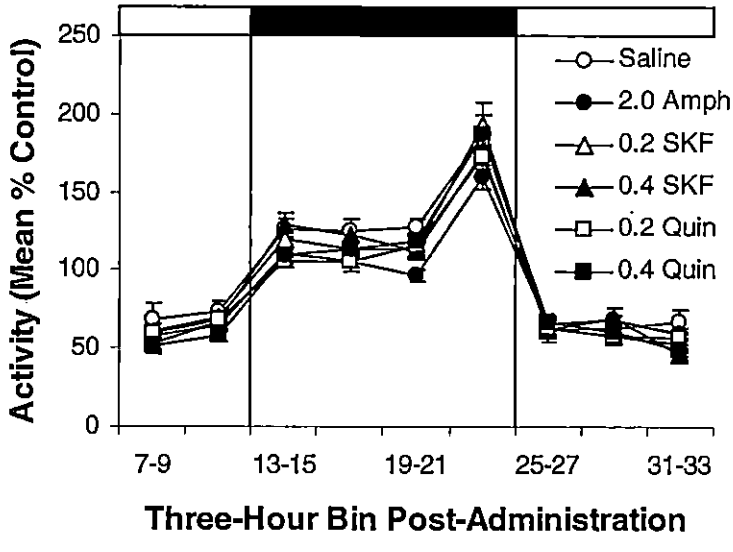


*Figure 7.* Mean activity during hr 7-33 post-administration averaged into 3-hr bins for saline, amphetamine, 0.2 mg SKF, 0.4 mg SKF, 0.2 mg Quinpirole and 0.4 mg Quinpirole groups during drug cycles. Light/ dark bars across top indicate lights-on/ lights-off. ± *SEM* bars are shown.

dark-period bins (13-15, 16-18, 19-21 and 22-24 hr bins) compared to the light-period bins, *ps* < .0001.

Figure 7 shows mean activity (as a percentage of control) averaged across 3-hr bins for all 6 groups during the treatment cycles. Again, activity appeared to be lower during the light-period bins than during the dark-period bins and appeared to be elevated during the last dark-period bin. However particular groups appeared to differ during the dark period. Specifically, the activity of the amphetamine group appeared to decline from the 13-15 hr bin to the 19-21 hr bin. This apparent decline in activity was not seen in the other groups. Another six (drug treatment groups) by nine (post-treatment hour bins) mixed two-way ANOVA was conducted. No significant effect of group was obtained, $F(5, 56) = 2.230$, $p > .05$. A significant effect of hour and a significant group by hour interaction were obtained, $F(8, 448) = 313.917$, $p < .0001$ and $F(40, 448) = 1.518$, $p < .05$.

One-way ANOVAs were conducted between groups at each of the 3-hr dark-period bins, and additional Fisher's post hoc analyses were conducted to examine significant effects. Group differences were obtained for the 13-15 hr bin, $F(5, 6) = 3.233$, $p < .05$. The amphetamine and two Quinpirole groups displayed less activity than the saline group, *ps* < .05. Groups did not differ in activity during the 16-18 hr bin, $F(5, 56) = 2.231$, $p > .05$. However, differences in activity were obtained between groups during the critical 19-21 hr bin, $F(5, 56) = 3.392$, $p < .01$. The activity of the amphetamine group was found to be significantly less compared to saline, 0.2 SKF, 0.2 Quinpirole and 0.4 Quinpirole groups during the 19-21 hr bin, *ps*

< .05, and trends were obtained for the 0.4 SKF group, $p = 0.06$. No other differences between groups were obtained. By the 22-24 hr bin, no differences between groups were obtained, $F(5, 56) = 1.179$, $p > .05$.

Additional, separate one-way within group ANOVAs were conducted for each treatment group across time. Significant differences were obtained for all groups across time, $F(8, 72) = 35.836$, $p < .0001$, $F(8, 88) = 66.06$, $p < .0001$, $F(8, 56) = 57.443$, $p < .0001$, $F(8, 56) = 43.177$, $p < .0001$, $F(8, 88) = 89.887$, $p < .0001$ and $F(8,88) = 57.519$, $p < .0001$, for saline, amphetamine, 0.2 SKF, 0.4 SKF, 0.2 Quinpirole and 0.4 Quinpirole groups, respectively. For the amphetamine group, activity declined from the 13-15 hr bin to the 19-21 hr bin. The SKF, Quinpirole and saline groups did not display significant differences in levels of activity across the 13-15, 16-18 and 19-21 hr bins, *ps* > .05. Activity for all groups was less during the 13-15, 16-18 and 19-21 hr bins than during the 22-24 hr bin, *ps* < .0001. To summarize, the main difference compared to the control condition was that the amphetamine treated group first showed a decrease in dark period activity through hours 19-21 and then showed a normalization of activity afterwards.

*Results summary.* Figure 8 shows mean activity (as a percentage of control) for the 19-21 hr bin post-treatment for all 6 groups during the control (left) and treatment (right) cycles. The amphetamine group alone appeared to decrease in activity during the 19-21 hr bin from the control cycles to the treatment cycles. A six (treatment groups) by two (treatment conditions) mixed two-way ANOVA was conducted. No significant effect of group was obtained, $F(5, 56) = 1.557$, $p > .05$. A

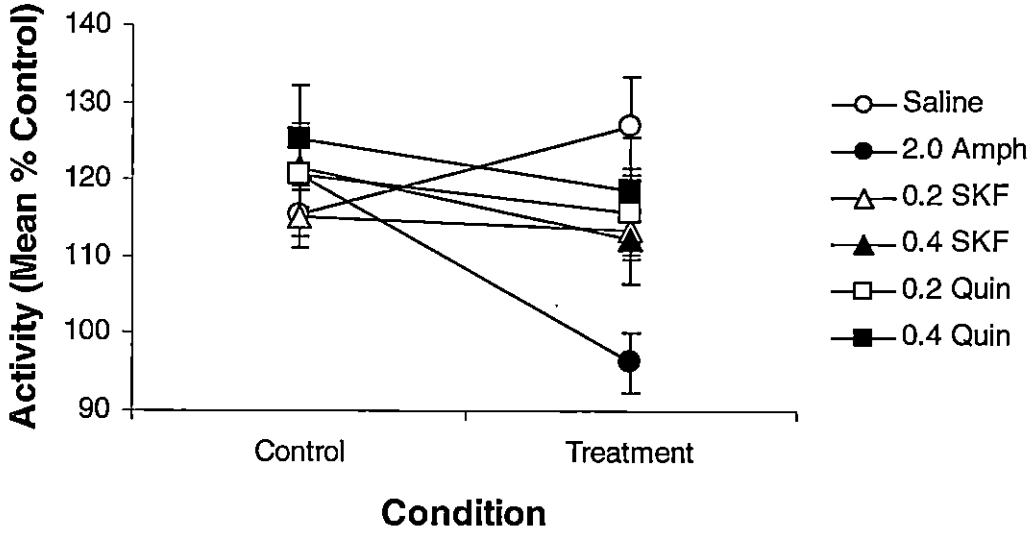


*Figure 8.* Mean activity during hr 19-21 post-treatment of control and treatment cycles for saline, amphetamine, 0.2 mg SKF, 0.4 mg SKF, 0.2 mg Quinpirole and 0.4 mg Quinpirole groups. ± *SEM* bars are shown.

significant effect of treatment and a significant group by treatment interaction were obtained, $F(1, 56) = 4.427$, $p < .05$ and $F(5, 56) = 3.087$, $p < .05$.

To investigate the basis of the interaction, a paired *t*-test was conducted on the data for each treatment group. Only the amphetamine group showed a significant decline in activity from the control to the drug cycles, $t(11) = 6.547$, $p < .0001$. No other group differences were obtained, all *ps* >.05.

### *Conclusions and Discussion*

The purpose of Experiment 1 was to investigate whether stimulation of dopamine receptor subtypes had the ability to produce hypo-activity 20 hr later. SKF 81297, a selective D1 receptor agonist, Quinpirole, a selective D2 receptor agonist, amphetamine, or saline were administered to rats, and locomotor activity was monitored for the next 33 hr. If a selective receptor agonist were to produce hypo-activity similar to that produced by amphetamine, then amphetamine might produce the hypo-activity via that receptor subtype.

Groups did not differ following control treatments. Consequently, the functions provided a good baseline with which to assess the capacity of the different drugs to produce significant time- or dose-dependent effects. Drug treatments did produce differences in activity. During the first few hours post-treatment, the 0.2 and 0.4 SKF, the 0.2 and 0.4 Quinpirole, and the 2.0 mg/kg amphetamine groups produced very different profiles of immediate period activity. However, the activity profiles for all drugs were typical for these particular doses. During the longer-term,

different groups again produced differences in activity patterns. Only the amphetamine group showed hypo-activity 20 hr post-treatment. Similar to White and White (in press) our 2.0 mg/kg amphetamine treatment produced a reduction in activity around hour 20, as well as a recovery in activity shortly afterwards in Wistar rats. The SKF and Quinpirole treatments did not produce hypo-activity at this time: Instead these treatments produced a level of activity during the 19-21 hr bin that was comparable to that produced by saline treatment.

The inability of the selective agonists to produce an activity profile similar to that produced by amphetamine suggests that amphetamine-induced hypo-activity is not produced by initial stimulation of either the D1 or the D2 receptor subtype alone. Thus, our hypotheses were not confirmed.

Certain differences in the effects produced by amphetamine and the selective dopamine agonists probably did not account for the failure of the agonists to produce hypo-activity. Amphetamine produced hyperactivity in the short-term, but neither SKF nor Quinpirole did. Conceivably, the initial hyperactivity may have produced fatigue and later hypo-activity. Neither selective agonist produced hyperactivity, so there was no fatigue and hypo-activity.

However, some results are inconsistent with this interpretation. 1.0 mg amphetamine produces a greater amount of short term locomotor activity than a 2.0 mg dose in Wistar rats. However, a 1.0 mg dose does not produce hypo-activity 20 hr later, whereas a 2.0 mg dose does (White & White, in press). Therefore, amphetamine-induced hypo-activity does not appear to be due to short term

hyperactivity that produces a delayed fatigue effect. Similarly, the inability of the selective agonists to produce short term hyperactivity may not account for their inability to produce hypo-activity.

Amphetamine and the selective dopamine receptor agonists probably differ in half life. Some symptoms of amphetamine-induced acute withdrawal might be seen when amphetamine levels fall to a critical level. For example, low doses of dopamine agonists activate ventral tegmental area (VTA) auto-receptors and produce catalepsy, daytime sleepiness, and REM sleep (Fletcher & Starr, 1988; Wanibuchi & Usuda, 1990). Perhaps the selective agonists did not produce hypo-activity near hour 20 because they fell to the critical level with a different time course.

However, a couple of observations are inconsistent with the possibility that the timing of hypo-activity is related in some simple way to drug half-life. First, the half-life of amphetamine in rats is about 2 hours, depending on state factors: Therefore, essentially no drug is present after 20 hr. Second, the timing of hypo-activity seems to be dose independent over a wide rage of doses, from 2.0 mg to 10 mg amphetamine (White & White, 2004). These observations may be more consistent with a model of amphetamine induced hypo-activity involving short term saturation of some mechanism. Similarly, the inability of the dopamine agonists to produce hypo-activity may not have been due to their differences in half-life.

Amphetamine and the selective agonists may also have differed in receptor activation. SKF and Quinpirole may not have produced hypo-activity, because they did not produce the same level of short term receptor stimulation that the dose of

amphetamine did. Ideally I would have wanted to use doses of SKF and Quinpirole that were comparable to amphetamine in D1 or D2 activation. Bringing about this comparability would be difficult to do, primarily because the modes of action of these three drugs are very different. Amphetamine is an indirect agonist of dopamine, whereas SKF and Quinpirole are direct agonists of D1 and D2 receptors, respectively. The 0.4 doses of both agonists that I used are considered moderately high. By using this dose I hoped to produce sufficient receptor activation. In a pilot study involving the same animals, we administered 0.8 mg/kg Quinpirole and again did not observe hypo-activity 20 hours post-administration. Future studies could use a higher dose range more systematically.

Finally, selective agonists may not have produced hypo-activity around hour 20 because the cascade of events leading to hypo-activity may be activated by an initial D1 and D2 receptor interaction. Dopamine D1 and D2 receptors need to be co-stimulated in order to produce a wide range of dopamine related behaviors, including locomotion.

### Experiment 2: Combined Dopamine Receptor Activation

Hypo-activity 20 hr after amphetamine administration is dependent upon some sort of initial effect. In Experiment 1, initial stimulation of either D1 or D2 receptors alone did not produce this hypo-activity. However, in a prior study, administration of the non-selective dopamine receptor agonist apomorphine did (White et al., 2002). The apomorphine result, combined with the results of

Experiment 1, indicated that initial stimulation of both D1 and D2 receptors may be necessary to produce hypo-activity. Many immediate dopaminergic effects depend upon an interaction between D1 and D2 receptors, and these immediate effects could produce cascades resulting in longer term effects. Therefore, in Experiment 2, I tried to determine whether short-term D1 and D2 receptor co-stimulation could produce hypo-activity 20 hr later.

To justify this approach, in this section I will review D1 and D2 receptor interaction. First, to indicate when an interaction is absent, I will illustrate the concept of double dissociation and of an additive relation between treatments. Next, I will explain when an interaction is indicated -- an interaction involves a violation of double dissociation -- and then I will review the two kinds of synergistic interactions. Because my dependent measure is activity, I will then outline the evidence that activity involves an interaction of D1 and D2 receptors and then describe possible mechanisms that might be involved. Next, I will review evidence that other behaviors also depend on a D1 and D2 receptor interaction. Finally, I will describe the evidence that justifies the particular dose combinations that I chose for Experiment 2.

*Hypothesis.*

- Combined dopamine D1 and D2 receptor activation with agonists SKF 81297 and Quinpirole will, in a dose dependent manner, produce hypo-activity 20-hr post-treatment.

The ability of the selective agonists (SKF 81297 and Quinpirole) in combination to produce hypo-activity would support the hypothesis that short term stimulation by amphetamine of both D1 and D2 dopamine receptor subtypes is necessary to produce hypo-activity 20 hr later.

*Absence of interaction.* The double dissociation technique has been used to provide evidence that different mechanisms are responsible for different processes. In behavioral pharmacology, a double dissociation would be indicated if agonist and antagonist manipulations suggested that receptor subtypes made independent contributions to a particular behavior. The pattern of results in the following hypothetical example would be consistent with a double dissociation.

1. A control treatment produced a grooming behavior value of 0.
2. Treatment with a D1 agonist produced a grooming behavior value of 4.
3. Treatment with a D2 agonist produced a grooming behavior value of 2.
4. Combined treatment with the D1 and D2 agonists used above produced a value of 6: This result would be consistent with an additive relationship.
5. Combined treatment with the D1 agonist and a D2 antagonist produced a grooming value of 4, that is, the D2 antagonist did not alter the effect of the D1 agonist.
6. Combined treatment with the D2 agonist and a D1 antagonist produced a grooming value of 2, that is, the D1 antagonist did not alter the effect of the D2 agonist.

The pattern of results in this example suggests that D1 and D2 receptor subtypes make independent contributions to the same behavior (grooming).

*Interaction.* An interaction of receptor subtypes is indicated when agonist and antagonist manipulations suggest that the effect of one receptor subtype depends on the co-activation of another receptor subtype. Manipulations that produce exceptions to the double dissociation rule indicate an interaction. For example, an interaction is indicated when treatment effects are non-additive or when the effects of stimulating one receptor subtype are altered synergistically by administering antagonists of a different receptor subtype. Many dopamine-dependent behaviors, including various forms of activity, require an interaction of D1 and D1 receptors.

*Types of synergistic interactions.* A couple of different types of synergistic interactions have been defined. A synergistic interaction is present when the effect of giving receptor subtype agonists in combination is different than the sum of giving the receptor subtype agonists separately. A synergistic interaction can either be cooperative or oppositional in nature. A cooperative synergism is present when the combination produces an effect that is greater than the sum of the agonists given separately, whereas an oppositional synergism is present when the combination produces an effect that is smaller.

The following results illustrate the different synergistic interactions by quantifying stereotypy. A D1 agonist treatment produces a stereotypy value of 2. A D2 agonist treatment produces a stereotypy value of 6. If combined treatment with D1 and D2 agonists produces a value significantly greater than 8, such as 12, a

cooperative synergistic interaction has occurred. Alternatively, if combined treatment with D1 and D2 agonists produces a value significantly less than 8, such as 1, an oppositional synergistic interaction has occurred.

*Interaction and activity.* As mentioned, the expression of many dopamine mediated behaviors requires D1 and D2 receptor interaction. Various forms of activity are examples. In a comprehensive review, Waddington (1993) concluded that motor behavior depended upon D1 and D2 receptor interaction. Forms of activity including locomotion, sniffing, jaw movement, stereotypy and turning behavior show a cooperative synergism when D1 and D2 agonists are co-administered (Adachi, Ikeda, Hasegawa, Nakamura, Waddington & Koshikawa, 1999; Molloy & Waddington, 1985; Robertson & Robertson, 1986; Starr, 1988). Additionally, Koller and Herbster (1988) found that D1 agonists combined with D2 agonists elicited levels of grooming and sniffing that were similar to those elicited by non-selective dopamine agonists. Combined with the previous findings concerning amphetamine and apomorphine, these results suggest that longer-term hypo-activity depends on a short term interaction of D1 and D2 receptors.

*Interaction and mechanism.* Dopamine D1 and D2 receptors are found in many brain regions. Within some neural structures, D1 and D2 receptors are found together, while within other neural structures the receptor subtypes are found separately. Thus, two different hypotheses have been proposed regarding the manner in which the subtypes interact mechanistically--the co-localization hypothesis and the neuro-integrative hypothesis.

The co-localization hypothesis proposes that D1 and D2 receptors residing on the membrane of the same neuron interact to produce a particular behavior. Therefore interaction occurs directly via a unitary mechanism. Studies involving intracerebral injections into the striatum, nucleus accumbens, ventral striatum, and globus pallidus have indicated that co-localized D1 and D2 receptors may interact to affect various measures of activity (Waddington & Daly, 1993). Furthermore, electrophysiological research has shown that cell firing in the striatum, nucleus accumbens and globus pallidus may be due to D1/D2 interaction. Guanine nucleotide-binding proteins, levels of Na+/K+-ATPase activity and arachidonic acid release within the striatum and nucleus accumbens may also be due to D1/D2 interaction (reviewed in Waddington & Daly, 1993).

The neuro-integrative hypothesis proposes that D1 and D2 receptors that reside on distant neurons interact to produce a particular behavior. Therefore interaction occurs indirectly via collaterals or efferents of different neurons (Robertson & Robertson, 1987). In particular, D1 receptors in the globus pallidus and the substantia nigra pars reticulata (areas of the basal ganglia involved in motor output) may interact with D2 receptors in the striatum (although they never specify which particular nuclei of the striatum). Whether D1 and D2 interaction within the striatum is involved in the expression of a particular behavior may depend on the behavior. The neuro-integrative hypothesis, like the co-localization hypothesis, can account for regulation of adenylyl cyclase, as well as ascorbic acid release within the striatum (Waddington & Daly, 1993).

The co-localization and neuro-integrative hypotheses are still being debated.

*Interaction and other behaviors.* In addition to activity being affected by dopamine D1/D2 receptor interaction, other behaviors and processes also appear to depend upon an interaction. Dopamine is involved in motor behavior and in reward and learning processes, thus it is plausible that many behaviors and processes are influenced by the interaction of D1 and D2 receptors.

Dopamine receptor interaction modulates arousal and sleep (Ongini, 1993). D1 agonists induce EEG arousal and enhance wakefulness, whereas D1 antagonists induce sedation and sleep. Furthermore, D2 agonists induce arousal, but D2 antagonists have no effect on sleep. An interaction between D1 and D2 receptors may induce arousal and sedation, but REM sleep appears to be regulated by just D1 receptors.

Dopamine receptor interaction is involved in aspects of food intake (Cooper & Al-Naser, 1993). D1 receptor agonists do not appear to affect the rate of eating, but they do reduce the frequency of feeding. Alternatively, D2 receptor agonists appear to reduce the rate of eating. Moreover, the effects are synergistic when agonists are combined. An anorectic effect is elicited when D1 and D2 receptors are stimulated simultaneously. This potentiation in food reduction is found with both selective and nonselective, as well as with direct and indirect, dopamine agents. Furthermore, the effect also appears to be dose dependent.

On the other hand, drug discrimination does not appear to depend on dopamine receptor interaction (Nielsen, 1993). D1 agonists failed to substitute for a

D2 cue, and, similarly, D2 agonists failed to substitute for a D1 cue. Different direct and indirect mixed D1/D2 agonists could be discriminated (amphetamine, apomorphine, cocaine). The findings suggested that D1 and D2 receptors are non-interacting in the drug discrimination model. In other words, a D1 and D2 interaction is not necessary for drug discrimination.

The relation between D1/D2 interaction and behavior is complicated. Certain behaviors depend on simultaneous stimulation of receptor subtypes, whereas others do not. Furthermore, the effects appear to be dose dependent as well as dependent on the sensitivity of the behavioral measure.

*Interaction and dose issues.* The interaction of D1 and D2 receptors appears to depend upon certain threshold levels of activation for each receptor subtype. Braun and Chase (1986) suggested that concurrent stimulation of both D1 and D2 receptors is necessary for the expression of behaviors that are seen with non-selective agonists such as apomorphine and amphetamine. The study suggested that the nature or the quality of the behavioral response is a function of the ratio of D1 and D2 receptor stimulation. Furthermore, the response depends upon the level of the underlying D1 receptor tone. The authors suggest that "in order to investigate the dopaminergic contribution in a particular behavior, one must fix the dose of the D2 stimulation and use a range of D1 stimulation." Waddington and Daly (1993) also suggested that D1 receptor stimulation is needed in order to obtain expression of D2 mediated behaviors. The level of D1 tone appears to exert important qualitative and quantitative synergistic control of D2-stimulated motor activity. That is, increases and

decreases in D1 stimulation appear to enhance and attenuate the intensity of D2 agonist-induced behaviors such as stereotypy.

The concept that one receptor subtype gates or enables the function of the other appears in much of the D1/D2 interaction literature, although the reason for this segregation of receptor subtype function is still unclear. Behavioral and physiological explanations have been given for why a tonic dose is important in D1/D2 interaction. Waddington (1986, 1993) suggested that the D2 system influences the mode of expression for a behavior, whereas the D1 system influences the level of D2 activity. Thus, D1 receptor stimulation can enhance or block normal behaviors. Similarly, when a D2 agonist is given, a stereotyped mode of behavior is selected that can then be either enhanced or blocked by D1 agonists or antagonists. Waddington also suggested that the normal level of tonic D1 activity is high. Other researchers such as LaHoste and Marshall (1990) suggested that the effects of D1 tonic activation may be due to interactions with GABA. GABA activity is modulated by D1 receptors in the substantia nigra pars reticulata (SNr), which in turn influences motor activity. The only way for a D2 agonist to activate GABA is by first stimulating D1 receptors in the SNr. Therefore, the researchers suggested that GABA might regulate D1 tone and D1 behavioral influence.

Many dopamine mediated effects depend on threshold doses of agonists and an appropriate balance of receptor activation. The reason why a tonic dose of D1 activation is necessary for the expression of behavior is not understood. However, to increase the likelihood of using a combination that has these properties and to elicit

hypo-activity around hour 20, I used an increasing dose of SKF in combination with a fixed, moderately-high dose of Quinpirole.

## *Methods*

### *Subjects*

The subjects were 32 male rats of the Wistar strain, purchased from Harlan (Indianapolis, IN). As in Experiment 1, prior to the start of the study all rats were housed in pairs in the departmental colony in a temperature of 20°C – 22°C and on a 12-hr light/ 12-hr dark cycle. Rats had free access to food (5001 Rodent Diet, Lab Diet) and water and weighted between 300 and 400 g at the start of the experiment. Animals were treated in accordance with ethical guidelines established by the National Institutes of Mental Health.

### *Apparatus*

Eight of the sixteen stations used in Experiment 1 were utilized in Experiment 2. The stations were housed in one of the rooms from that experiment. The same control equipment was used.

### *Drugs*

0.2 mg/ml, 0.4 mg/ml or 0.8 mg/ml SKF 81297 (Sigma) was mixed with 0.4 mg/ml Quinpirole (Sigma) in saline. All drugs were injected subcutaneously in the back of the neck in a volume of 1.0 ml/kg. Saline was used as the control treatment.

| | | | L/D cycle | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Group | Cycle | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| 1 | 1 | Saline1 | | | | | | | | | | | I | | T | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | | | | | | | | | | | | O | M |
| 3 | 2 | Saline1 | | | | | | | | | | | I | | T | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | | | | | | | | | | | | | O | M |
| 5 | 1 | Saline2 | | | | | | | | | | | I | | T | | | | | | | | | | | |
| 6 | | | | | | | | | | | | | | | | | | | | | | | | | O | M |
| 7 | 2 | Saline2 | | | | | | | | | | | I | | T | | | | | | | | | | | |
| 8 | | | | | | | | | | | | | | | | | | | | | | | | | O | M |
| 9 | 1 | Drug1 | | | | | | | | | | | I | | T | | | | | | | | | | | |
| 10 | | | | | | | | | | | | | | | | | | | | | | | | | O | M |
| 11 | 2 | Drug1 | | | | | | | | | | | I | | T | | | | | | | | | | | |
| 12 | | | | | | | | | | | | | | | | | | | | | | | | | O | M |
| 13 | 1 | Drug 2 | | | | | | | | | | | I | | T | | | | | | | | | | | |
| 14 | | | | | | | | | | | | | | | | | | | | | | | | | O | M |
| 15 | 2 | Drug2 | | | | | | | | | | | I | | T | | | | | | | | | | | |
| 16 | | | | | | | | | | | | | | | | | | | | | | | | | O | M |

I In apparatus
T Treatment
O Out of apparatus
M Maintenance
System recording
Animals handled

*Figure 9.* Diagram of procedure used in Experiment 2 showing habituation (Hab), control (Saline), and treatment (Drug) cycles for the 2 groups. Light/ dark hr shown across top with days going down.

*Procedure*

Figure 9 displays a schematic diagram of the basic procedure. Once a day for 3 days prior to the start of each study animals were handled, weighed and vigorously rubbed on the nape of the neck for several seconds. Each study involved 2 groups run in the stations during alternating administration cycles. During a typical administration cycle animals in the first group were taken out of the colony and placed in individual stations 2 hr prior to lights on. At lights on, each animal was briefly removed from the station and treated. Treatment involved subcutaneous injection into the nape of the neck. After administration, the rat was placed back into the apparatus where activity could be monitored for the next 33 hr. After 33 hr animals were removed from the stations and placed back into the departmental colony. At this time, maintenance of the apparatus occurred. Maintenance consisted of wiping down each pan, adding new top soil, and replenishing food and water. The second group was then exposed to the same conditions beginning the next day. All rats were housed on the same 12-hr light/ 12-hr dark cycle either in the departmental colony on non-testing days or in the apparatus on testing days. Throughout the experiment, animals had free access to food and water.

Each group received 2 administration cycles involving control treatments followed by 2 administration cycles involving drug treatments. Consequently, the 2 groups were alternated in the stations for a total of 4 administration cycles each. Treatments for a particular group were always separated by at least 96 hr. During the control treatment cycles all animals were treated with 0.5 ml saline. During the drug

treatment cycles animals were treated with 1.0 ml/kg of different combinations of drug. For each group the dose of Quinpirole was fixed, but across groups the dose of SKF 81297 with which Quinpirole was combined varied. The 4 treatment groups were 0.0 SKF/ 0.4 Quinpirole, 0.2 SKF/ 0.4 Quinpirole, 0.4 SKF/0.4 Quinpirole, and 0.8 SKF/ 0.4 Quinpirole.

*Data Analysis*

The tracking software was again used to quantify total distance moved for each rat into 10-min bins. For each rat activity was quantified beginning with the 10-min bin following treatment. Again, data were then combined into 33 1-hr bins for each rat. Similar to Experiment 1, all hourly activity totals were expressed as a percentage of each rat's mean control value. Hourly percent control values were then averaged for each rat across the 2 control cycles and the 2 treatment cycles. Activity was also averaged into 3-hr bins for each rat. Activity was then averaged across subjects in a treatment group.

Changes in immediate activity (hours 1-6 post-treatment) and longer-term activity (hours 7-33 post-treatment averaged into 3-hr bins) were analyzed with within-subjects analysis of variances (ANOVAs). One-way within subjects ANOVAs were performed on significant main effects. All significant results were followed-up with Fisher's PLSD post hoc analyses or paired *t*-tests.

*Results*

*Immediate effects.* Figure 10 shows mean activity (as a percentage of control) for the first 6 hr post-treatment for 0.0 SKF/ 0.4 Quinpirole and 0.2 SKF/ 0.4 Quinpirole combination groups during control and drug cycles. Figure 11 shows similar data for 0.4 SKF/ 0.4 Quinpirole and 0.8 SKF/ 0.4 Quinpirole groups. In order to evaluate how activity changed from control to drug cycles, a series of analyses were done on the data for each group. First, to assess overall effects, a two (treatment condition [control or drug]) by six (post-treatment hour) within subjects two-way ANOVA was performed ("overall analysis"). Next, to investigate changes in activity across post-treatment hours, two separate one-way within subjects ANOVAs were done on control and treatment data ("time analysis"). Fisher's PLSD post hoc analysis was used to examine significant effects. Finally, to compare activity during control and drug cycles at each hour post-treatment, six separate within subjects *t*-tests were carried out ("condition analysis").

*0.0 SKF/ 0.4 Quinpirole group.* In the overall analysis no significant effect of condition was obtained, $F(1, 7) = 1.873$, $p > .05$. A significant effect of hour and a significant condition by hour interaction were obtained, $Fs(5, 35) = 15.874$ and 4.527, $ps < .0001$ and .01. The time analyses showed that, for both control and drug cycles, activity changed significantly over time, $F(5, 35) = 23.638$, $p < .0001$ and $F(5, 35) = 8.446$, $p < .0001$. Activity during control cycles was elevated during the first hour compared to all other hours post-treatment, $ps < .0001$. Activity during drug cycles

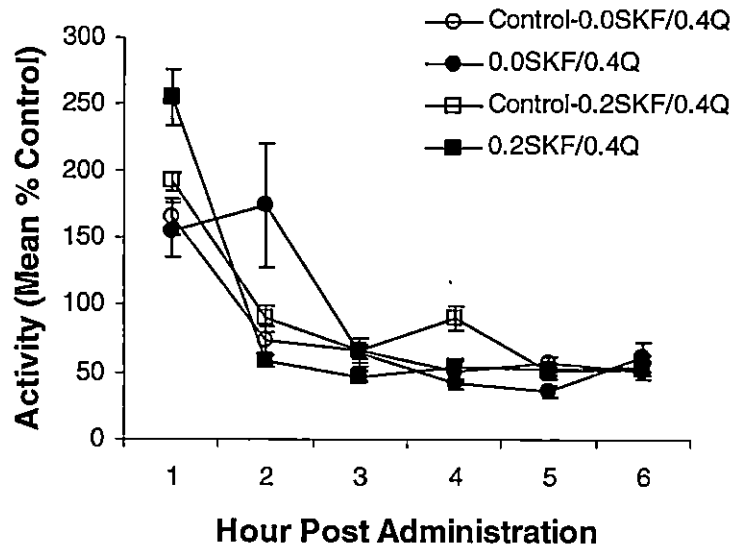


*Figure 10.* Mean activity during hr 1-6 post-administration for 0.0 SKF/ 0.4 Quinpirole and 0.2 SKF/ 0.4 Quinpirole groups during control and drug cycles. ± *SEM* bars are shown.

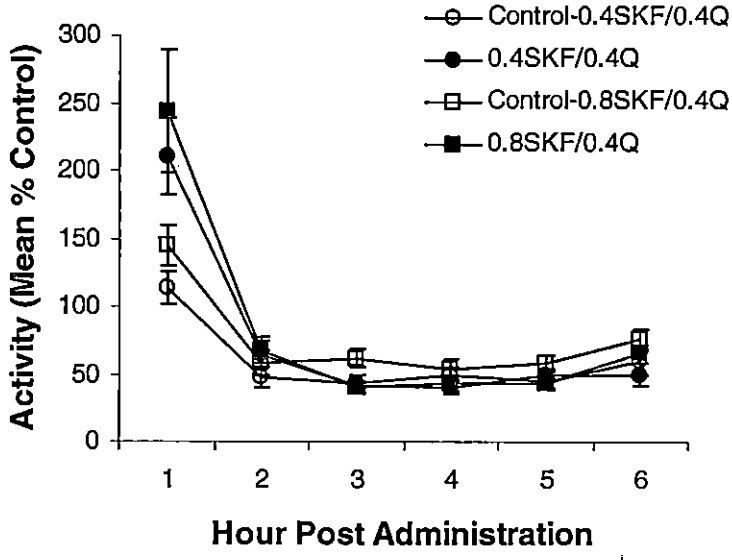


*Figure 11.* Mean activity during hr 1-6 post-administration for 0.4 SKF/ 0.4 Quinpirole and 0.8 SKF/ 0.4 Quinpirole groups during control and drug cycles. ± *SEM* bars are shown.

was elevated during hours 1 and 2 compared to all other hours post-treatment, *ps* < .01. The condition analysis showed that activity was elevated during control cycles relative to drug cycles during hour 5 post treatment, $t(7) = 3.147$, $p < .05$

*0.2 SKF/ 0.4 Quinpirole group.* In the overall analysis no significant effect of condition was obtained, $F(1, 7) = 0.033$, $p > .05$. A significant effect of hour and a significant condition by hour interaction were obtained, *Fs*(5, 35) = 31.149 and 51.805, *ps* < .0001. According to the time analysis, for control and drug cycles, activity changed significantly over time, $F(5, 35) = 51.805$, $p < .0001$ and $F(5, 35) = 78.6060$, $p < .0001$. Activity during control cycles was elevated during the first hour compared to all other hours post-treatment, *ps* < .0001. Activity during hour 2 was elevated compared to hours 3, 5 and 6 post-treatment, *ps* < .05. Furthermore, activity during hour 4 was elevated compared to hours 3, 5 and 6 post-treatment, *ps* < .05. Activity during drug cycles was elevated during the first hour compared to all other hours post-treatment, *ps* < .0001. According to the condition analysis, differences in activity were obtained during hours 1, 3 and 4 post-treatment, *ts*(7) = -2.379, 2.915 and 3.381, *ps* < .05. Activity of drug cycles was higher during the first hour, and activity of control cycles was higher during hours 3 and 4.

*0.4 SKF/ 0.4 Quinpirole group.* In the overall analysis no significant effect of condition was obtained, $F(1, 7) = 4.074$, $p > .05$. A significant effect of hour and a significant interaction were obtained, *Fs*(5, 35) = 53.944 and 2.746, *ps* < .0001 and .05. According to the time analysis, for both control and drug cycles, activity changed significantly over time, $F(5, 35) = 11.833$, $p < 0001$ and $F(5, 35) = 25.526$, $p < .0001$.

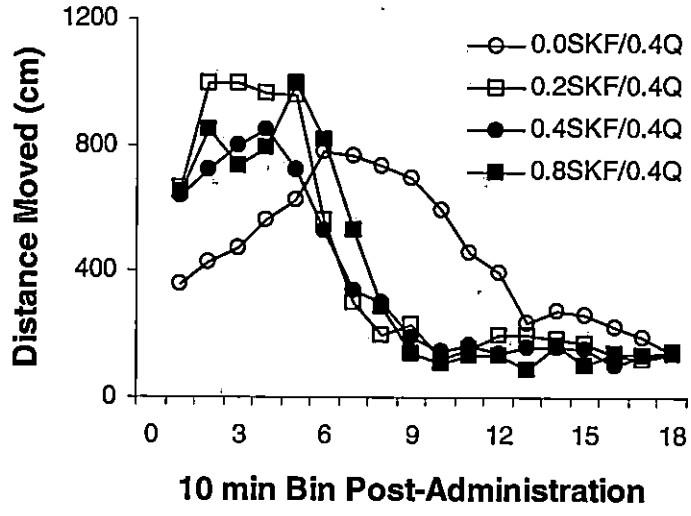


*Figure 12.* Mean distance moved in 10-min bins during first 3 hr post-administration for 0.0 SKF/ 0.4 Quinpirole, 0.2 SKF/ 0.4 Quinpirole, 0.4 SKF/ 0.4 Quinpirole and 0.8 SKF/ 0.4 Quinpirole groups during drug cycles.

Activity during control cycles was elevated during the first hour compared to all other hours post-treatment, *ps* < .0001. The same was true for drug cycles, *ps* < .0001. According to the condition analysis a difference in activity between control and drug treatments was obtained during the first hour, when activity of drug cycles was elevated, $t(7) = -3.136, p < .05$.

*0.8 SKF/ 0.4 Quinpirole group.* In the overall analysis no significant effect of condition was obtained, $F(1, 7) = 1.183, p > .05$. A significant effect of hour and a significant interaction were obtained, $Fs(5, 35) = 29.260$ and 5.026, *ps* < .0001 and .01. According to the time analysis, for control and drug cycles, activity changed significantly over time, $F(5, 35) = 18.833, p < .0001$ and $F(5, 5) = 17.497, p < .0001$. Activity during control cycles was elevated during the first hour compared to all other hours post-treatment, *ps* < .0001. The same pattern was obtained during drug cycles, *ps* < .0001. According to the condition analysis, a difference in activity was obtained during hour 3 post-treatment, when activity of control cycles was elevated, $t(7) = 2.448, p < .05$.

Figure 12 shows activity (as centimeters moved) in 10-min bins for the first 3-hr post-treatment. The figure shows activity for each group during drug cycles. All functions were biphasic. Adding each dose of SKF seemed to partially alleviate the hour 1 inhibition of activity produced by Quinpirole.

*Longer-term effects.* Figure 13 shows mean activity (as a percentage of control) averaged across 3-hr bins for hours 7-33 post treatment for 0.0 SKF/ 0.4 Quinpirole and 0.2 SKF/ 0.4 Quinpirole combination groups during control and drug

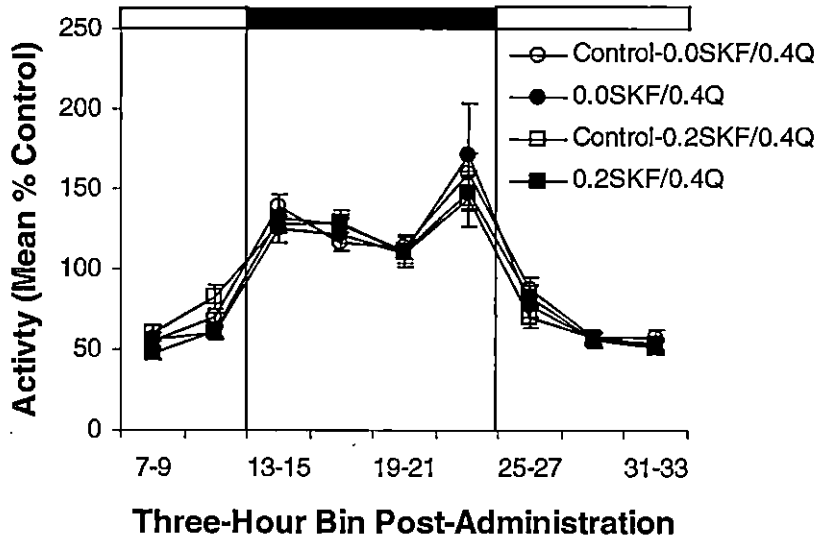


*Figure 13.* Mean activity during hr 7-33 post-administration averaged into 3-hr bins for 0.0 SKF/ 0.4 Quinpirole and 0.2 SKF/ 0.4 Quinpirole groups during control and drug cycles. Light/ dark bars across top indicate lights-on/ lights-off. ± *SEM* bars are shown.

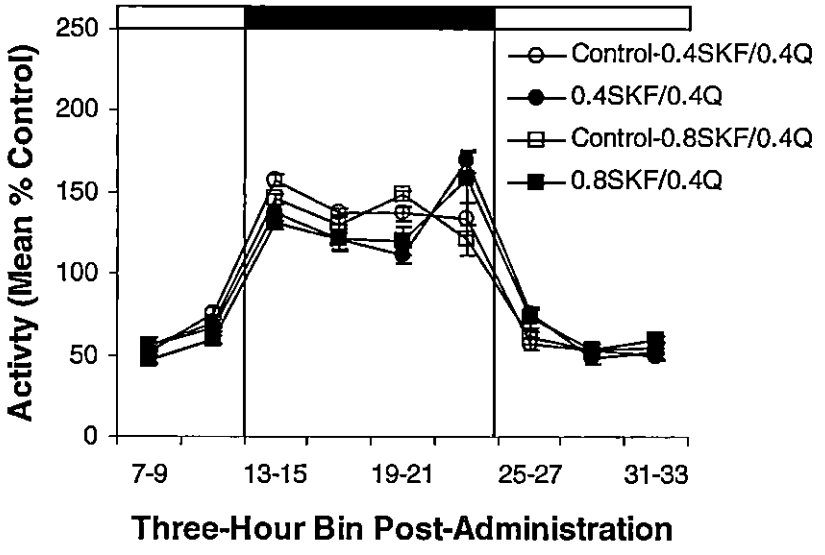


*Figure 14.* Mean activity during hr 7-33 post-administration averaged into 3-hr bins for 0.4 SKF/ 0.4 Quinpirole and 0.8 SKF/ 0.4 Quinpirole groups during control and drug cycles. Light/ dark bars across top indicate lights-on/ lights-off. ± *SEM* bars are shown.

cycles. Figure 14 shows the same data for 0.4 SKF/ 0.4 Quinpirole and 0.8 SKF/ 0.4 Quinpirole groups. In order to evaluate how activity changed from control to drug cycles, a series of analyses, similar to those done on immediate period activity, were planned. For the overall analysis a two (treatment condition) by nine (3-hr bin post-treatment) within subjects two-way ANOVA was performed. When a significant effect of treatment or a significant treatment by bin interaction was obtained, time analysis and condition analysis were done. In the time analysis, to investigate changes in dark-period activity across 3-hr bins, two separate one-way within subjects ANOVAs were done on control and treatment data. Fisher's PLSD post hoc analyses were used to interpret significant effects. In the condition analysis, activity for control and drug cycles was compared at each of the 3-hr dark-period bins with four separate within subjects *t*-tests.

*0.0 SKF/ 0.4 Quinpirole group.* In the overall analysis no significant effect of condition and no significant condition by hour interaction were obtained, $F(1, 7) = 0.469$, $p > .05$ and $F(8, 56) = 0.621$, $p > .05$. A significant effect of hour was obtained, $F(8, 56) = 21.143$, $p < .0001$. Fisher's PLSDs were used to interpret the significant main effect of 3-hr bin. Activity was elevated during dark-period bins relative to light-period bins, and it was elevated during bin 22-24 relative to other dark-period bins, $ps < .05$.

*0.2 SKF/ 0.4 Quinpirole group.* The same pattern of results was seen. In the overall analysis no significant effect of condition and no significant interaction of condition and 3-hr bin were obtained, $F(1, 7) = 0.484$, $p > .05$ and $F(8, 56) = 1.787$, $p$

> .05. A significant effect of hour was obtained, $F(8, 56) = 32.366$, $p < .0001$. Again, activity was elevated during dark-period bins relative to light-period bins, and it was elevated during bin 22-24 relative to other dark-period bins, $ps < .05$.

*0.4 SKF/ 0.4 Quinpirole group.* In the overall analysis no significant effect of condition was obtained, $F(1, 7) = 1.939$, $p > .05$. A significant effect of 3-hr bin and a significant condition by 3-hr bin interaction were obtained, $Fs(8, 56) = 145.688$ and 9.562, $ps < .0001$. In the time analysis, for control and drug cycles, activity changed significantly over time, $F(8, 56) = 83.748$, $p < .0001$ and $F(8, 56) = 90.518$, $p < .0001$. For control cycles activity was elevated during the 13-15 hr bin compared to the 16-18, 19-21 and 22-24 hr bins, $ps < .01$. For drug cycles, activity was elevated during the 13-15 hr bin compared to the 16-18 and 19-21 hr bins, $ps < .05$ and .001. Activity was also elevated during the 22-24 hr bin compared to all other dark period bins, $ps < .0001$. According to the condition analysis, compared to control, drug cycle activity was lower during the 13-15, 16-18 and 19-21 hr bins, $ts(7) = 2.858$, 2.60 and 4.247, $ps < .05$ - .01, and higher during the 22-24 hr bin, $t(7) = -3.367$, $p < .05$. To summarize, during the drug condition activity appeared to decline during the 19-21 hr bin and to rebound during the 22-24 hr bin

*0.8 SKF/ 0.4 Quinpirole group.* According to the overall analysis no significant effect of condition was obtained, $F(1, 7) = 0.246$, $p > .05$. A significant effect of 3-hr bin and a significant interaction of condition and 3-hr bin were obtained, $Fs(8, 56) = 75.277$ and 8.102, $ps < .0001$. According to the condition analysis, for both control and drug cycles, activity changed significantly over time,

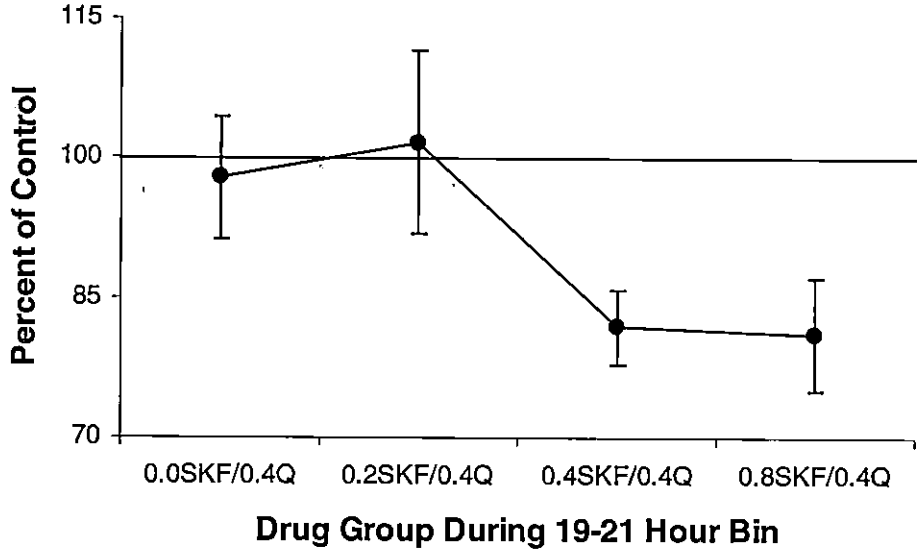


*Figure 15.* Mean activity as a percent of control during the 19-21 hr bin for 0.0 SKF/ 0.4 Quinpirole, 0.2 SKF/ 0.4 Quinpirole, 0.4 SKF/ 0.4 Quinpirole and 0.8 SKF/ 0.4 Quinpirole groups during drug cycles. ± *SEM* bars are shown.

$F(8, 56) = 69.927$, $p < .0001$ and $F(8, 56) = 42.415$, $p < .0001$. For control cycles activity was elevated during the 13-15 hr bin compared to the 16-18 and 22-24 hr bins, *ps* < .05 and .01. Additionally, activity was elevated during the 19-21 hr bin compared to the 16-18 and 22-24 hr bins, *ps* < .05 and .001. For drug cycles, activity was elevated during the 22-24 hr bin compared to all other dark period bins, *ps* < .005. The condition analysis indicated that activity was lower during drug cycles compared to control cycles during the 13-15 and 19-21 hr bins, *ts*(7) = 4.229 and 3.196, *ps* < .01 and .05. Activity then increased during drug cycles compared to control cycles during the 22-24 hr bin, $t(7) = -2.433$, $p < .05$.

*Results summary.* Figure 15 shows activity for all combination groups during the 19-21 hr bin post-treatment. Drug data were expressed as a percentage of control for each subject, and *t*-tests were used to see if groups' means were significantly different from 100%. The 2 high dose combination groups had decreased activity during the 19-21 hour hr bin, *ts*(7) = -4.510 and -3.137, *ps* < .01 and .05, whereas the 2 low dose combination groups did not, *ts*(7) = -0.333 and 0.171, *ps* > .05.

### *Conclusions and Discussion*

The purpose of Experiment 2 was to investigate whether co-stimulating D1 and D2 receptor subtypes had the ability to produce hypo-activity 20 hr later. A range of doses of the D1 agonist SKF 81297 was combined with a dose of the D2 agonist Quinpirole. Different groups of rats were given 0.0 SKF/ 0.4 Quinpirole, 0.2 SKF/ 0.4

Quinpirole, 0.4 SKF/ 0.4 Quinpirole or 0.8 SKF/ 0.4 Quinpirole, and activity was then monitored for 33 hr. The ability of combined agonists to produce hypo-activity would be consistent with the possibility that concurrent stimulation of D1 and D2 receptors induces amphetamine-induced hypo-activity.

With respect to short term effects, only the 0.0 SKF/ 0.4 Quinpirole treatment group showed an effect of drug on activity through hour 2 post-administration. The function for this combination group was biphasic, which is typical of a 0.4 mg/kg dose of Quinpirole. The other three combination groups (0.2 SKF/ 0.4 Quinpirole, 0.4 SKF/ 0.4 Quinpirole and 0.8 SKF/ 0.4 Quinpirole) had a high level of activity only during the first hour post-treatment

Groups showed different patterns of longer term activity following treatment compared to control. Specifically, the two lower dose combination groups (0.0 SKF/ 0.4 Quinpirole and 0.2 SKF/ 0.4 Quinpirole) did not display hypo-activity during the 19-21 hr bin. However, the two higher dose combination groups (0.4 SKF/ 0.4 Quinpirole and 0.8 SKF/ 0.4 Quinpirole) did display such hypo-activity. Additionally, during drug cycles the two higher dose combination groups also showed levels of activity that were elevated during the 25-27 hr bin. This pattern of hypo-activity around hour 20 and of enhanced activity near hour 25 is very similar to the activity pattern seen following amphetamine administration. These results obtained with the high dose combination groups suggest that amphetamine-induced hypo-activity may require initial co-activation of D1 and D2 receptors. Additionally, the inability of the 0.2 SKF/ 0.4 Quinpirole combination to produce hypo-activity suggests that this

behavior is dependent not only on co-activation, but also on dose or amount of receptor stimulation.

Interestingly, these data from Experiment 2 suggest that an oppositional interaction in the short-term may be correlated with hypo-activity in the longer-term. Compared to the Quinpirole-alone group, the 0.4 SKF/ 0.4 Quinpirole group had a higher initial level of activity. The function for this dose combination had an earlier asymptote and a quicker decline to zero. I will have more to say about the implications of short term receptor interaction in the general discussion when I make comparisons across experiments.

The results of Experiment 2 provide further evidence regarding the processes and mechanisms that might be responsible for amphetamine-induced hypo-activity.

The high dose treatment combinations did not produce levels of immediate activity like that of amphetamine. The levels of activity produced by these combinations might not be expected to produce fatigue. And yet, like amphetamine, these combinations did produce longer-term hypo-activity. Thus, amphetamine-induced hypo-activity may not be due to a fatigue effect.

The pharmacokinetic profiles of the agonists used in this research are not known. Given the nature of their short term effects on activity, they probably have short half-lives that are very different from one another and from the half-life of amphetamine. The fact that both the combined agonists and amphetamine can produce hypo-activity 20 hr after administration provides further evidence that expression of hypo-activity is not related to drug half-life.

In Wistar rats, 2.0 mg/kg amphetamine is needed to produce hypo-activity 20 hr after administration. In the present experiment, the 0.4 SKF/ 0.4 Quinpirole combination produced a similar effect. Perhaps amphetamine produces hypo-activity when it brings about a level of D1 and D2 receptor activation that is similar to that produced by a 0.4 SKF/ 0.4 Quinpirole dose combination.

*General Discussion*

Amphetamine administration produces hypo-activity 20 hr later in rats (White et al., 2004; White & White, in press). The purpose of this research was to investigate whether specific dopaminergic mechanisms might contribute to this amphetamine-induced effect. Two experiments were conducted in order to carry out this purpose. The locomotor activity of rats was recorded after administration of drug. SKF 81297 and Quinpirole, D1 and D2 receptor agonists, respectively, were administered. In Experiment 1, dopamine receptor subtype agonists administered alone did not produce hypo-activity 20 hr later. In Experiment 2, the dopamine agonists administered in combination did produce such hypo-activity. Co-stimulating D1 and D2 receptors produced a longer-term activity pattern that was similar to that produced by amphetamine administration.

An oppositional interaction reflected in short-term activity may be correlated with hypo-activity in the longer-term. Less short term activity occurred following the 0.4 SKF/ 0.4 Quinpirole combination (Experiment 2, Figure 12) than would be expected by summing the effects of the agonists given separately (Experiment 1,

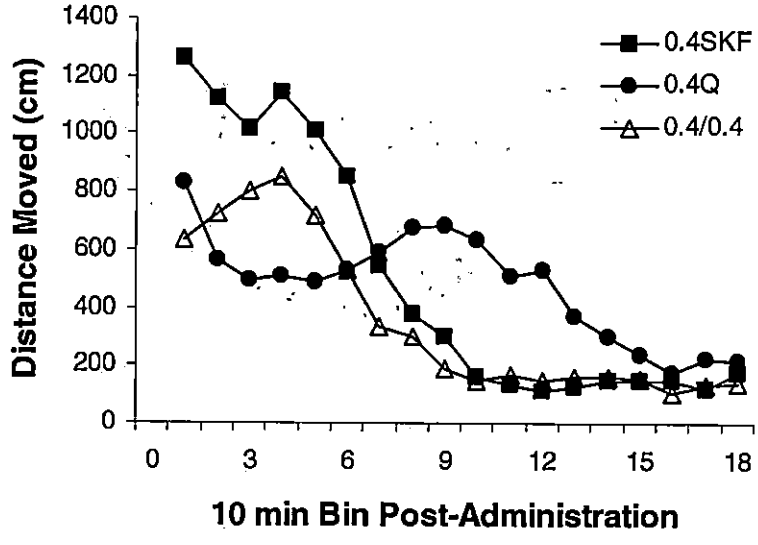


*Figure 16.* Activity during first 3 hr post-administration in 3 min bins for 0.4 mg SKF and 0.4 mg Quinpirole groups from Experiment 1 and 0.4 mg SKF/ 0.4 mg Quinpirole group from Experiment 2 during drug cycles.

Figure 5). The relevant data are re-plotted in Figure 16. The figure shows centimeters moved every 10 min following treatment with 0.4 mg/kg SKF, 0.4 mg/kg Quinpirole, or the combination. The fact that short term D1/ D2 synergism might have longer term consequences for behavior seems to be a novel finding. The functions in Figure 16 were from different experiments entailing different methods, so conclusions are tentative.

As of now, the location of the mechanism involved in the induction of hypo-activity is not known. This research suggests that the location is an area where the dopamine receptor subtypes converge. Furthermore, the area of convergence may be involved in motor control. Potential areas include the nucleus accumbens, striatum, cingulate gyrus and substantia nigra.

Treatments that produce hypo-activity in the longer term produce enhanced activity in the short term. Opponent process theory provides one explanation for this reversal in measure. According to opponent process theory, a treatment that produces an intense state later produces a rebound state having many of the opposite characteristics. Amphetamine treatment produces an intense state characterized by hyperactivity and hedonic effects. The hypo-activity observed later may reflect a rebound state having the opposite characteristics, that is, it may indicate the presence of a "crash" or acute withdrawal.

According to the theory, the initial response to drug would produce neuro-adaptations that outlast the initial drug response and that mediate this crash. In the case of amphetamine, the initial response and the neuro-adaptations might occur in

the reward system and involve dopamine. The processes of within system adaptation and between systems adaptation suggest further details.

In a within system adaptation overlapping mechanisms mediate the initial state and the rebound state. In Experiment 2, higher dose combinations produced increased activity in the shorter term and hypo-activity in the longer term. This result may have reflected a within system adaptation. For example, the site of D1 and D2 receptor convergence may have contributed to both short term hyperactivity and longer term hypo-activity. In this case, the short term D1 and D2 receptor synergism with respect to activity might not just be correlated with later hypo-activity but may be causally related to it. Amphetamine may produce hypo-activity via such a within system adaptation.

Alternatively, higher dose combinations may have induced hypo-activity via a between systems adaptation. In a between systems adaptation different mechanisms mediate the initial state and the rebound state. For example, the initial state produced by the higher dose combinations in Experiment 2, and indicated by hyperactivity, may have been mediated by the reward system. On the other hand, the rebound state, indicated by hypo-activity, may have been mediated by the sleep system.

Amphetamine and apomorphine have both been shown to produce hypo-activity 20 hr post-administration. The results of this research suggest that D1 and D2 receptor co-activation may be necessary to induce this effect. Several symptoms of acute withdrawal are present 20 hr after amphetamine receipt, including a Haloperidol-like cue state, decreased motivation to obtain natural reward, REM sleep

rebound and hypothermia (Barr & Phillips, 1992; Barret et al., 1992; Edgar & Seidel, 1997; Eikelboom & Stewart, 1981). Whether D1 and D2 receptor co-activation is necessary to produce these symptoms is unknown. Hypo-activity may be a correlate of acute withdrawal.

If hypo-activity is indeed an indicator of acute withdrawal, then the methods used here may provide a convenient animal model with which to study the determinants of this state.

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