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DISSERTATION

COCAINE IMPAIRS WORKING AND REFERENCE COMPONENTS OF SPATIAL MEMORY IN LABORATORY RATS

Submitted by

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In partial fulfillment of the requirements

for the degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring, 1999

COLORADO STATE UNIVERSITY

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We hereby recommend that the dissertation prepared under our supervision by Phillip L. Quirk entitled "Cocaine Impairs Working And Reference Components Of Spatial Memory In Laboratory Rats" be accepted as fulfilling in part requirements for the degree of Doctor of Philosophy.

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ABSTRACT OF DISSERTATION

COCAINE IMPAIRS WORKING AND REFERENCE COMPONENTS OF SPATIAL MEMORY IN LABORATORY RATS

In the United States the illegal use of cocaine is prevalent across socioeconomic levels and age groups. Since cocaine has been shown to cause significant disruptions of normal functioning in many bodily systems and causes deficits in cognitive functions such as memory, it is important to investigate the extent of these effects in systematic, controlled studies. Because it is not ethically possible to do this using human subjects, an animal model of chronic cocaine abuse has been employed to determine the effects of daily cocaine exposure on reference and working memory. Forty-eight male Sprague-Dawley rats were given daily injections of saline, 20mg/kg cocaine, or 40mg/kg cocaine and their performance swimming in a Morris water maze was assessed. Animals were required to locate a hidden platform either without prior experience in the maze, or after four days of cued trials training in the maze prior to being required to find the hidden platform. Animals in all treatment groups learned to locate the hidden platform, but the efficiency with which they learned was affected by

cocaine. A dose-dependent increase in escape latency was observed in the animals that received cued trial training prior to being required to locate the hidden platform. Cocaine also caused an increase in escape latency in animals that were not trained on cued trials prior to hidden platform trials. This increase, however, was not dose dependent. Furthermore, the animals receiving cued trial training prior to hidden trial training had shorter escape latencies than animals that had no initial cued trial training regardless of treatment condition.

The results of this investigation indicate that cocaine produces memory deficits in laboratory rats, but that the animals are still capable of learning. In addition, it is apparent that the amnestic effect of cocaine is determined, in part, by the learning history of the subject.

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Introduction

Cocaine: Consumption and properties

Cocaine, a substance derived from the coca shrub (Erythroxylon coca), has been used for centuries by peoples indigenous to the high Andean regions of Bolivia and Peru. Typically, coca leaves are processed by mixing them with wood ash in order to obtain a pharmacologically efficacious alkali extract that, when chewed, produces euphoric and stimulatory effects. The use of cocaine is thought to be popular in these agrarian societies because it increases productivity by reducing fatigue and appetite; thereby increasing the number of hours worked per day, thus, reducing the number of work stoppages for rest and consumatory behavior. In addition, the chewing of coca leaves during spiritual rituals has also been documented (Cintron, 1986).

It has been estimated that the average daily dose of cocaine produced when the coca plant is chewed reaches approximately 200 mg (Julien, 1998). Paley, Van Dyal, Jatlow, Cabieses, & Byck (1979) have reported that after 3 hours of chewing coca leaves Peruvian farmers had plasma concentrations of cocaine reaching several hundred nanograms per milliliter. For comparison, if the free-base form of cocaine is smoked plasma levels of over 400 nanograms per milliliter of plasma can be reached after 3 minutes (Paley, Jatlow, Van Dyke, Raul Jeri & Byck, 1982). While the use of cocaine by Andean Indians is frequent

and pervasive, the amounts of cocaine consumed and the resulting plasma levels obtained by chewing coca leaves pale in comparison to those found in U.S citizens who abuse cocaine.

Although the benefits of cocaine have been known to the native populations of South America for centuries, its potential as a clinically useful drug has only been known to modern scientists for about 130 years. The German scientist Albert Niemann first isolated cocaine from the coca shrub in 1860, and serendipitously discovered its local anesthetic property while tasting the purified substance. In 1884 cocaine was used clinically by Carl Koller as a topical anesthetic in opthalmological surgery. Cocaine was then administered by Halstead in infiltration and conduction block anesthesia (Catteral and Mackie, 1996).

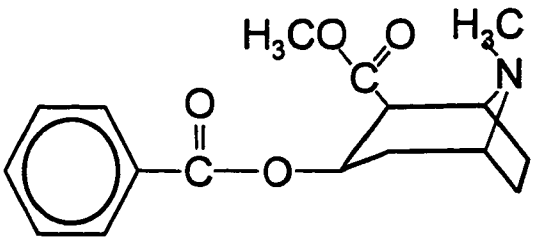
Many of the physiological and psychological effects of cocaine were studied by Freud, who initially claimed it to be a "cure-all" drug that could be used to treat a variety of ailments including fatigue, general malaise, and clinical depression. Freud himself was afflicted with clinical depression and used cocaine to treat it. Of course, after realizing the negative effects of cocaine use, such as dependence, tolerance, and psychosis, Freud reversed his position and labeled cocaine the "third scourge of humanity," with alcohol and heroine comprising the first two (as cited in Julien, 1998).

Due to its potential for toxicity and addiction, the use of cocaine in clinical applications began to decline, and by 1892 suitable synthetic alternatives were being sought. The search for a synthetic substitute for cocaine, that had similar

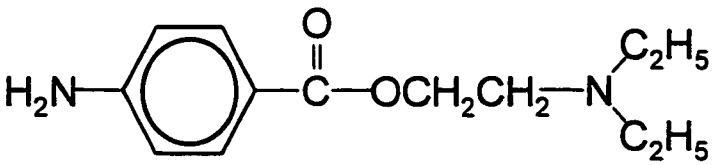
local anesthetic properties without the potential for addiction, came to fruition in 1905 with the synthesis of procaine by Einhorn (Catteral and Mackie, 1996). Since then many other local anesthetics, including lidocaine, benzocaine, and bupivacaine have been synthesized. Procaine, however, was the template used for development of these other anesthetics, and cocaine provided the basic blueprint for procaine; thus, the origins of most modern synthetic local anesthetics can be traced to cocaine. This chemical relationship between cocaine and other local anesthetics becomes quite apparent by comparing the chemical structures of the various substances (Figure 1).

By 1885, the negative aspects of the clinical use of cocaine were known, but it was still marketed to the general public in tonics and soft drinks (Coca Cola contained cocaine until 1903) to relieve fatigue and bolster a sense of well being. This practice was discontinued in 1914 when the Harrison Narcotic Act (as cited in Das, 1993) banned cocaine in patent medicines and beverages. It is interesting to note, however, that while the use of cocaine is today viewed by most people as an insidious blight on society that breaks up families, destroys careers, and ends lives, it is likely that those same people would be unwilling have a dentist drill their teeth without the comfort gained by exploitation of the chemical structure of cocaine.

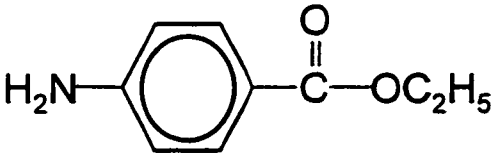
Cocaine is an ester of benzoic acid and the complex alcohol 2-carbomethoxy, 3-hydroxy-tropane. Like many other local anesthetics, it contains hydrophobic and hydrophilic regions separated by an ester linkage. The hydrophobic property of cocaine allows it to readily diffuse across cellular



COCAINE



PROCAINE



BENZOCAINE

Figure 1. Chemical structures of cocaine and closely-related local anesthetics.

membranes in most biological tissue. This is important for local anesthetic action because the binding site for these anesthetic compounds is thought to consist of a segment of hydrophobic amino acids within the transmembrane pore near the intracellular surface of voltage-gated Na^+ channels (Courtney and Strichartz. 1987). It is through blockade of these voltage-gated Na^+ channels that cocaine exerts its local anesthetic effect.

This hydrophobic property is also one reason why cocaine is a widely abused psychostimulant. The ease with which it crosses cellular membranes allows it to rapidly pass into the blood stream and then into brain tissue. Within the brain, cocaine acts as an indirect dopamine agonist. Its primary mechanism of action is to inhibit reuptake of monoaminergic neurotransmitters (dopamine, in particular) by blocking presynaptic reuptake transport proteins (Iverson, 1974; Koe, 1976; Zahniser, Gerhardt, & Cass, 1995). The subjective effects of cocaine intoxication are thought to be largely the result of this pharmacological action taking place in the nucleus accumbens, an area of the striatum that receives rich dopaminergic innervation from the ventral tegmental area. The nucleus accumbens has been shown to be involved with the reinforcing effects of many abused substances including cocaine (Cunningham, Dworkin, & Smith, 1992; Roberts, 1992).

It has been shown in numerous experiments that cocaine administration increases extracellular dopamine concentrations in the nucleus accumbens. For example, Finlay, Fiberger, Blaha, & Phillips (1988) used in vivo, electrochemical

detection to show that i.v. self administration of cocaine in rats increased dopamine levels in the nucleus accumbens. Hurd, Weiss, Koob, Anden and Ungerstedt (1989), and Pettit and Justice (1989), obtained similar results using in vivo microdialysis. Although there were differences in the results obtained between these laboratories due to the parameters of the experiments, the general finding was that cocaine administration increased dopamine in the nucleus accumbens. It should be noted, however, that other substances, such as GBR 12909, also bind to the dopamine transporter and block dopamine reuptake, but do not share the reinforcing efficacy and potential for abuse that cocaine has been shown to posses (Chait, Uhlenherth, & Johanson, 1987; Rothman, 1990). The mechanism by which this action takes place is unclear and is in need of further research, but the pharmacodynamics of these substances makes them interesting candidates for study in an effort to develop pharmacological treatments for cocaine abuse. The rationale for these studies would be that cocaine analogues could be used to treat cocaine addiction in the same way that methadone has been used to treat opiate addiction.

The use of cocaine is prevalent

It has been estimated that as many as 30 million Americans have tried cocaine at least once (Ableson & Miller, 1985), and approximately 8 million Americans use it regularly (Stone et al., 1984). According to a 1993 report by the United States Department of Justice, the number of Americans who used cocaine within the month preceding the survey numbered about 1.3 million.

Occasional users, those who reported using cocaine less often than monthly, numbered approximately 3 million (down from 8.1 million in 1985). The number of weekly users has remained about 0.5 million since 1983. As self-report surveys of illicit activity are suspect in terms of generating true estimates of illegal activity, it can be assumed that the actual number of cocaine users in this country is somewhat higher.

While these statistics are alarming, the prevalence of cocaine use in this country becomes an even more important issue in light of the fact that the detrimental effects of cocaine on physiological and/or cognitive processes are not limited to the adult population. Examination of the latest data available from the National Institute on Drug Abuse reveals that there is a large population of school-age children who use cocaine. It was reported that in 1996, 4.5% of eighth-grade children, and 7.1% of twelfth-graders in this country had tried cocaine (NIDA, 1998). It is alarming to note that a population whose neurological and cognitive development is not fully complete is using cocaine, a substance known to have widespread effects on neural activity. This situation serves to illustrate the urgency with which a full understanding of the potential for cocaine to disrupt neurophysiological and cognitive processes should be sought. Fortunately, due to its widespread use, the effects of cocaine on physiological and psychological systems, especially those involved with learning, have become the focus of considerable research.

Cocaine causes alterations in human neuroanatomy and neurophysiology

Numerous researchers have shown that habitual use of cocaine by humans is associated with a variety of physiological, neurological, and neuropsychological deficits and abnormalities. The medical complications involved with cocaine use include hypertension, myocardial infarction, cardiac arrhythmia, and hyperthermia (see review by Cregler & Mark, 1986). Cocaine precipitates seizures, optic neuropathy, cerebral and myocardial infarction leading to subarachnoid and intracerebral hemorrhage, and multifocal cerebral ischemia (Daras, Tuchman, & Marks, 1991; Fredricks, Lefkovits, Challa, & Troost, 1991; Pascual-Leone, Dhuna, & Anderson, 1991a; Strickland, Miller, Kowell, & Stein, 1998). Cocaine is also implicated in increased ventricular brain ratio, cortical atrophy (Pascual-Leone, Dhuna, & Anderson, 1991b; Morgan, Cascella, Stapleton, Phillips, Yung, Wong, Shaya, & London, 1993), and alterations in EEG and evoked potentials (Herning & King, 1996). For example, Pascual-Leone, et al. (1991a) reported that the brains of subjects who had a history of chronic-habitual cocaine abuse (at least 4 times/week for at least 2 years) showed significant cortical atrophy compared to the brains of abstinent controls or first-time cocaine abusers.

In EEG studies Pascual-Leone, et al. (1991a) found that chronic-habitual cocaine abusers had significantly decreased relative alpha (8-12Hz) power in the fronto-temporal and temporo-parietal regions of the brain while the relative powers of beta (12-20Hz), theta (4-8Hz), and delta (1-4Hz) bands were

significantly increased. The investigators suggested that these results might indicate a propensity toward fronto-temporal dysfunction in chronic-habitual cocaine abusers. Cocaine possesses the ability to significantly affect dopamine transmission by antagonizing the dopamine reuptake transporter protein. Since there are numerous structures in the frontal and medio-temporal regions, such as the mesolimbic and mesocortical dopamine systems, that receive dopaminergic projections, it would seem reasonable that these areas would be affected by chronic cocaine use.

Pascual-Leone, et al. (1991b) also generated data regarding the effect of cocaine on neuroanatomy. CT scanners were used to collect information on neuroanatomical alterations due to the effects of cocaine. The researchers calculated global brain atrophy indices that took into account the width of the frontal horn of the ventricles, the intercaudate distance, the frontal brain width, and the maximal brain width. Brain atrophy indices were significantly higher in chronic-habitual users than they were in first-time users or abstinent controls. Another notable finding in their study was that first-time abusers' indices of brain atrophy did not differ significantly from the indices of abstinent controls. This may indicate that there is some level of cocaine exposure that is safe. Thus, it is possible that the so-called "recreational" cocaine user may not suffer long-lasting detrimental effects after limited cocaine use. Of course, it would be irresponsible to assert that any level of cocaine use is safe when there is overwhelming evidence that cocaine produces detrimental effects. However, the fact that limited use may not result in permanent injury is encouraging in terms of the

prognosis for individuals seeking medical or psychological counseling after experimenting with cocaine.

Cocaine has negative effects on human cognition

There is clear evidence that cocaine can affect neurophysiology, and there is evidence that the effects of chronic cocaine abuse on human cognition are also numerous and varied. Researchers have shown that cocaine use affects auditory recall, concentration, reaction time, attention, arithmetic performance, short-term memory, and problem solving (Ardila, Rosselli, & Strumwasser, 1991; Herning, Glover, Koepel, Weddington, & Jaffe, 1990; O'Malley, Adamse, Heaton, & Gawin, 1992; Weinrieb & O'Brien, 1993). In addition, cocaine abuse has been linked to affective disturbances such as depression, anhedonia, and paranoia (O'Malley, et al., 1992; Gawin, 1991).

While the list of structures and systems affected by cocaine is extensive [see Hammer (Ed.), 1995], the effects on structures and processes involved with learning and memory are of particular interest to behavioral scientists. This is, in part, because in order for a species to thrive it must have the ability to respond to stimuli in an adaptive manner. In other words, it must be able to learn. Thus, it is important to understand the range of effects of any substance that has the potential to compromise learning.

Data generated in studies of the effects of cocaine on human cognition provide a good indication that it is a substance deserving further evaluation in terms of its effects on human learning. However, there are several serious

disadvantages to engaging in research on the effects of cocaine using drug-abusing humans as subjects. One problem with using humans in cocaine research is that conclusions drawn from data generated from drug-abusing humans are suspect since there are no reliable ways to assess and compare the cognitive condition or level of performance of drug abusers prior to their cocaine-abusing situation. Another problem is that drug abusers rarely limit themselves to one drug or neurotoxic substance. It is common for cocaine abusers to also expose themselves to THC, ethanol, and/or nicotine; or to use opiates and barbiturates to avoid the negative effects of withdrawal from cocaine.

The confounding influence of polydrug use is an inherent liability when drug-abusing humans are used as subjects. In addition to the effects of polydrug use, the general lifestyle of drug abusers undoubtedly has a profound effect on their physical and psychological well being. This further confounds studies in which they are subjects. People who chronically abuse drugs often have less than optimal general health, are poorly nourished, have altered sleep patterns, and expose themselves to many substances and situations that can affect their performance on laboratory assessment procedures.

A further problem that arises when human subjects are used in such research is that the actual level of drug ingested is difficult to ascertain. Even if a cocaine abuser was able to provide an accurate estimate of the amount of cocaine used during a particular period of time, it would be impossible to determine the actual amount of active drug consumed. "Street cocaine" is not cocaine in its pure form. There is a wide range in the level of purity of commonly

available forms of cocaine. The content of this cocaine, particularly when purchased as a powder, commonly consists of a number of adulterants which were added to increase weight and volume, thus increasing profit margin for the seller. These adulterants, which range from seemingly innocuous substances such as sodium bicarbonate and manitol to psychoactive substances such as methamphetamine, can have effects of their own, or synergistic effects that can alter the degree to which cocaine affects an individual.

For all of the above-mentioned reasons it is clear that in order to conduct meaningful research into the effects of cocaine exposure on learning, it is of paramount concern to use a population of subjects with a documented history whose immediate environment can be monitored and controlled. Additionally, it is necessary for tightly controlled doses and routes of administration to be used. For obvious ethical reasons the only feasible method to conduct such research is to use non-human animal models.

Cognitive Studies Of Cocaine In Non-Human Animals

Simple tasks

Non-human animals have been used to examine the effects of cocaine and other psychoactive substances on certain aspects of learning (review by Kosman & Unna; 1968; Moresbaecher, Boren, Schrot, & Simoes, 1979; and review by Thompson & Moersbaecher, 1978). These early studies on the effects of cocaine on learning were designed primarily to investigate the effects of drugs on fairly simple learning processes such as place preference conditioning,

light/dark visual discriminations, and passive avoidance behavior (Castallano, 1973; Nomikos & Spyraki, 1988).

Unfortunately, it is difficult to understand fully the effects of cocaine on these learning processes because the variety of experimental designs, species of subjects, behavioral tasks, routes of administration, and dose ranges that were used varied greatly. Thus, the results of these experiments are not typically comparable. There are, however, several important methodological issues that became apparent in these early experiments, as well as some features of cocaine-induced behavior that are consistent across studies.

Probably the most important information gained from early animal research into the effects of cocaine on learning is that the route and pattern of administration can have profound effects on behavioral outcomes. For example, Nomikos & Spyraki (1988) found that rats given i.v. injections of cocaine hydrochloride at low doses (0.5-2.5 mg/kg) developed a conditioned place preference for the drug-paired side of an apparatus, but animals given high doses i.v. (5 and 10 mg/kg) did not develop such a preference. In addition, animals that were given i.p. injections of cocaine required higher doses and more trials before conditioned place preferences were observed. More recently researchers studying the effects of cocaine on learning have focused their attention on cocaine's effects on cognitive processes involving more complex behaviors and memory function (e.g. Cutler, Wilkerson, Gingras, & Levin, 1996; Hutchings & Dow-Edwards, 1991).

Complex tasks

One common strategy for assessing memory function in non-human animals is to examine their ability to perform certain types of conditional discriminations. Tranberg and Rilling (1980) have suggested that the delayed matching-to-sample (DMTS) procedure provides a particularly sensitive measure of short-term memory. In the standard DMTS procedure a 'sample' stimulus is presented and the subject is required to emit an observing response that is appropriate to the species and the procedure. Upon completion of the observing response, the sample stimulus is removed for a specified interval that is followed by presentation of two or more 'comparison' stimuli, one of which is identical to the sample stimulus. A response to the comparison stimulus that is identical to the sample is reinforced. Thus, with an analysis of the proportion of correct responses, it is possible to determine whether an animal is capable of using a previously experienced stimulus to guide its response in the presence of other stimuli.

The DMTS procedure allows considerable flexibility when investigating memory function in that there are several parameters inherent to the procedure that can be manipulated in order to examine specific aspects of memory. For example, the schedule of reinforcement during observing responses and the length of delay between sample and comparison can be manipulated, as can the modality used to sense both sample and comparison stimuli. The value of this flexibility in studies of drug effects was illustrated by Branch and Dearing (1981) who showed that acute doses of cocaine decreased the accuracy of pigeons on

a delayed matching to sample task. This finding is interesting in itself, but by manipulating the length of the delay the researchers were further able to characterize the effects of cocaine on memory. They showed that the deficits in learning produced by cocaine are sensitive to changes in the temporal relationship between sample and comparison stimuli. In most species the proportion of correct responses on DMTS trials decreases as an exponential function of the delay between sample and comparison stimuli. While errors increased with delay for all animals in Branch and Dearing's study, the difference in performance decrements between experimental and control group animals were greater under long delay conditions. In other words, the effect of cocaine was more pronounced if the delay between sample and comparison was long; therefore, it was concluded that cocaine increases the efficacy of delay in disrupting performance.

In other studies of the effects of cocaine on non-human memory, Wenger and Wright (1990) and Hudzik and Wenger (1992) have shown that acute doses of cocaine can affect the performance of pigeons and squirrel monkeys exposed to titrating matching-to-sample procedures. The titrating matching-to-sample procedure is similar to DMTS except that the length of the delay is not fixed; it is dependent upon the animal's performance on previous trials. In general, with this procedure each correct response emitted by the animal results in an incremental increase in the sample-comparison interval, and incorrect responses result in incremental decreases, but the same increment is not necessarily used for increasing and decreasing the delay interval. The primary variable of interest in

this procedure is the maximum delay interval tolerated by an animal. In studies of behavioral effects of psychoactive drugs on memory, the maximum delay tolerated provides a measure of resistance to amnestic effects of the drug while avoiding ceiling effects that can limit the descriptive power of the standard DMTS procedure.

Another behavioral procedure developed for the study of learning and memory in non-human animals is the Morris water maze (MWM) task. Sometimes referred to as the swimming maze, or spatial navigation task, the MWM (Morris, 1981) was initially designed to study how rats use environmental cues to navigate from place to place. Essentially, the task is used to study the ability of rats to use distal environmental cues to locate a hidden platform in a pool of water, and to analyze the strategies they employ to complete the task. In most published research in which the MWM is used a standard procedure is employed. Animals are typically given three trials each day where the subjects are placed in the water along the edge of the pool in one of three different start locations on each of the three trials. The rats are allowed to swim until the hidden platform is located or a predetermined interval has passed. The platform usually remains in the same location for all trials over the course of the experiment for each animal, but the platform location is varied randomly between animals. The question of interest is whether the animals' ability to locate the platform improves over successive days. Reductions in latency to find the platform, or 'escape latency', across successive days of training is considered

evidence of learning the spatial relationship between the platform location and extramaze visual cues.

Since its introduction the MWM has been used as a tool to study a variety of issues regarding animal learning and memory. The MWM has been used to study reference and working memory in normal rats and mice. Additionally, the maze has been used to examine the effects of brain lesions and drug treatments (see review by Brandeis, Brandys, and Yehuda, 1989) on learning and memory.

Variations on the standard MWM procedure have also been used to assess the ability of rats to learn visual and spatial discriminations (Morris, 1983; 1984; Morris, Hagan & Rawlings, 1986), and to form spatial learning sets (Wishaw, 1984). Morris (1983) has developed a procedure to study what he termed 'spatial matching-to-sample'. In its simplest form the matching-to-sample procedure requires an organism to emit one response in the presence of one particular stimulus environment and to emit a different, usually similar, response in the presence of a different stimulus environment. Because the proper response is conditional upon the initial stimulus presented the procedure falls under the general category of conditional discrimination.

According to Morris, spatial matching-to-sample is similar to the standard DMTS procedure in that a conditional discrimination must be learned. Information regarding one set of stimuli, the stimulus complex of extramaze cues and proprioceptive feedback gained on the first trial, must be retained over a specified interval. This information must then be reaccessed and used to guide

the responses emitted in the presence of novel stimuli. These novel stimuli comprise the stimulus complex present on the second trial.

In Morris' procedure one pair of trials is run each day with the hidden platform placed in the same location for both trials within a day, but different locations are selected on successive days. The question of interest is whether rats are able to use information gathered on the first trial to improve platform-locating performance on the second trial each day. While it could certainly be argued that simple repetition of a previously emitted behavior does not constitute a conditional discrimination, it is not unreasonable to consider the two-trial task as an example of successive, identity matching-to-sample. In this case the sample stimulus includes all visual cues and proprioceptive feedback experienced on the first trial, and the comparison stimulus includes the visual cues and proprioceptive feedback available on the second trial. To make an appropriate response would require an animal to compare visual and proprioceptive information occurring on the second trial with that experienced on the first; then to match, movement-for-movement, second trial with first trial behavior. While terminology might be argued, the important issue is that Morris' procedure provides a powerful tool to study spatial memory in a controlled and systematic way.

A key feature of Morris' procedure is that it allows for measures of both working and reference memory. Analysis of the working memory component of the task involves assaying the difference in escape latency between the first and second trials on each day. If escape latency for the second trial is lower than

escape latency on the first trial, it is an indication that working memory is intact. That is, the animal retained specific information about the first trial and used that information to improve performance on the second trial. If second trial latency does not differ significantly from first trial latency, it can be argued that there is some disruption in working memory.

A measure of reference memory in the MWM involves changes in escape latency over successive days. If memories regarding the task on one day are successfully encoded, stored, and retrieved the next day, it would be expected that escape latencies would decline as animals refine their search strategy and become more practiced at the task. A failure of escape latency to decline over successive days of training may be indicative of reference memory errors, especially if the first vs. second trial data indicate that working memory is intact. In other words, if an animal can improve performance between trials, but fails to improve performance between days it may be that working memory is in tact, but that reference memory has failed. In Morris' (1983) study it was reported that normal rats improved intraday and interday performance. This indicates that rats utilize processes analogous to human working and reference memory, and that these processes can be measured using the MWM.

Wishaw (1984) used a similar MWM procedure to study spatial learning, but each subject was allowed eight trial pairs each day. The start location was varied between four different places within days such that the rats started from each of the four start locations four times on each day for a total of 16 trials per day. Because the escape platform was positioned in a different location on each

day, and the rats started from different start points within days, the rats were required to learn new responses (or problems) each day. For this reason Wishaw viewed the procedure as a 'spatial learning-set' task, rather than a spatial matching to sample task.

As was the case with Morris' use of terminology, it could be argued that Wishaw's procedure is not a true example of learning set because the same extramaze cues are present throughout testing; they are merely in a different relative location. Traditionally, leaning set implies that learning about the relationship between the items in one set of stimuli transfers to a novel set of stimuli and improves learning the relationship between the items in the second set of stimuli. Wishaw considered viewing extramaze cues from a novel start location and swimming to a new platform location sufficiently different, when viewed from each start point, to consider the stimulus complex novel. Perhaps it would be most appropriate to refer to performance on these types of tasks within days as spatial matching-to-sample, as no novel stimuli are presented, and performance across days, when the platform location is changed, as spatial learning-set. Regardless, whether termed spatial matching-to-sample or spatial learning-set, decreases in escape latency between the first trial of a pair and the second trial of a pair are indicative of a rat's ability to use information gained on the first trial to facilitate location of the escape platform on the second trial. This is the point of the procedure. Again, the terminology can be argued, but the fact remains that the procedure provides a flexible test of memory function that should be sensitive to the effects of putative amnestic substances.

Since the tasks involved in both matching-to-sample and learning set procedures are fairly complex, requiring an animal to use information gathered from previous experience to maximize performance on a trial, it is reasonable that performance on these tasks would be particularly susceptible to disruption from exogenous chemical insult. For this reason alone a water maze conditional discrimination would seem an appropriate behavioral task to study the cognitive effects of daily cocaine exposure on learning (to date no such study has been published). However, there is another reason why such a study would be logical and valuable. This has to do with the effects of cocaine on a type of synaptic plasticity commonly referred to as hippocampal long-term potentation (hLTP).

Long-term potentiation is a form of synaptic plasticity, initially characterized by Bliss and Lomo (1973), in which the magnitude of some post-synaptic potentials is enhanced as the result of presynaptic stimulation. Long-term potentiation can be generated in cortex and some other brain structures, but hippocampal long-term potentiation is probably the most well-studied. Long-term potentiation in the hippocampus is of interest in the present study because it has been shown that hLTP plays a significant role in spatial memory processes.

It has been shown that AP-5, a substance that interferes with in vitro hLTP in brain slice preparations and in vivo hLTP in anesthetized rats, also inhibits MWM performance when injected into the cerebral ventricles of rats (Davis, Butcher, & Morris, 1992). Similar effects on MWM performance were obtained when dextromethorphan, also an hLTP inhibitor, was delivered to rats systemically via i.p. injection. For example, Bane, Rojas, Indermauer, Bennett, &

Avery (1996), using a MWM, showed that the latency to find a hidden platform increased in a dose-dependent fashion when rats were given systemic injections of dextromethorphan.

It has been suggested that dextromethorphan exerts its amnestic effect by inhibiting N-methyl-D-aspartate (NMDA) glutamate receptors in the hippocampus, thus decreasing conductance of Na^{+} and Ca^{++} through the associated ion channels (Bane, et al., 1996). Functional activity of these ionotropic Na^{+}/Ca^{++} channels is a necessary constituent of hLTP. In light of the evidence that substances that inhibit hLTP in vitro or in vivo also decrease the ability of rats to perform well on spatial memory tasks, it is logical to asses the effects of amnestic substances on behavior mediated by hLTP processes (i.e. the MWM).

Furthermore, Smith, Browning, and Dunwiddie (1993) have demonstrated that cocaine inhibits hLTP in vitro, and Heyser, Spear, and Spear (1995) have shown that prenatal exposure to cocaine disrupts performance of male rats in the MWM. Therefore, it is of particular interest to determine whether daily doses of cocaine will disrupt the performance of adult rats on a MWM task. Since cocaine inhibits in vitro hLTP, and substances that inhibit hLTP interfere with spatial learning tasks, it would seem likely that daily doses of cocaine would cause disruptions in the performance of rats on a MWM task.

Cocaine and MWM Performance in Adult Rats

The purpose of the present study is to determine if exposure to daily doses of cocaine will affect the ability of rats to locate a hidden platform in a MWM. In addition, other measures of performance will be taken to further characterize the effects of cocaine administration on water maze performance. These additional measures may determine whether any effects of cocaine on MWM performance are due to errors in working or reference memory. Measures of potential changes in performance due to psychomotor effects or alterations in reinforcement efficacy will also be recorded.

Since working memory processes must be used to find the location of the platform on the second run of each standard trial in an efficient manner, and because cocaine has been shown to affect short term memory in pigeons (Branch and Dearing, 1981; Wenger and Wright,1990), monkeys (Hudzik & Wenger, 1992), and humans (Rosselli & Ardila, 1996), it is expected that animals in the experimental groups will require more time than animals in the control group to find the hidden platform on each second run. However, since there is no consistent evidence that reference memory processes are affected by substances that inhibit hLTP, it is anticipated that escape latencies on both runs in the second and subsequent trials within days will decrease in relation to escape latencies on Trial 1. Furthermore, it is predicted that escape latencies for all standard trials will decrease over the course of training. In other words it is

expected that there will be a dose-dependent effect of cocaine on working memory, as indicated by intraday performance between treatment conditions.

In addition to determining the effects of cocaine on working memory, reference memory effects will also be evaluated. In the standard MWM task normal rats show decreases in escape latency over successive days of training. This finding has been replicated in an unpublished pilot study using a variation of Morris' (1983) and Wishaw's (1984) procedures (Quirk, unpublished data). Morris (1983), with regard to rat performance in a radial arm maze, suggested that the ability of animals to successfully locate the arms of the maze and continue to run in them to search for food is an indication that the (reference) memory of "how" to solve the maze is intact. In the MWM decreases in latency across days is a similar indication of functional reference memory.

In addition to this standard reference memory assay, however, a further measure of reference memory will be made. During pilot research it was noted that on the first run of the first trial on any given day rats appeared to spend more time swimming in the quadrant of the maze that contained the escape platform on the previous day than in any other quadrant. Although this behavior did not persist past the first run, it seems a valid indication that rats had remembered something about the escape platform location from the previous day, which would indicate reference memory activity. To systematically analyze this datum, a measure of perseverative behavior, dwell time in the previous day's platform quadrant on the first trial of the second and subsequent days, was analyzed. Dwell ratio was calculated by dividing the time spent in the previous day's

platform quadrant by total swim time. If no spatial bias exisits, the proportion of time spent in any one quadrant would be 0.25. Dwell ratios in the previous day's platform location that significantly exceed 0.25 would be an indication of functional reference memory. Conversely, if dwell time is equal to or less than 0.25 it would be an indication that no information regarding the location of the platform on the previous day was successfully accessed, an indication that some step in the encoding-to-recall process of spatial reference memory has failed.

As cocaine is a potent dopamine agonist, it could be expected that increases in available extracellular dopamine in the striatal or mesolimbic dopamine systems might result in variations in swim speed. Since swim speed may be correlated with escape latency, changes in swim speed could potentially mask cocaine's effects on escape latency. Therefore, the effects of cocaine on escape latency were assessed while statistically controlling for the effects of swim speed.

Cocaine binds to the presynaptic dopamine transporter protein making it unavailable for dopamine reuptake. This causes facilitation of dopaminergic activity. The two brain systems that would likely be affected most by this action are the mesocorticolimbic and the nigrostriatal dopaminergic pathways as they account for the majority of dopaminergic activity in the brain. Facilitation of dopaminergic activity in either of these areas could be expected to decrease escape latency in the cued, and perhaps even the standard trials. Dopamine activity in the nigrostriatal pathway is associated with motor activity, so facilitation of this system may decrease escape latency by increasing swim speed.

Dopaminergic activity in the mesocorticolimbic system, primarily in the dopaminergic projections from the ventral tegmental area to the nucleus accumbens, is necessary for a stimulus to have reinforcing properties. Thus, if escape is reinforcing, its efficacy as a reinforcer may increase for the experimental groups. This may be manifested by increased swim speeds due to enhanced dopaminergic activity in the nucleus accumbens.

By comparing escape latencies with swim speeds some conclusion may be drawn regarding this issue. If latencies are reduced, but swim speeds increase, it may be the result of enhanced motor performance or reinforcer efficacy. However, if escape latency decreases and swim speed remains unaffected or decreases, it would indicate that the animals have learned a more efficient search strategy or the specific location of the platform on a given trial.

By allowing measures of both working and reference memory processes, the present procedure will provide a systematic study of the effects of daily cocaine exposure on two aspects of memory. At this point such a study, with all the controls inherent in laboratory research using non-human subjects, has not been reported. While there has been research on the effects of cocaine on human memory, studies with humans are contaminated with numerous uncontrolled extraneous and confounding variables as described previously. Therefore, the use of an animal model is warranted. As cited before, there has been some research into the effects of cocaine on learning and memory using non-human animals, but to date there have been no systematic studies of daily doses of cocaine on both working and reference memory processes.

Method

Subjects

Forty-eight male Sprague Dawley rats acquired from Charles River Laboratories, Wilmington, MA, served as subjects. Each subject weighed between 238-352g at the beginning of the study. Upon arrival subjects were housed individually in plastic hanging cages containing wood shavings as bedding material. Subjects were allowed ad lib access to lab chow for an initial period of five days, and free access to H_2O throughout the experiment. During the initial five days each rat was handled for five minutes each day. A continuous 12:12h light/dark cycle was maintained in the room where the subjects were housed. Experimental manipulations began at the end of the dark cycle each day (06:00).

Apparatus

All animals were tested in the same room (approximately 12 x 20ft) and in the same apparatus. The MWM, a circular, galvanized steel trough, 183cm in diameter and 61 cm deep was used to test the animals. The maze was filled to a depth of 30 cm with 18°C water. The escape platform used for standard trials was constructed of black-painted PVC pipe tube. To construct the platform, an 8.8cm (diameter) section of PVC tube 27cm in length was fitted with a 14cm diameter PVC base. The tube was filled with pea gravel to counter buoyancy and the open end was then fitted with a PVC end-cap and closed with glue. The

PVC end-cap was 10 cm in diameter and served as the hidden escape platform onto which the rats could climb.

The platform apparatus was placed on the floor of the tank so that the escape platform remained approximately 1 cm below the surface of the water. On each day of standard trials the platform was placed randomly in the center of one of four quadrants. Each quadrant comprised 90° section of the maze. Thus, the center of the platform was located approximately 46 cm from the wall of the maze.

A nearly identical platform was constructed for use in the cued trials. This platform was constructed in the same manner as the platform used in the standard trials except that the PVC tube was 2 cm longer. Thus, it remained above the surface of the water when placed on the bottom of the maze during cued trials. The cued-trial platform also differed from the standard trial platform in that it was not painted black. The color of the cued trial platform was not altered from the stock off-white of common, commercially available PVC pipe.

The platforms weighed approximately 8 kilograms and were sufficiently stable so that they did not move with movement of the animals. The platforms were also easily portable, which facilitated the changing of platforms between different trial types.

A video tracking system was used to monitor and record the swim path, path distance and latency to find the platform. Swim speed was calculated by dividing swim distance by escape latency. The testing environment also contained multiple extramaze visual cues including a desk, the video tracking

system, and art which was mounted on two of the walls. Extraneous noise in the test environment was minimal.

Drug injection procedure

After five days subjects were randomly assigned to the 20mg/2cc/kg cocaine group (C20), the 40mg/2cc/kg cocaine group (C40), or the 2cc/kg saline group (SAL). Cocaine was injected in a 2cc/kg volume rather than the standard 1cc/kg in order to reduce the probability of necrotic skin lesions occurring due to the vasoconstricive effects of cocaine. Each animal in the SAL group was matched by weight to an animal in the C40 group and pair-fed the amount of food that was consumed by its C40 counterpart on the previous day. This was done to control for any effects of decreased food intake due to the anorectic properties of cocaine. Any effects on performance as the result of the anorectic effect of cocaine would presumably be more apparent in the C40 group. Thus, it was decided that pair-feeding the SAL group to the C40 group would accomplish the goal of providing a conservative control without the increase in subjects that would be necessary to include a SAL control group that was pair-fed to the C20 group.

Beginning on Day 6 all animals received daily injections of 0.9% saline solution (2 cc/kg s.c.) for four days prior to drug exposure. This was done to habituate any behavioral or physiological response to the handling and injection procedure. Daily drug delivery began on the tenth day after the rats' arrival and was continued throughout the experiment. Beginning on Day 10 either 20 or

40mg/2cc/kg cocaine hydrochloride (courtesy of NIDA), or an equivalent volume of sterile physiologic saline was injected. Daily injections were given s.c. using 27 g-0.5 in needles. As cocaine is an efficient monoamine agonist in the periphery, subcutaneous injections commonly result in localized necrotic lesions at injection sites due to the vasoconstricive effect of increased norepinepherine release. In order to reduce the probability of lesions injection sites were varied on a daily basis. On each day the injection sites were the same between animals; however, new injection sites on the dorsal surface contralateral to the previous day's site were used on consecutive days. Behavioral testing began on the eighth day of drug exposure and continued for eight days for each animal.

Procedure: Behavioral testing

Two types of trials occurred during the experiment; standard trials, and cued trials. On standard trials the hidden platform was placed in the maze and rats were required to swim until the platform was located or 60 s elapsed, whichever came first. In this situation no intramaze cues were available to the animals that could indicate the location of the hidden platform. During cued trials the taller, white platform replaced the hidden platform so that a distinct visual intramaze stimulus was available for the rats to use as a cue in order to locomote directly to the escape platform.

It was necessary to include cued trials in this study because cocaine is a highly effective central nervous system dopamine (and other biogenic amine neurotransmitters) reuptake inhibitor. As such, it increases the levels of

extracellular dopamine in the major dopaminergic pathways in the brain, the mesocorticolimbic system and the nigrostriatial system. Since these brain regions are important for motivation and motor behavior respectively, it is possible that alterations in swimming behavior may occur due to the modulation of dopaminergic transmission that is not necessarily involved with spatial memory per se, but is linked to motivational and/or motor system output. For example, increases in nigrostriatal dopamine could affect motor responses, thus resulting in decreased escape latencies because of increased swim speed. Also, alterations in the reinforcing efficacy of escape may result from the effects of increased dopamine activity in the mesocorticolimbic system concomitant with animals boarding the platform. This could also potentially affect swim speed, and thus escape latency.

Inclusion of cued trials allows any variations in escape latency due to changes in swim speed to be detected. Since animals in the cued conditions typically learn to swim directly to the visible platform in a few trials a comparison of swim speeds of animals in cued trials across drug treatment conditions should allow for a measure of potential changes in behavior caused by factors other than spatial memory.

Four days of cued trials preceded four days of standard trials for half the animals. These animals are referred to as the Cued First (CF) group. The remaining animals completed four days of standard trials prior to four days of cued trials. This group of animals is referred to as the Standard First (SF) group. This procedure not only allowed detection of alterations in escape latencies

caused by factors not involved with spatial memory, but also allowed an analysis of the effects of prior training on water maze performance.

For each rat, training consisted of three trials each day with two runs in each trial for a total of six runs each day. For each run a rat was placed in the water at one of the four randomly assigned start locations. Start locations were defined as the center of one of the non-platform quadrants along the circumference of the maze, approximately 3 cm from and facing the wall. The subject was released and allowed to swim for 60 s or until the platform was found. Subjects that failed to locate the platform within 60 s were placed on the platform for a 30 s platform interval. Rats that successfully located the platform were allowed to remain on the platform for 30 s. The second run of each trial was started immediately upon completion of the 30 s platform interval. For both runs within a trial the start point was unchanged. Between trials rats were placed under a heat lamp for a two-minute intertrial interval (ITI), during which feces were removed from the maze and the water was stirred to eliminate the possibility of olfactory cues which might bias the swim path of the animals.

The second and third trials were run in the same manner as the first. However, the start locations were changed according to a random sequence that allowed each animal to start from each of the three possible start locations (quadrants not containing the platform) on each of the four days of testing.

On each day the platform remained in the same location on all trials for each rat, but the platform location was varied randomly across rats. Each day the platform was moved to a different location for each rat such that the platform

was located in each of the four quadrants for one day for each rat. This procedure resulted in each rat starting from, and swimming to, each of the four quadrants an equal number of times.

The same procedure was used for standard and cued trials during which one half the animals swam in cued trials first and the other half swam in standard trials first as previously described. Training continued until all rats completed six runs with the platform in each of the four quadrants under both standard and cued conditions. Each rat swam a total of 48 runs.

Data analysis

In analyzing the difference between CF and SF groups it was determined that latency data within each group lacked homogeneity of variance. Therefore, these data were analyzed by Kolmogorv-Smirnov analysis, which allows comparisons of means without assuming homogeneity of variance. In subsequent analyses, where data were subjected to further partitioning, within-cells variance did not violate the homogeneity assumption required for parametric analysis of variance; thus, standard statistical analyses were performed.

Results are reported as escape latency in seconds, path length in pixels, and swim speed in pixels/second. These data were analyzed in separate repeated measures analyses of variance ([ANOVA];Treatment condition x Day x Trial x Run). For all escape latency analyses swim speed was entered as a covariate. Post hoc comparisons were performed using Tukey's HSD.

Dwell ratios were analyzed for each group, CF or SF, separately. An initial one-sample t-test was performed to determine if the group mean was significantly different from .25. This test was followed by a one-way ANOVA to determine if dwell ratios were different among conditions.

Results

Cued First vs Standard First – Standard trials

Upon initial inspection of the data (Figure 2), it was clear that there was a difference in the ability of rats to locate the hidden platform. This difference was apparently dependent on whether they had been previously exposed to the MWM procedure via training on cued trials (CF animals), or were required to locate the hidden platform without prior experience in the apparatus (SF animals). Animals in the CF group took less time to locate the hidden platform than animals in the SF group. The difference in escape latencies between the two groups was found to be significant via Kolmogorv-Smirnov analysis for two independent samples, $Z(1152)=3.95$, $p<.001$. Due to the difference in mean escape latencies between the CF and SF groups all subsequent analyses were performed separately on each group.

Swim speed

Since it was a concern that cocaine treatment may affect escape latency by influencing systems other than those involved with memory (i.e. sensory-

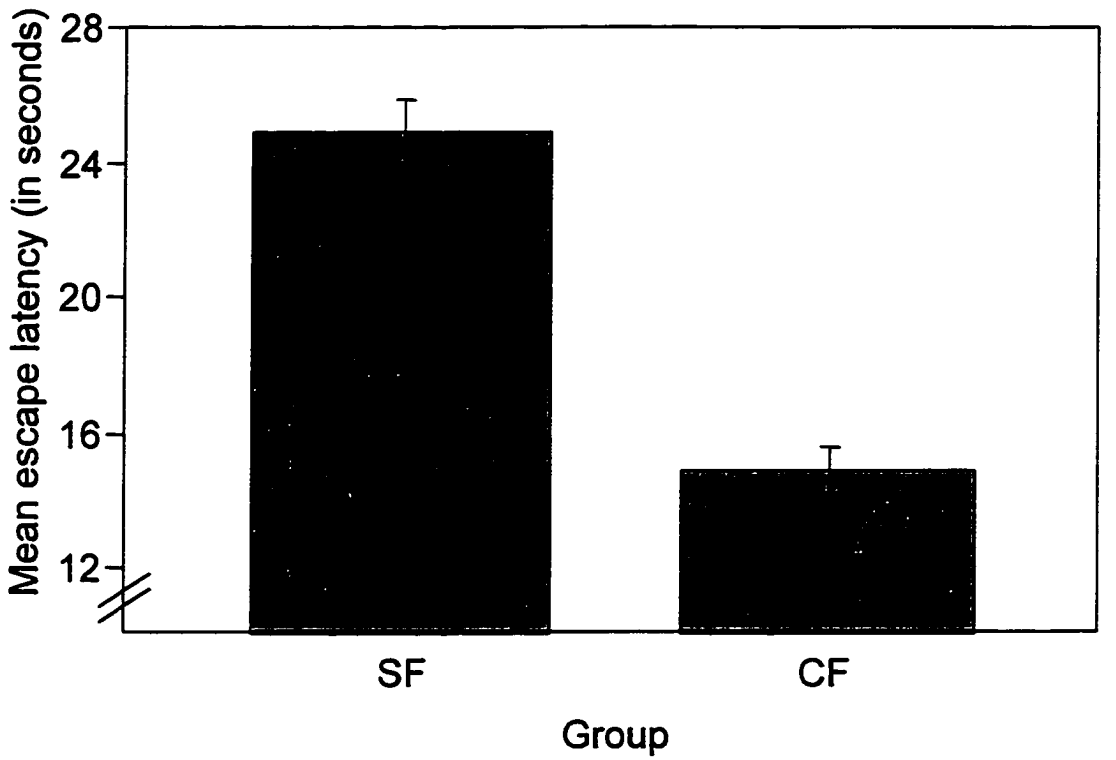


Figure 2. Mean escape latency of the Standard Trials First (SF) group was significantly longer than that of the Cued Trials First (CF) group on standard trials ($p<0.001$). Error bars represent S.E.M.

motor or motivational), an initial analysis was performed to determine if there were systematic variations in swim speed across treatment conditions that could have affected escape latency. The initial analysis was performed on data generated under cued trial conditions since it was believed that rats would employ a more direct swim path to the platform which would contain fewer direction changes and, thus allow a more accurate estimate of swim speed.

All swim speed data are reported as pixels/second (pix/s), which is the unit of measure utilized by the tracking software. This measure represents the number of computer monitor pixels darkened along the swim path in one second as the computer tracking system followed a rat's movement. While it would have been possible to convert these data into centimeters/second, or some other common unit of measure, doing so would provide no additional useful information regarding differences in swim speed between groups since swim speed is only important in terms of absolute variations among treatment groups. For this reason pixels/second has been used as the unit of measure for all analyses of swim speed data.

Two separate one-way repeated measures analyses of variance were performed to compare swim speeds; one on mean swim speed for cued trials in the SF group, and one on mean swim speed for cued trials in the CF group. In the SF group, a significant difference in swim speed was detected, $F(21,552)=6.75$, $p<.001$. In post hoc comparisons significant differences were found between the C20 and C40 groups, but no significant differences were found between the SAL and C40 or SAL and C20 groups. Animals in the C40

group swam faster than SAL animals, and animals in the SAL condition swam faster than the animals in the C20 condition. Thus, low-dose cocaine decreased swim speed while high-dose cocaine increased swim speed relative to controls.

Analysis of cued trial CF group swim speed data also showed a significant effect of Treatment condition, $F(21, 552)=7.78$, $p<.001$, on swim speed. Post hoc analysis detected a difference between SAL and C20 swim speeds, with SAL animals swimming significantly faster than C20 animals, but no other significant pairwise comparison was detected. In the CF group animals in the cocaine treatment groups swam slower than the SAL animals, but it was not a dose-dependent effect.

While finding differences in swim speed between treatment groups is interesting, and represents an important parameter of the present investigation, the purpose of analyzing these data was to determine whether treatment-related changes in swim speed could affect subsequent analyses involving escape latency. It was clear that the speed with which rats swam in the MWM was affected, to some extent, by cocaine treatment. Since speed is inherently linked to escape latency, all remaining analyses included swim speed as a covariate to control for differences in escape latency due to variations in swim speed.

Standard Trials First Group – Standard trials

In order to analyze differences in escape latency due to cocaine treatment, repeated measures analyses of variance were performed on the SF and CF groups separately with Treatment condition, Day, Trial, and Run coded

as potential sources of variance and Speed coded as a covariate. As was the case with swim speed data analysis, repeated measures analyses accounted for within-subject variability in the Treatment condition source; hence, Subject was not included as a separate factor in the model.

In the SF group, analysis of escape latency means revealed significant main effects for Treatment condition, $F(21,482)=7.79$, $p<.001$; Day, $F(3,482)=69.85$, $p<.001$; Trial, $F(2,482)=75.77$, $p<.001$; and Run, $F(1,482)=40.05$, $p<.001$. A significant Trial x Run interaction, $F(2,482)=23.56$, $p<.001$, was also found.

For the Treatment condition effect, Tukey's multiple comparison procedure indicated that significant differences in escape latency existed between the SAL and C20 groups and the SAL and C40 groups. No significant difference in mean escape latency was found between the two cocaine groups (Figure 3). Animals in both treatment conditions took longer to locate the escape platform than saline control animals.

Significant differences between escape latencies across the four days of standard trials were found to exist between Days 1 and 2, 1 and 3, 1 and 4, and 2 and 4. of training. As can be seen in Figure 4, this represents an approximately linear decrease in escape latency over days that occurred regardless of treatment condition. This is a typical result in MWM studies. Most of the learning occurred over the first day or two of conditioning. It should be noted that escape latencies for SAL animals were those of C20 and C40 animals in over all four days.

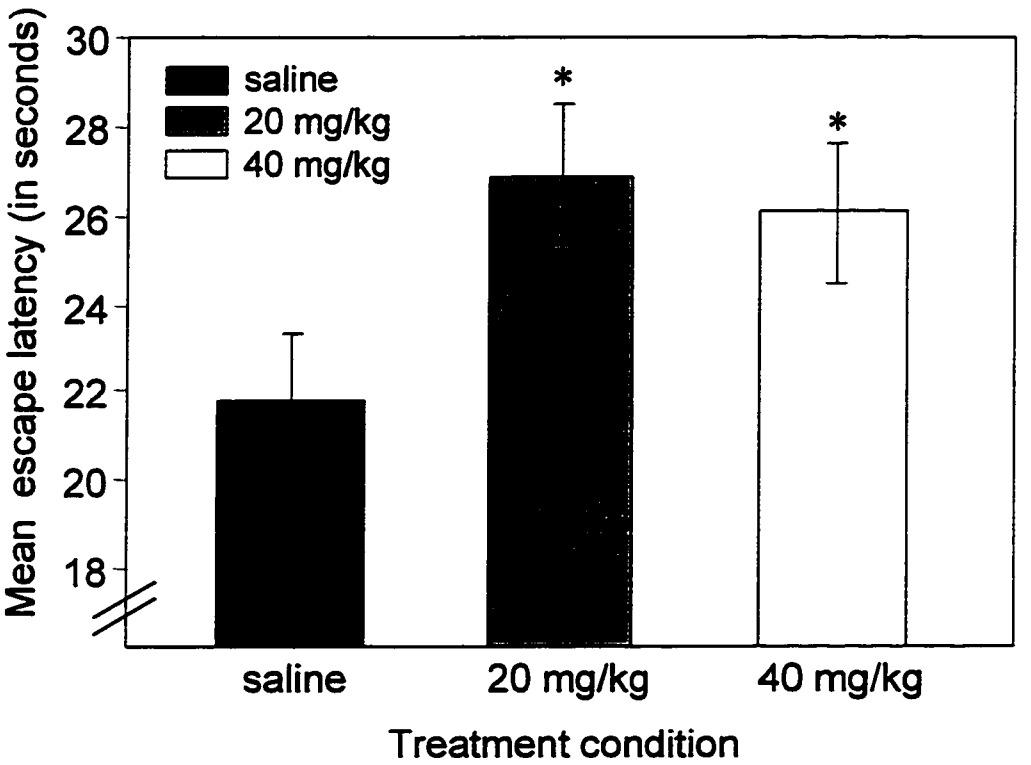


Figure 3. Mean escape latency across treatment groups for SF animals on standard trials. Error bars represent S.E.M. An asterisk (*) represents values significantly different from saline control ($p<0.001$).

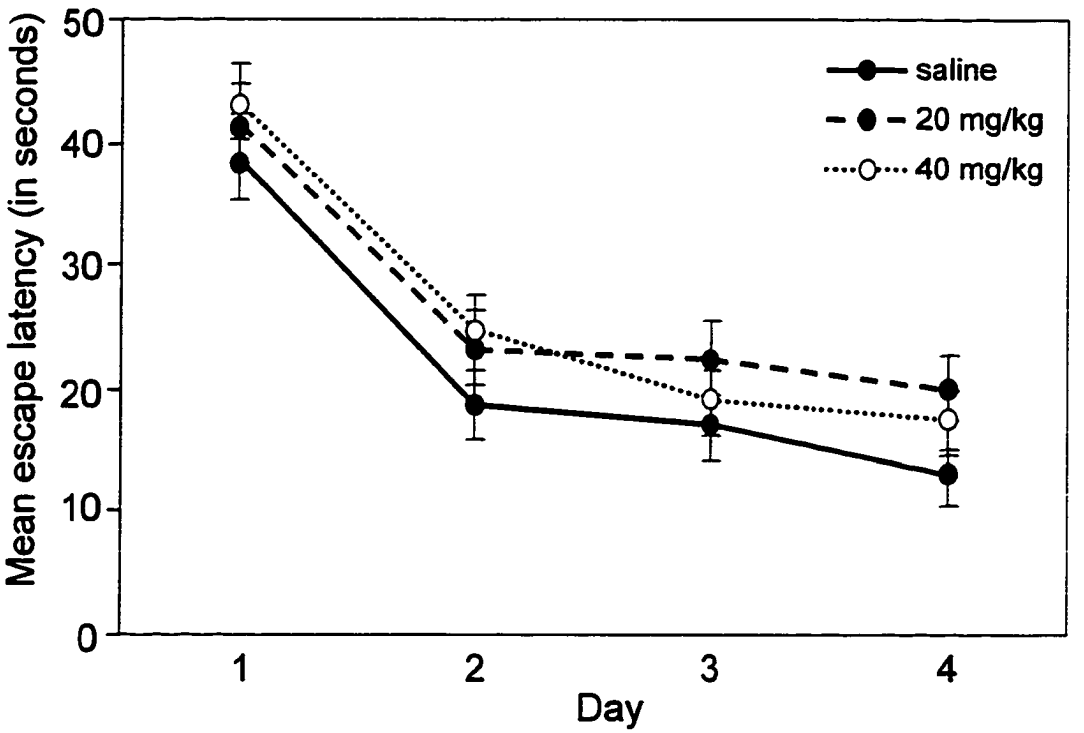


Figure 4. Mean escape latency as a function of days of training for animals in the SF group on standard trials. Data for all runs and trials are collapsed across days. Error bars represent S.E.M.

Linear decreases in escape latency were also seen across trials within days (Figure 5). Tukey's multiple comparison procedure revealed that there was a significant decrease in escape latency between each of the possible combinations of trials, indicating a significant linear improvement in escape latency on each trial, indicating effective working memory

A linear improvement in escape latency similar to that seen over trials was also seen over runs. The main effect for Run, depicted in Figure 6, was due to the fact that mean escape latency was lower on the second run of each trial regardless of treatment condition.

The analysis of path distance data for the SF group, using the same repeated measures strategy used for the escape latency data, revealed significant main effects for Treatment condition, $F(2,482)=7.28$, $p<.001$, Day, $F(3,482)=54.24$, $p<.001$, Trial, $F(2,482)=73.56$, $p<.001$, and Run $F(1,482)=43.33$, $p<.001$. A significant Trial x Run interaction, $F(2, 482)=20.62$, $p<.001$, was also detected.

Significant pairwise differences between Treatment conditions included SAL vs C20 and SAL vs C40, but no difference was detected between the two cocaine treatment conditions (Figure 7). Comparisons of distance over Day (Figure 8) revealed differences between Day 1 and Days 2, 3, and 4, but no significant pairwise differences between any other pairs of days. Pairwise comparisons between Trials (Figure 9) were all significant, as was the difference between Run 1 and Run 2 (Figure 10).

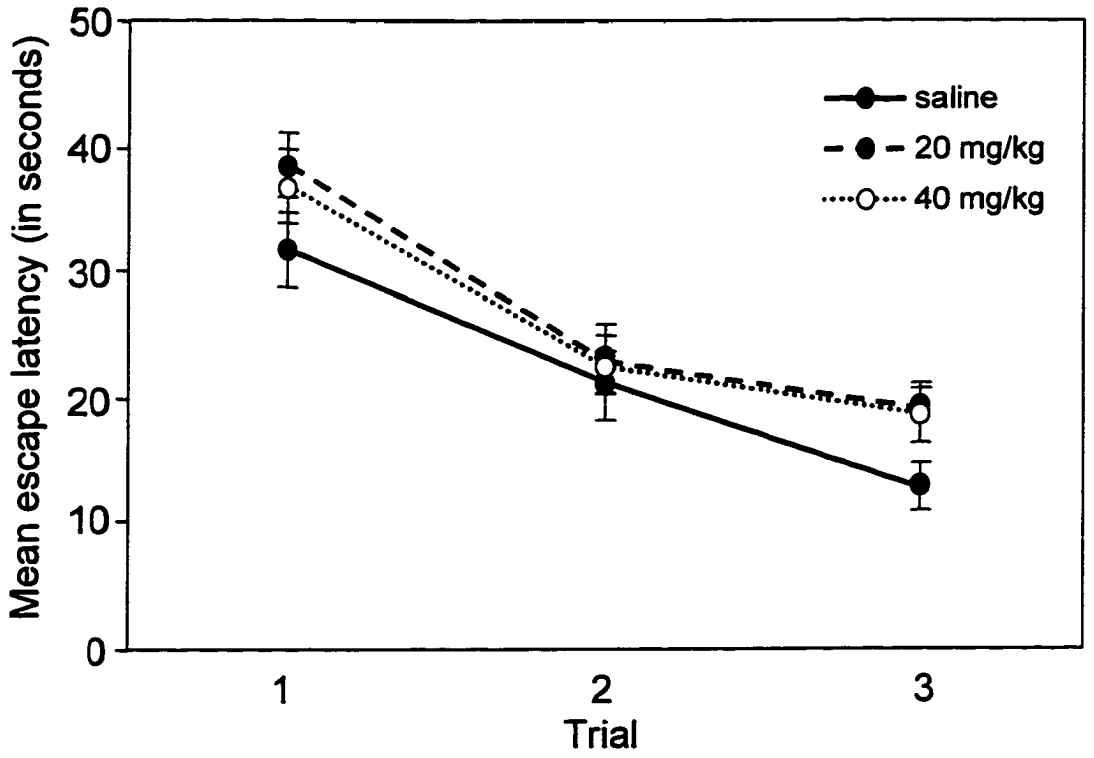


Figure 5. Mean escape latency as a function of trial on standard trials. Data from all four days of training are collapsed across trials for the SF group. Error bars represent S.E.M.

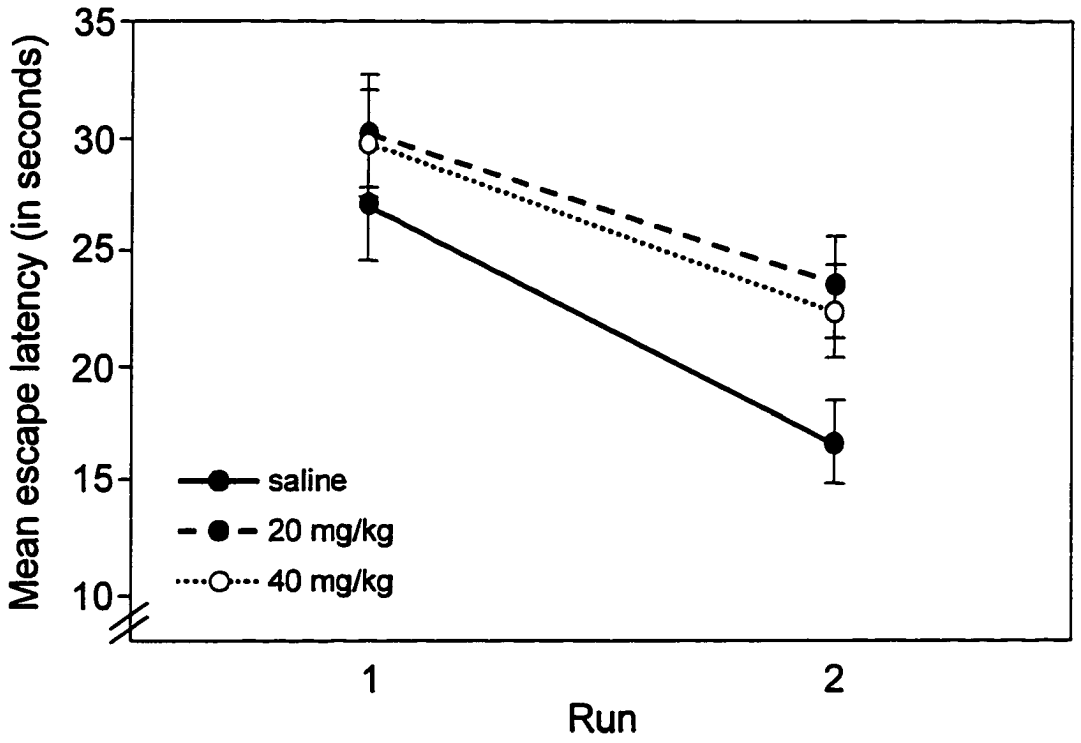


Figure 6. Mean escape latency as a function of run for standard trials. Data from all days and trials were collapsed across run for the SF group. Error bars represent S.E.M.

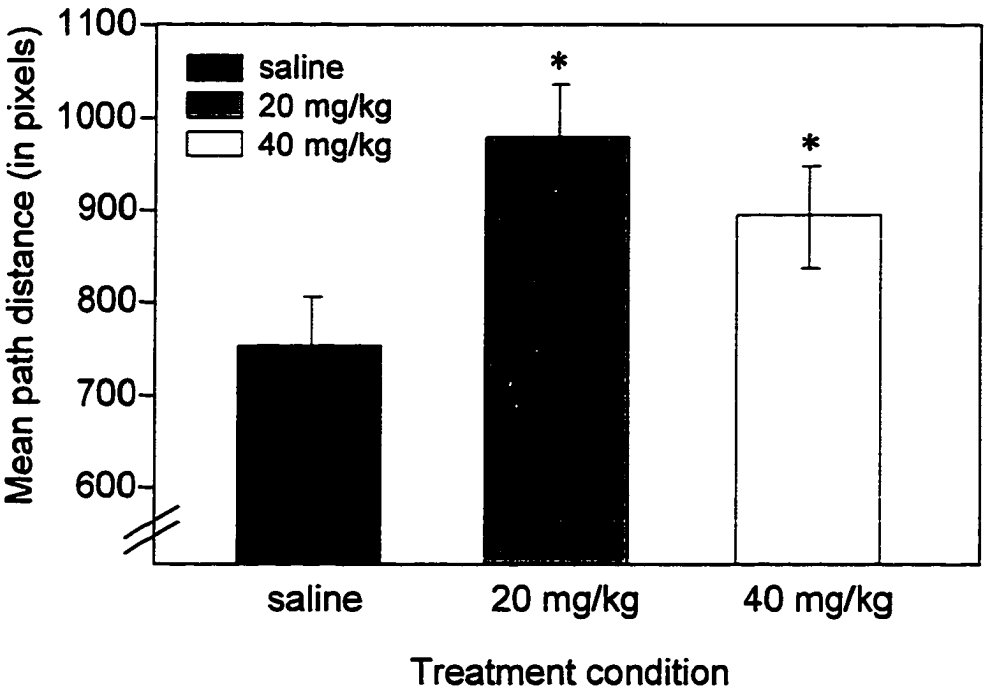


Figure 7. Mean path distance as a function of treatment condition for SF animals on standard trials. Error bars represent S.E.M. An asterisk (*) represents values significantly different from saline control ($p<0.001$).

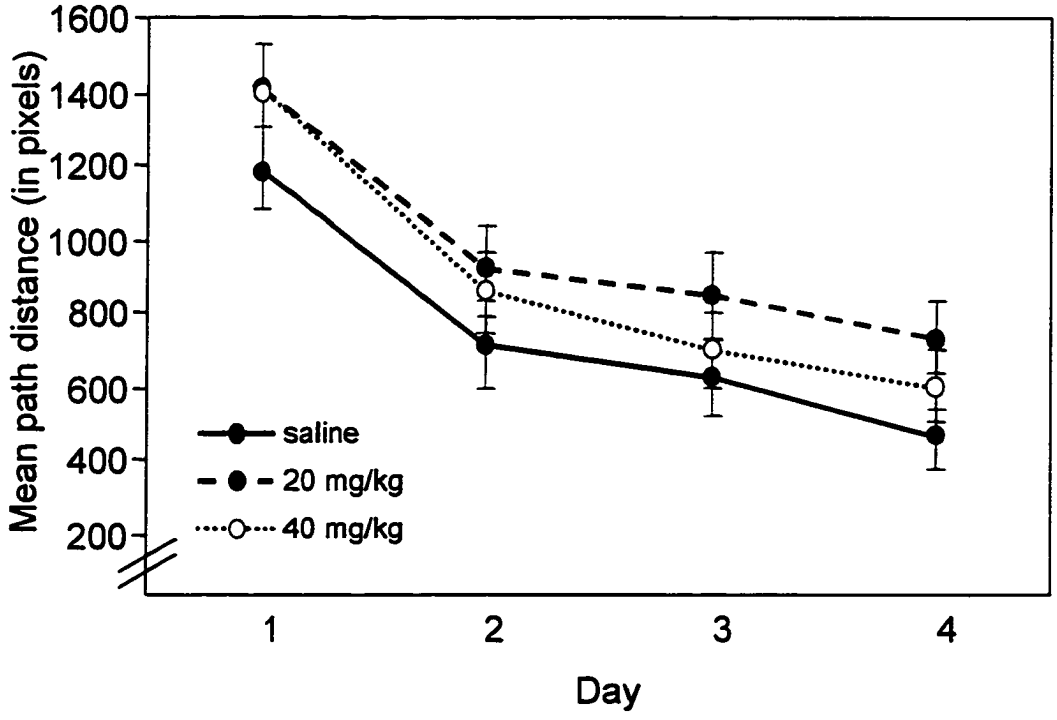


Figure 8. Mean path distance as a function of day for SF animals on standard trials. Error bars represent S.E.M.

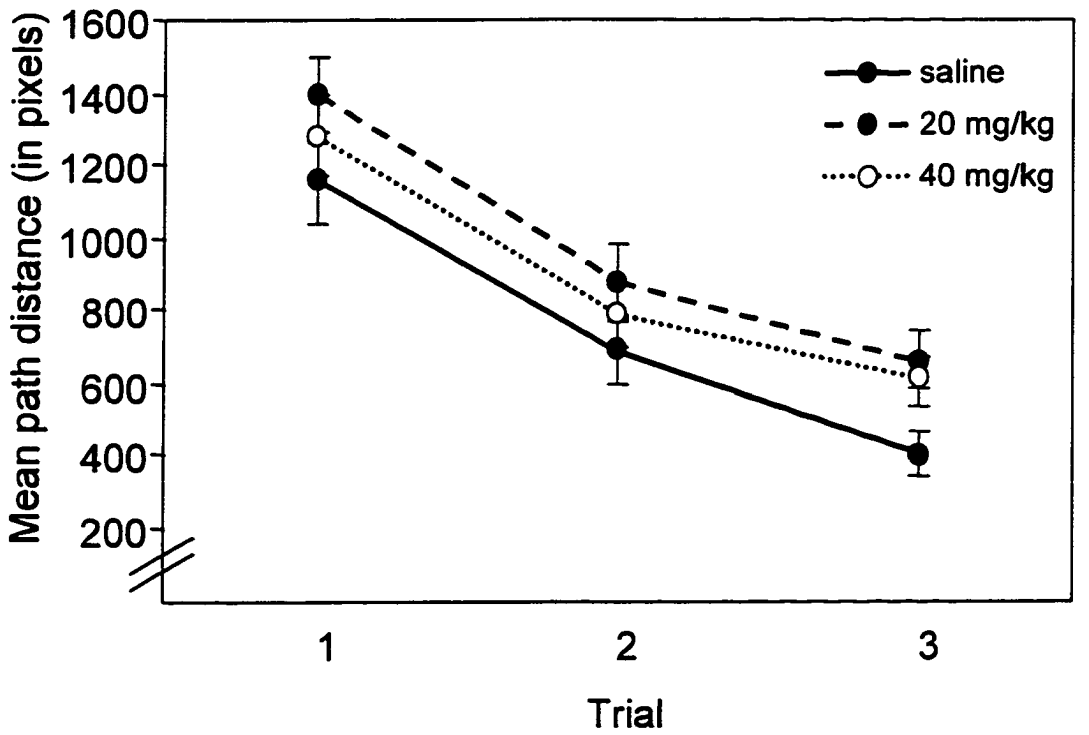


Figure 9. Mean path distance as a function of trial for SF animals on standard trials. Data from all days and runs are collapsed across trials. Error bars represent S.E.M.

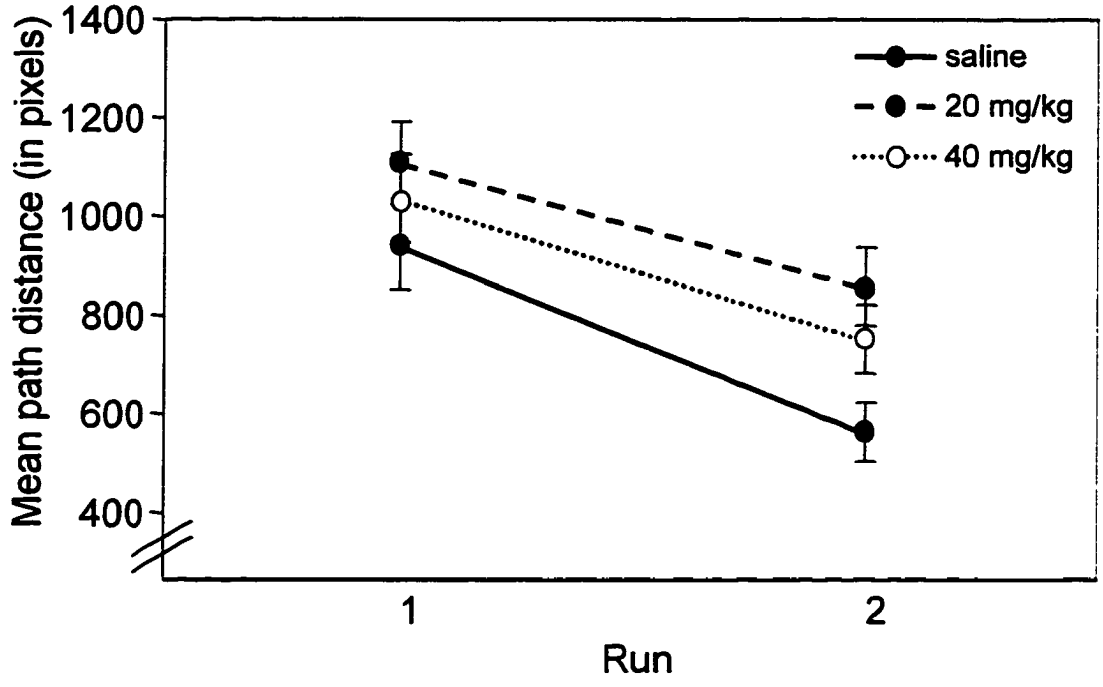


Figure 10. Mean path distance as a function of run for SF animals on standard trials. Data from all trials and days are collapsed over run. Error bars represent S.E.M.

To determine whether dwell ratios were significantly different from .25, a one-sample t-test was performed on data from days 2,3, and 4. It was determined that the group, as a whole, showed a preference for the previous day's quadrant, $t(71)=4.06$, $p<.000$ (mean=.31 ± .01) A separate repeated measures one-way ANOVA was also performed to determine differences among treatment groups. No significant differences were detected, $F(2,21)=.391$, $p=.681$.

Cued First Group – Standard trials

In the CF group, significant main effects for escape latency were found for Treatment condition, $F(21,482)=2.00$, $p=.006$, Day, $F(3,482)=8.31$, $p<.001$, Trial, $F(2, 482)=64.02$, $p<.001$, and Run, $F(1,482)=21.64$, $p<.001$. Significant Treatment condition x Day, $F(6,481)=3.11$, $p=.003$, and Trial x Run $F(2,483)=7.53$, $p=.001$, interactions were also found, as was a significant three-way interaction among Treatment condition, Day and trial, $F(12,483)=2.01$, $p=.02$.

Tukey's multiple comparisons indicated significant differences in mean escape latency between the SAL and C40 conditions, and between the C20 and C40 conditions (Figure 11). No significant difference in escape latency was found between the Saline and C20 conditions.

Post hoc tests of the Day effect revealed differences in escape latency between Day 5 and 6, Day 5 and 7, and Day 5 and 8. As can be seen in Figure

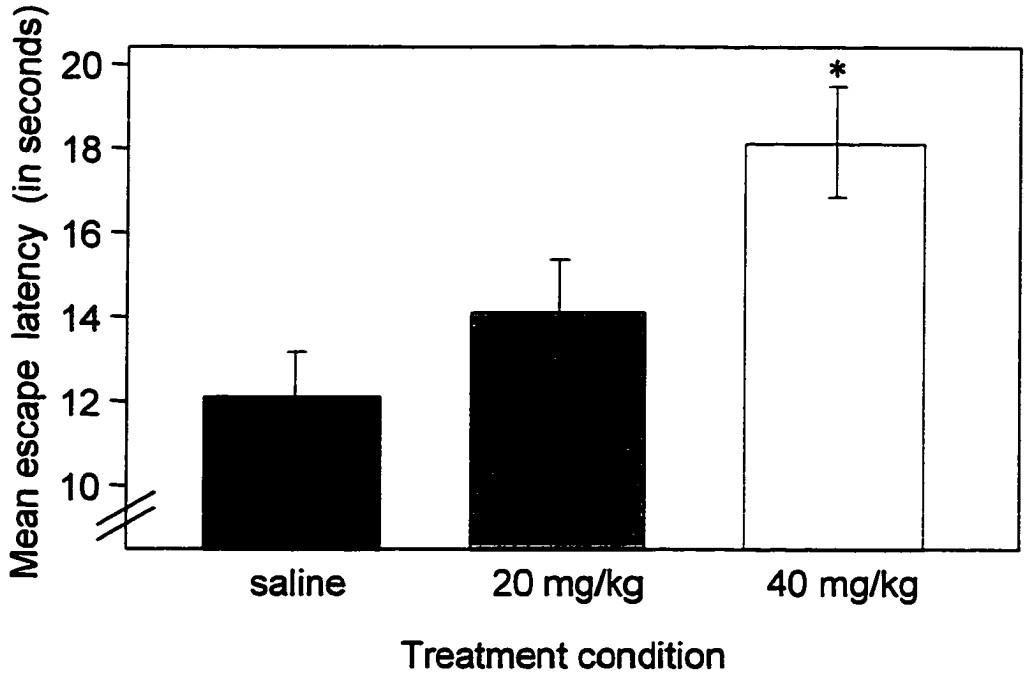


Figure 11. Mean escape latency across treatment conditions for CF animals on standard trials. Error bars represent S.E.M. An asterisk (*) represents values significantly different from other treatment conditions ($p<0.001$).

12, it is apparent that most of the learning in the CF group occurred between the first and second days of standard trial training.

Analysis of the differences between escape latency over trials using Tukey's multiple comparisons test indicates significant differences among all pairwise combinations of trials. As is seen in Figure 13, escape latency decreased across the three trials, and the significant main effect for Run was the result of decreases in escape latency in the second run of each trial relative to the first (Figure 14).

The analysis of variance performed on distance data for the CF group resulted in significant main effects for Treatment condition, $F(21,482)=1.97$, $p<.006$, Day, $F(3, 482)=8.03$, $p<.001$, Trial, $F(2,482)=58.76$, and Run, $F(1,482)=22.38$, $p<.001$. Significant two-way Day x Condition, $F(6, 482)=3.25$, $p<.004$, and Trial x Run, $F(2,482)=8.28$, $p<.001$ interactions were also found, along with a significant three-way Day x Treatment condition x Trial interaction, $F(12,482)$ $p<.01$.

As was the case for distance data in the SF group Tukey's analyses were performed on the distance data for the CF group. Post hoc analysis revealed significant pairwise differences between the SAL and C40 conditions and the SAL and C20 conditions, but not between the C20 and C40 conditions (Figure 15). Animals in both C20 and C40 groups swam farther than SAL animals before reaching the platform. In addition, C20 animals swam farther than C40 animals, but this difference in path distance was not significant.

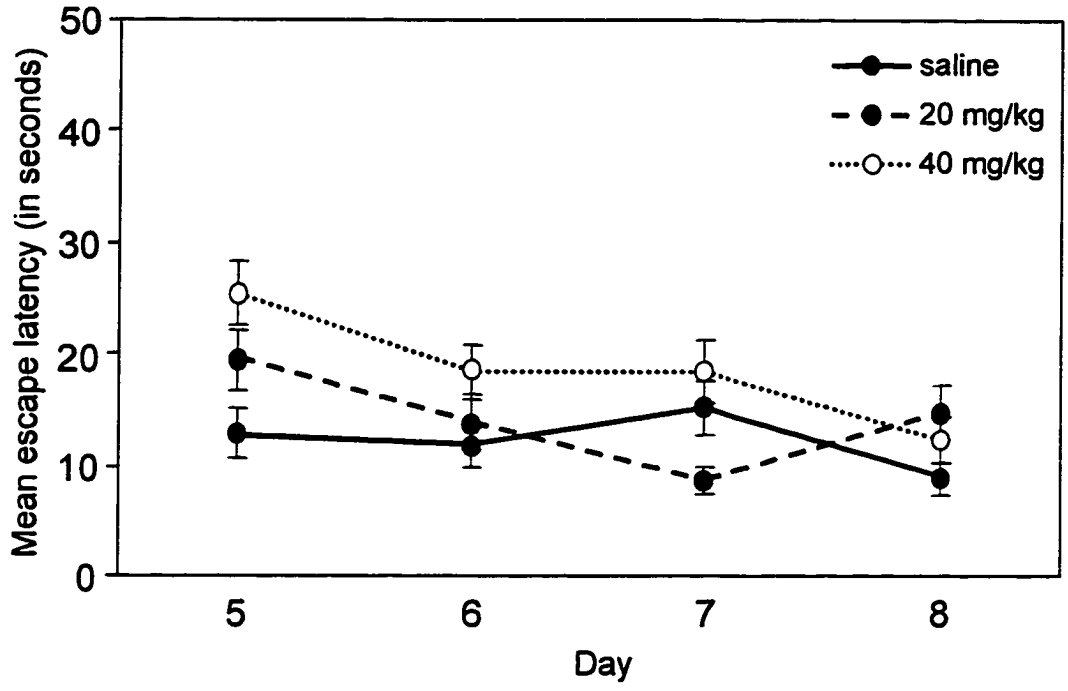


Figure 12. Mean escape latency as a function of day for CF animals on standard trials. Error bars represent S.E.M.

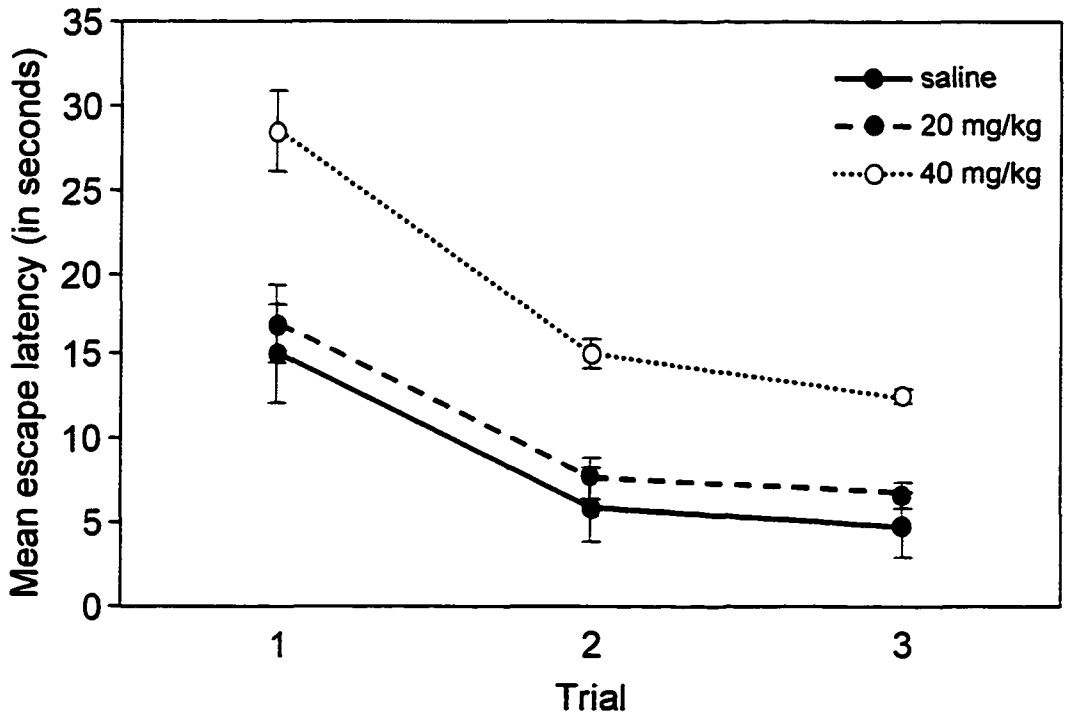


Figure 13. Mean escape latency as a function of trial for CF animals on standard trials. Data from all days and runs are collapsed across trial. Error bars represent S.E.M.

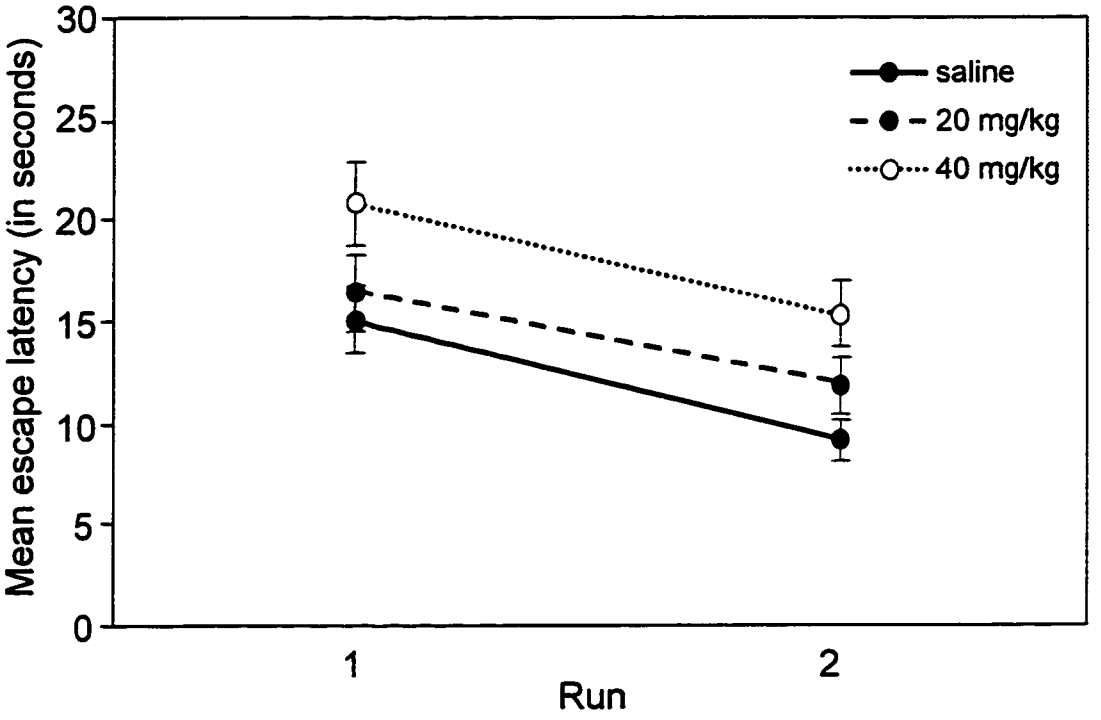


Figure 14. Mean escape latency as a function of run for CF animals on standard trials. Data from all days and trials are collapsed across run. Error bars represent S.E.M.

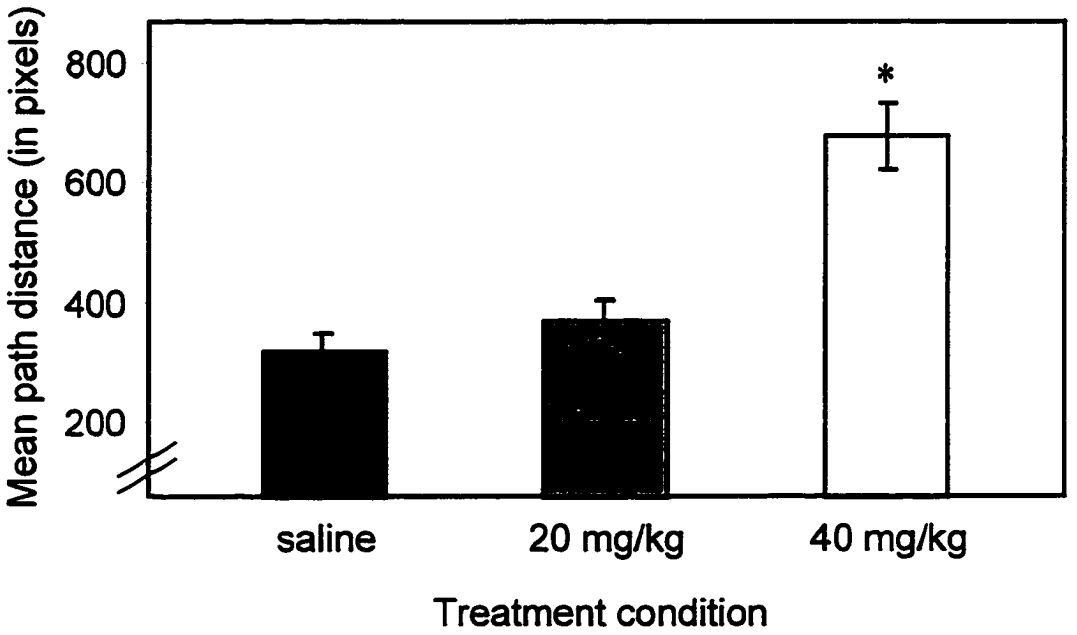


Figure 15. Mean path distance across treatment conditions for CF animals on standard trials. Error bars represent S.E.M. An asterisk (*) represents values significantly different from saline control ($p<0.001$).

Significant differences in swim distance only occurred between the first and second days of standard trial conditioning. As is illustrated in Figure 16, performance was inconsistent across days for the final three days of training. As was the case in the SF group, significant decreases in distance swum were seen between each of the three trials within days (Figure 17). Distance between Runs within trials for the CF group (Figure 18) was also consistent with that of the SF group. Distance decreased from the first to the second run in all cases.

A separate repeated measures analysis of variance was performed on data representing perseverative responding on standard trials in the CF group. The datum for this analysis was, as in the SF group analysis, derived from the amount of time spent in the previous day's platform quadrant on the first run of the second, and subsequent, days of standard trials (days 6,7 and 8). A one-sample t-test revealed that the group, as a whole, showed a preference for the previous day's quadrant, $t(72)=2.59$, $p=.012$ (mean=.34 ± .03). Reminiscent of SF group results, no significant differences among treatment conditions were found for this measure, $F(2,21)=.212$, $p=.811$.

Cued First vs Standard First – Cued trials

A Kolmogorv-Smirnov analysis for two independent samples performed on escape latency means for the CF and SF groups showed that there was a significant difference between the groups in terms of their ability to locate and swim to the visible platform, $Z(1152)=2.42$, $p<.001$. Animals in the SF group

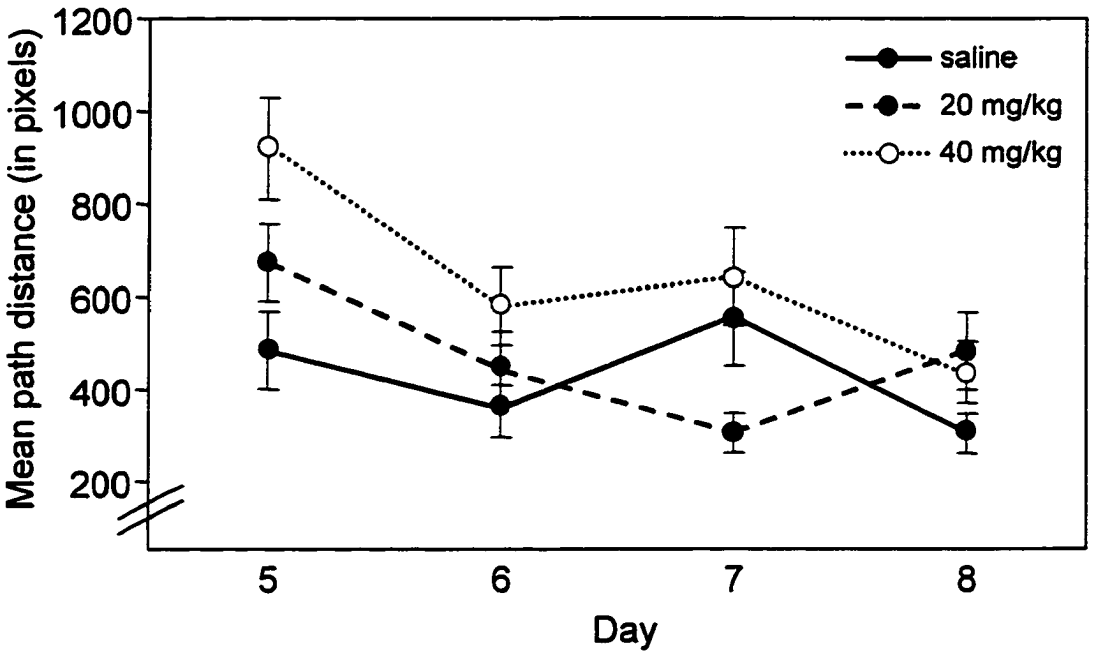


Figure 16. Mean path distance as a function of day for CF animals on standard trials. Data from all trials and runs are collapsed across day. Error bars represent S.E.M.

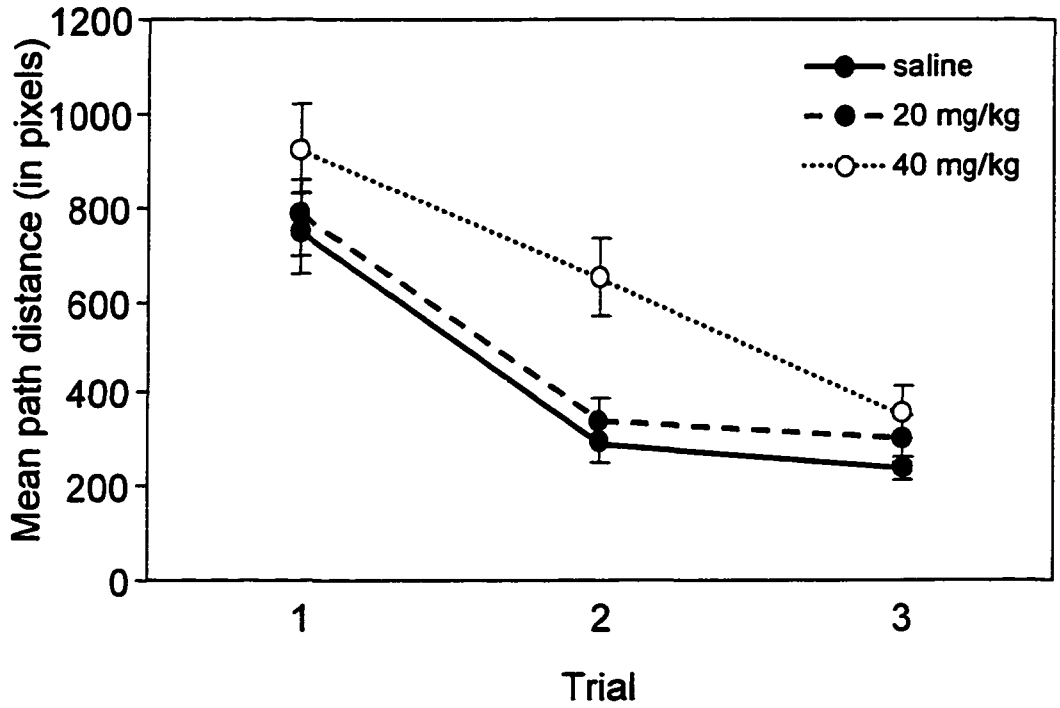


Figure 17. Mean path distance as a function of trial for CF animals on standard trials. Data from all days and runs are collapsed across trial. Error bars represent S.E.M.

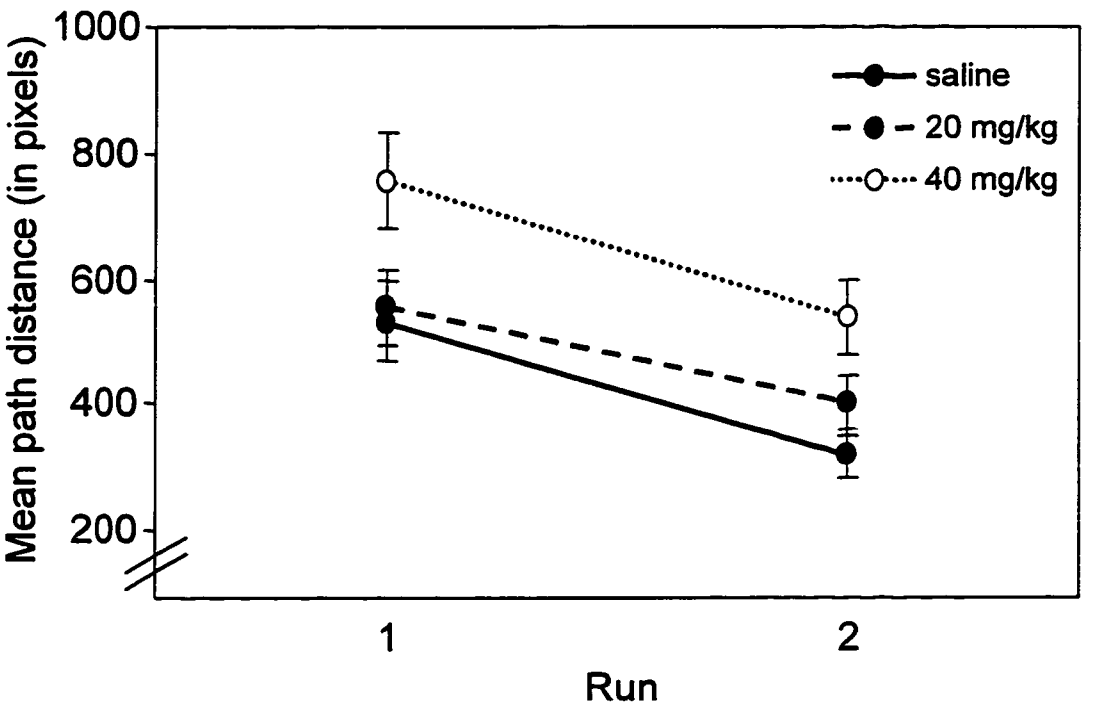


Figure 18. Mean path distance as a function of run. Data from all days and trials are collapsed across run. Error bars represent S.E.M.

were able to locate the platform faster than animals in the CF group, presumably because they had prior experience in the maze. These data are presented in Figure 19.

Standard First Group – Cued Trials

The repeated measures analysis of variance performed on cued trials escape latency data for SF animals revealed significant main effects for Treatment condition, $F(21,482)=14.89$, $p<.001$, Day, $F(3,482)=34.36$, $p<.001$, Trial, $F(2,482)=46.25$, $p<.001$, and Run, $F(1,482)=24.98$, $p<.001$. Significant two way interactions were found for Treatment condition x Day, $F(6,482)=4.99$, $p<.001$, Treatment condition x Trial, $F(4,482)=9.00$, $p<.001$, Treatment condition x Run, $F(2,482)=3.31$, $p=.04$, Day x Trial, $F(6,482)=6.14$, $p<.001$, and Trial x Run, $F(2,483)=9.21$, $p<.001$. A significant three way interaction was found for Day x Trial x Run, $F(6,483)=2.75$, $p=.012$. Post hoc analysis revealed significant differences between SAL and C40 groups with longer latencies in the C40 animals (Figure 20).

The same analysis run on Distance data revealed significant main effects for Treatment condition, $F(21,482)=13.51$, $p<.001$, Day, $F(3,482)=32.53$, $p<.001$, Trial, $F(2,482)=35.76$, $p<.001$, and Run, $F(1,482)=25.10$, $p<.001$. Significant two way interactions were found for Treatment condition x Day, $F(6,482)=5.00$, $p<.001$, Treatment condition x Trial, $F(4,482)=7.03$, $p<.001$, Treatment condition x Run, $F(2,482)=3.85$, $p=.02$, Day x Trial, $F(6,482)=5.06$, $p<.001$, and Trial x Run, $F(2,483)=8.41$, $p<.001$. A significant three way interaction was found for

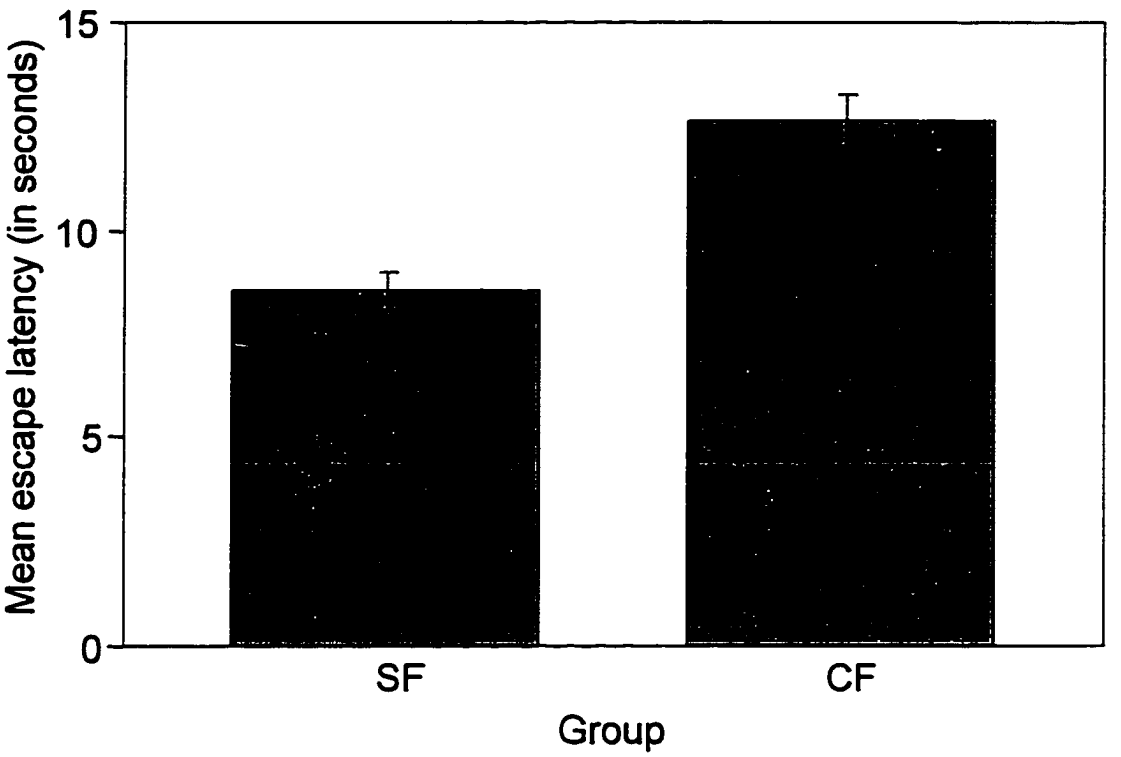


Figure 19. Mean escape latency of the SF group was significantly shorter than that of the CF group on cued trials ($p<0.001$).

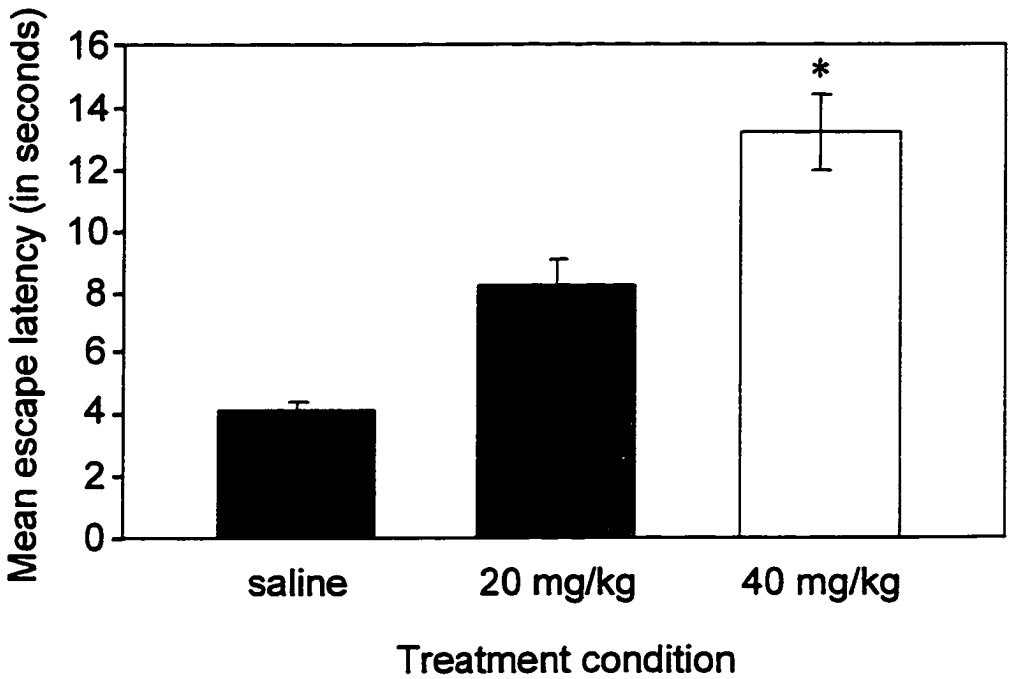


Figure 20. Mean escape latency across treatment conditions for SF animals during cued trials. Error bars represent S.E.M. Asterisks (*) indicate values significantly different from saline control ($p>.001$).

Day x Trial x Run, $F(6,483)=2.38$, $p=.028$. Post hoc analysis revealed that C40 animals swam farther than SAL animals before finding the platform; these results are depicted in Figure 21.

A one-sample t-test performed on dwell ratio data indicated that the SF group showed no preference for the previous day's platform quadrant, $t(72)=1.93$, $p>.05$ (mean=.30 ± .03). Further, repeated measures ANOVA revealed no significant differences among treatment conditions, $F(2,21)=.231$, $p>.05$.

Cued First Group – Cued trials

Repeated measures analysis of variance performed on cued trials escape latency data for CF animals reveled significant main effects for Treatment condition, $F(21,482)=3.11$, $p<.001$, Day, $F(3,482)=71.85$, $p<.001$, Trial, $F(2,482)=60.74$, $p<.001$, and Run, $F(1,482)=13.27$, $p<.001$. Significant two way interactions were found for Treatment condition x Day, $F(6,482)=5.99$, $p<.001$, Treatment condition x Trial, $F(4,482)=9.00$, $p<.001$, and Trial x Run, $F(2,482)=12.26$, $p<.001$. A significant three way interaction was found for Day x Trial x Treatment condition, $F(12,482)=2.75$, $p=.012$. Post hoc analysis indicated that C40 animals had longer escape latencies than C20 or SAL animals, but C20 and SAL animals did not differ (Figure 22).

The repeated measures analysis of swim distance data detected significant main effects for Treatment condition, $F(21,482)=3.44$, $p<.001$, Day, $F(3,482)=54.15$, $p<.001$, Trial, $F(2,482)=51.65$, $p<.001$, and Run,

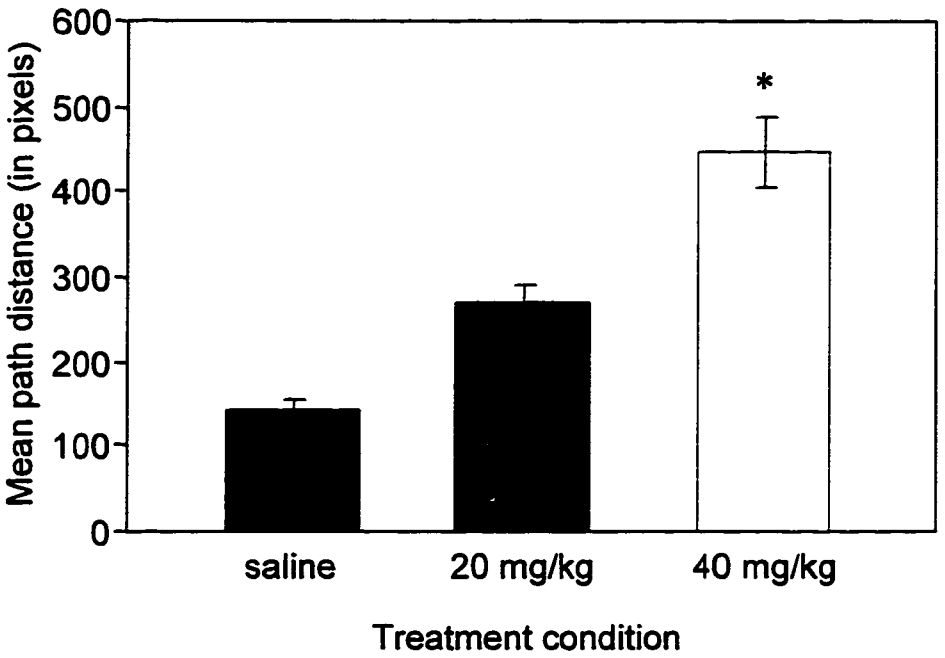


Figure 21. Mean path distance across treatment conditions for SF animals during cued trials. Error bars represent S.E.M. Asterisks (*) indicate values significantly different from saline controls ($p>.001$).

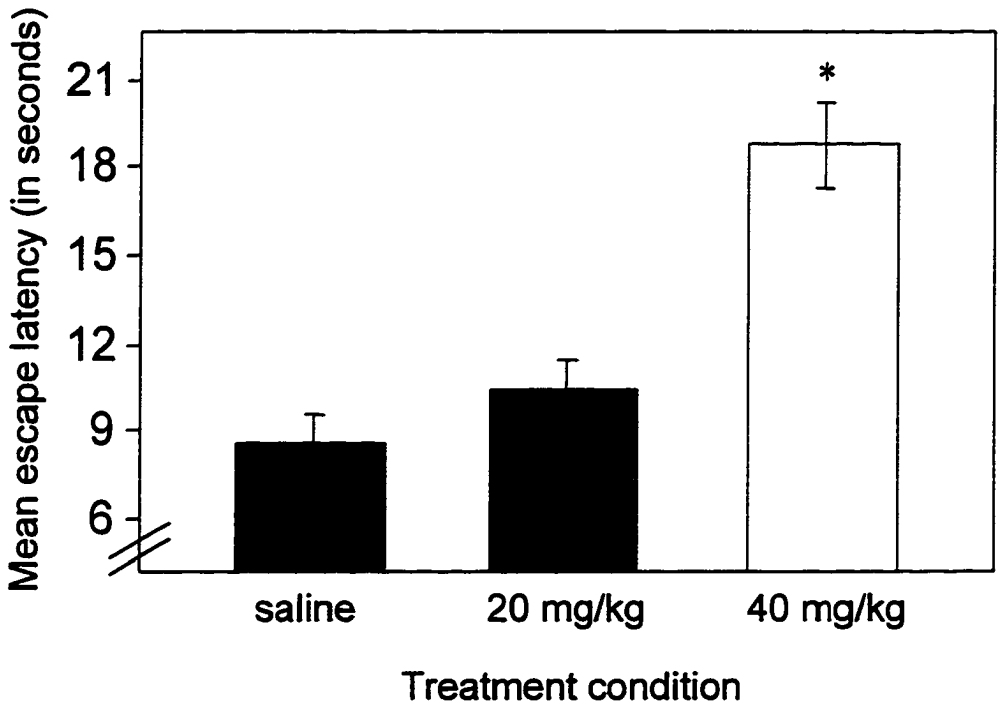


Figure 22. Mean escape latency across treatment conditions for CF animals during cued trials. Error bars represent S.E.M. Asterisks (*) indicate values significantly different from saline controls ($p>.001$).

$F(1,482)=11.30$, $p<.001$. Significant two way interactions were found for Treatment condition x Day, $F(6,482)=4.77$, $p<.001$, Day x Trial, $F(6,482)=6.03$, $p<.001$, and Trial x Run, $F(2,483)=10.12$, $p<.001$. A significant three way interaction was found for Day x Treatment condition x Trial, $F(12,483)=2.32$, $p=.007$. As was the case with escape latency, post hoc analysis revealed that C40 animals swam farther than C20 or SAL animals, but C20 and SAL animals did not differ (Figure 23).

A one-sample t-test performed on dwell ratio data indicated that the CF group showed no preference for the previous day's platform quadrant, $t(72)=1.33$, $p>.05$ (mean=.29 ± .02). Further, repeated measures ANOVA revealed no significant differences among treatment conditions, $F(2,21)=1.27$, $p>.05$.

Discussion

Effects of prior training (CF vs SF)

Traditionally in MWM studies standard trials escape latency is reported as the primary measure of performance. In the present study, comparison of standard trial performance between the CF and SF groups revealed a distinct difference in escape latency between the groups. Animals in the CF group, having had experience with the apparatus prior to being tested on standard trials, took less time to locate the hidden platform than their SF counterparts regardless of cocaine treatment condition. Of course, it should be noted that for rats in the

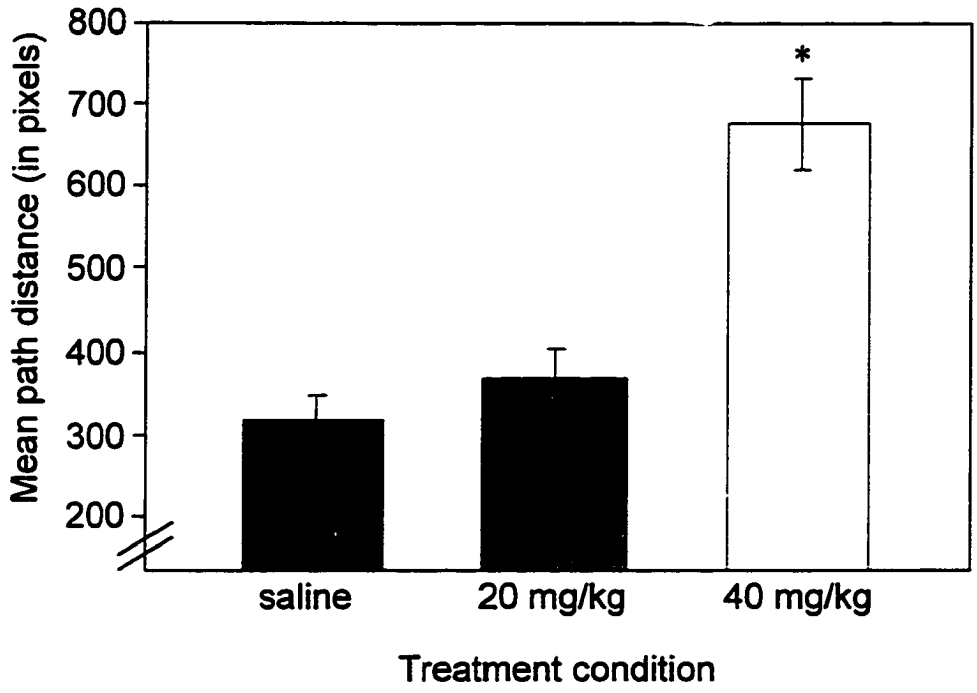


Figure 23. Mean path distance across treatment conditions for CF animals during cued trials. Error bars represent S.E.M. Asterisks (*) indicate values significantly different from saline controls ($p>.001$).

CF group the first day of standard trial training was their fifth day of swimming in the maze, and for the rats in the SF group standard trials were run beginning on their first day of swimming. This difference in training procedure between CF and SF groups appears to have had an important influence on the speed with which rats acquire the standard MWM task.

In studies where apparatuses are designed to assess learning it is important to realize that subjects are required to learn more than simply the parameters of the particular task in question. In a MWM task, before a rat can efficiently locate a hidden platform there are several prerequisites that must be mastered. First, the rats must learn to swim. Although evolution has provided rats with the means to become excellent swimmers, swimming is not (at least for Sprague Dawley rats) their primary mode of locomotion, so they require a certain amount of practice in order to maximize their efficiency through water.

In addition to learning to swim efficiently, rats must also learn that swimming along the perimeter of the pool (invariably a rat's initial response to being placed in the apparatus) will not result in escape from the water. Also, they must learn that there is one, and only one, place to which they can swim that will allow an opportunity for escape. Only after learning about these properties of the test environment can rats begin to use extramaze cues to hone their ability to reduce escape latency. This being said, it stands to reason that rats previously exposed to swimming and to using a platform to escape water, namely the CF group, should be better equipped than SF group animals to locate the hidden platform when initially exposed to standard trials. Animals required to learn

about swimming, search strategy, etc. while simultaneously learning to locate the hidden platform on standard trials, which was the case for animals in the SF group, were exposed to a more challenging set of circumstances upon their first exposure to the MWM task; therefore, it is not surprising that CF animals, learned to locate the hidden platform faster than SF animals.

In most research where the MWM is used to assess the effects of psychoactive drugs or brain lesions, cued trials are included as a way of indicating that subjects were not hindered by the effects of the independent variable in terms of their ability to locomote through the water to a particular place. The implication is that if animals in an experimental group can navigate to a platform as efficiently as control group animals, any significant difference in escape latency during standard trials must be due to the effects of the manipulation on memory, or some other cognitive process, rather than on visual or motor processes. Typically, these cued trials are introduced subsequent to collecting data on standard trials. While cued trials are a necessary component of a thorough MWM study, it seems that testing animals on cued trials only after they have mastered the task limits any analysis of the effects of the independent variable on learning per se, and only allows analysis of the effects of the manipulation on performance of a previously learned task.

Of course, moving the hidden platform to new locations, as is commonly done in MWM studies in so-called transfer tests, does require an animal to learn new variations of previously learned responses, but it does not facilitate analysis of the effects of a manipulation on an animal's ability to learn the task in the first

place. The present study provides an excellent example of the value of being able to analyze the effects of independent variables on both learning and performance in a MWM procedure.

This issue is particularly important because the effects of some drugs can vary with the testing environment. For example, it has been reported that prenatal exposure to cocaine does not result in significant impairment in the ability of offspring to learn in standard tests of operant or reflexive conditioning. However, when similar tests are performed under stressful conditions, offspring exposed prenatally to cocaine require more trials to reach criterion responding on a learning task (Spear, Campell, Snyder, Silveri, & Katovic, 1998).

It was previously noted that animals in the CF group found the hidden platform faster than the animals in the SF group which may be more indicative of increased ability of CF animals to escape due to prior learning than a decreased ability of SF animals to escape because of the effects of cocaine. While it could be argued that the SF animals suffered poor performance relative to the CF group because they differed in learning history, examination of the panels in Figure 24 reveals that cocaine may have a dose-dependent effect on learning. Comparisons of same-day performance across the panels of the figure show a discernible difference in escape latency between treatment conditions in the CF group on the first day of standard trial training (Day 5). This indicates that even though these animals had four days of previous training on cued trials, there was some effect of cocaine on escape latency during initial standard trials. When comparing the corresponding data across conditions in the SF group for their first

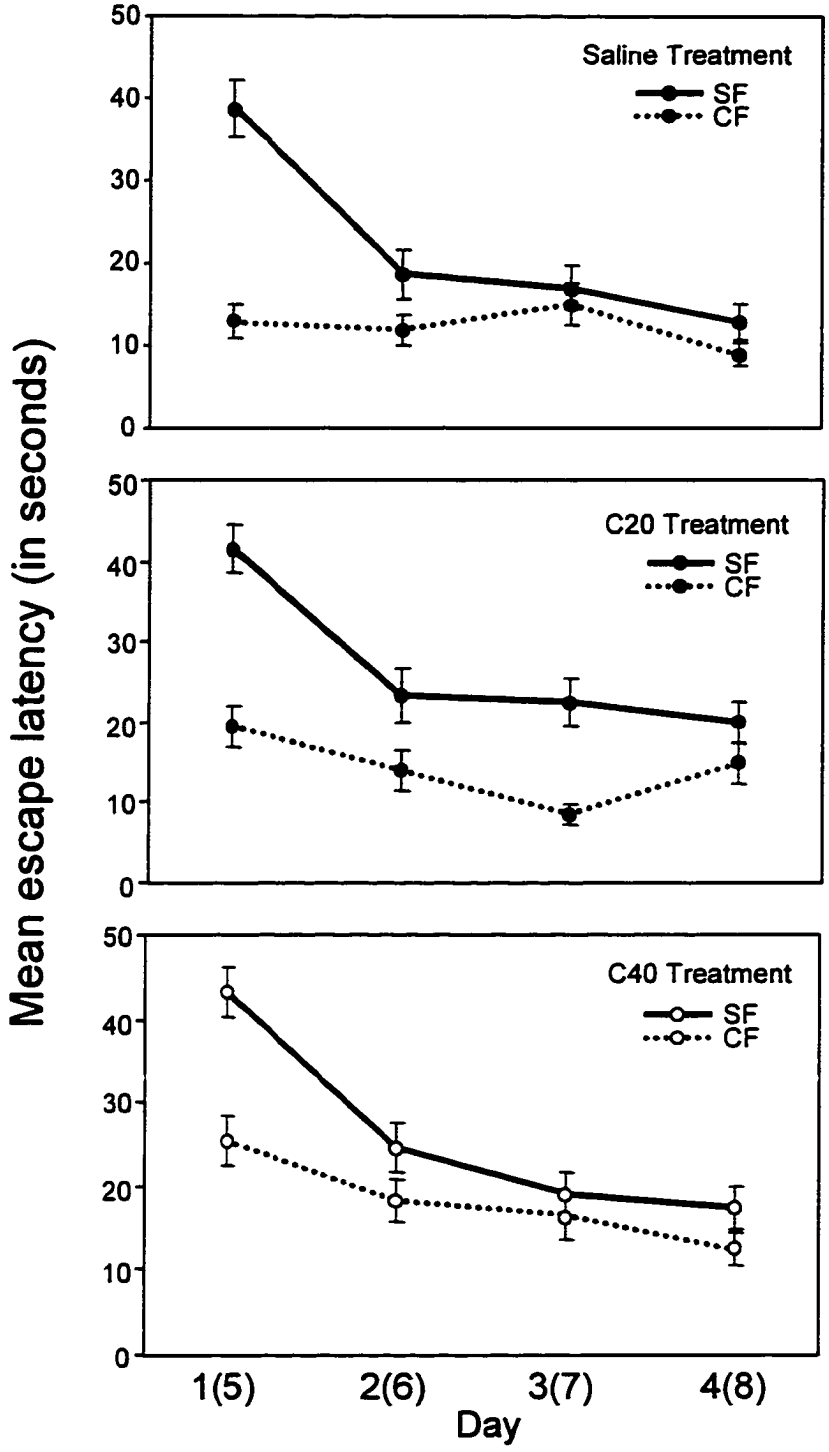


Figure 24. Escape latency on standard trials as a function of day for SAL, C20 and C40 groups. Data from all trials and runs are collapsed across days. Standard trials for SF animals occurred on days 1 through 4. Standard trials for CF animals occurred on days 5 through 8. Error bars represent S.E.M.

day of standard trials (Day 1) the effect of cocaine treatment condition is not readily apparent; the mean escape latency between treatment groups was similar for all the treatment conditions.

This comparison not only exhibits the effect of cocaine on learning in the present study, but also indicates that the effects of an independent variable, in general, can differ in a MWM study depending on whether the subjects have had previous experience with the apparatus and the behavioral task. The issue here is to understand that any analysis of a psychoactive substance on learning should be tested under as many different learning conditions as possible in order to elucidate as many manifestations of a drugs' effects as possible.

The present study is unique in that cued trials were employed prior to standard trials to systematically assess the effects of prior learning on MWM performance. In light of the differences between the SF and CF groups, it is suggested that future MWM studies include appropriate CF control groups. This may help determine whether the effects of an intervention are equivalent in both types of learning situations.

Standard Trials First Group (SF) – Standard trials

In reviewing the behavior of animals on MWM tasks it is customary to discuss performance in terms of improved escape latency over successive days. Improvement over days is attributed to reference memory. This Improvement is probably a function of the rats' knowledge of how to find the platform. Reference

memory included swimming skills, the use of extramaze cues as points of reference, and any other relatively permanent skills acquired through practice.

In the typical MWM procedure the platform is not moved to a different location each day, the animals must swim to the same location for several days. Thus, it is presumed that decreases in escape latency over successive days indicate an increase in an animal's ability to use extramaze cues for navigation to a particular location (Morris, 1981). Because an increase in general navigation ability, consolidated and re-accessed over relatively long periods of time, occurs this increase in performance may involve reference memory components. Thus, differences in escape latency among treatment groups across days are typically attributed to effects on reference memory.

In the present study there was a decrease in escape latency across days in each treatment group. Because of this, one might conclude that cocaine does not affect reference memory. However, when performance between treatment groups is considered, the situation is less clear. Since escape latency in the saline condition was significantly shorter than in the cocaine groups on most days, it would appear that, for the SF group, there is an effect of cocaine on reference memory. Although no dose-dependent effect occurred, it is apparent that cocaine does have an effect on measures of reference memory.

Measurements of reference memory have been used to elucidate the effects of pharmacological and surgical manipulations on performance in the MWM. In addition, the MWM is also useful for the exploration of working memory (Morris, 1983; Wishaw, 1984). Tasks that involve working memory rather than

reference memory are those that require a subject to access specific information about very recent events. The animals must use that information to respond in an appropriate manner with regard to the existing stimulus environment. For example, if a rat finds the hidden platform in a particular location on the first run of a trial, it must temporarily store information regarding the location of that platform by using extramaze cues. The animal must then access information about that location and apply it during the next run if it is to maximize escape efficiency. Failure to use information from one run to the next would result in failure to decrease escape latency between trials (and runs within trials). This would indicate a failure of working memory. Improvement of intraday performance when the hidden platform remains in the same location for all trials on a given day is evidence for the existence of functional working memory.

The results of the present study support those of Morris (1983) and Wishaw (1984) by making it clear that the MWM can be used for detailed analyses of the effects of various manipulations on working memory. In addition to assessing the effect of cocaine on learning across days, the reference memory component, it was possible to analyze within day performance to determine the effects of cocaine on behavior that would be considered under the control of working memory; that is, analysis of a rat's ability to know where the platform is on a given run or trial rather than how to find it.

When MWM performance is evaluated such that trials within days are analyzed (i.e. working memory), it can be seen that differences in escape latency between control and experimental animals across days, indicates drug-induced

disruptions of reference memory. In addition, working memory is also compromised. Saline control animals required less time to find the hidden platform than experimental animals across trials within days. This would seem to indicate that cocaine may have an effect on spatial memory that involves working memory components. However, since the difference between C20 and C40 conditions was not significant the disruption is not dose-dependent.

When analysis is such that performance within trials and between runs is assessed, there is an apparent disruption in working memory. Animals in the saline control group found the hidden platform faster than the animals in the cocaine conditions. It is important to note that the rats were not only required to locomote to the exact same place on both runs, but they began swimming from the same place on both runs. Therefore, the responses required to locate the platform on the second run of each trial were identical to those on the first. An animal was only required to remember what it had done on the preceding run. Since the location of all extramaze cues relative to the platform remained exactly the same between runs, and the number and pattern of right vs left turns required of the animals was also unchanged, an argument can be made that the difference in latency between Run 1 and Run 2 would be a conservative measure of the effects of cocaine on working memory.

This point is strengthened when temporal proximity between two runs of the same trial are considered. In theory, there were only 30s between the end of the first run and the beginning of the second, and the elapsed time between the end of the platform interval of Run 1 and the beginning of Run 2 was no more

than a few seconds. Since the effect of cocaine on working memory was apparent over successive runs in SF animals, the present study has shown that even conservative measures of cocaine effects are sensitive enough to detect amnestic effects of cocaine on working memory.

Although escape latency is the most frequently reported datum in MWM studies, measures of path distance probably reveal more about the drug effect. Increases in swim path directly translate into decreases in swim efficiency. In the present study measures of swim distance appeared to mirror the escape latency results. In all conditions, over days, trials, and runs, animals in the SAL condition found the hidden platform using a shorter swim path than either of the cocaine treatment groups. While there was not a clear dose-dependent effect, the presence of cocaine clearly affected the ability of rats to swim a direct path to the hidden platform.

While there was an effect of cocaine on swim distance, this measure did decrease across days, trials, and runs for all conditions. The implication is, once again, that cocaine does affect MWM performance, but the effect is not sufficient to alter the typical improvement seen over the course of many runs. In other words, cocaine disrupts reference and working memory in the MWM, but the drug does not prevent improvement in performance with practice.

Cued Trials First Group (CF) – Standard trials

As was true in the SF group, the reference memory measure of escape latency over days for the CF group showed that animals in all three treatment

conditions improved performance during the four days of standard trials conditioning. In addition, cocaine also affected performance in the CF group. Animals in the SAL condition had shorter escape latencies on the first day of standard trials. While this difference was also seen on the last day of standard trials the difference between treatment groups was not consistent across all four days, and was not dose-dependent. While there was a significant effect of cocaine on this measure of reference memory, escape latencies did decrease.

When working memory is considered, it appears that the performance of animals in the CF group was once again similar to that of animals in the SF group. In trials within days animals in the SAL group had consistently shorter escape latencies than animals in the cocaine groups. It is also noteworthy that on this measure there was a dose effect. Animals in the SAL and C20 groups showed a rapid decrease in escape latency from the first to the second trial, and then a more moderate decrease from the second to the third trial. The animals in the C40 condition, however, failed to show the rapid decrease in escape latency from the first to the second trial; rather, their performance improved in a gradual, linear manner across all three trials.

Latency within trials, Run 1 vs Run 2 comparisons, again showed that animals in all treatment conditions were able to use information gained on the first run to improve performance on the second run. In all treatment conditions latencies on second runs were shorter than those on first runs. However, cocaine did affect performance. Latencies for SAL animals were consistently shorter than latencies for animals injected with cocaine.

A further parallel between the CF and SF groups was apparent from analysis of distance data. As was the case for the SF group, swim distance in the CF group decreased over days, trials, and runs, and in all cases the SAL group subjects consistently swam a shorter path to the hidden platform than the animals in the cocaine groups. Again, this was not necessarily a dose-dependent effect, but there was clearly a general cocaine effect on the length of the swim path.

Cued trials

In some respects performance on cued trials may be the most valid indicator of drug effects. Since a subject is simply required to swim to the visible platform the animals are not required to encode and retrieve information regarding extramaze stimuli. Deficits in performance reflected in escape latency or distance data would most likely be indicative of drug effects on motor skills, motivational levels, or visual impairment. In the present research, significant, apparently dose-dependent, deficits in escape ability were seen on cued trials in both the CF and SF groups.

Due to the activational effects of cocaine on motor systems it is important to consider the contribution of variations in swim speed between groups to overall performance. Swim speed did not appear to play a role in overall cued trial performance. In general all animals increased swim speed over the course of the experiment, and no consistent dose-related variations were seen. In addition, measures were taken in the statistical analyses to control for swim

speed variations. Thus, it is suggested that any differences in performance during cued trials were not caused by the activational affects of cocaine on motor regions of the brain.

The differences in escape latency in cued trials among treatment conditions in both the CF and SF groups appeared to be a function of dose-dependent increases in path distance. In all treatment conditions SAL animals swam a more direct path to the platform than either of the drug treatment groups. Thus, there may be motivational and/or visual changes that account for the observed differences in performance among treatment groups. However, these effects may also be attributed deficits in reference memory resulting from cocaine exposure. In light of the results associated with cued trials, some conclusions regarding performance on standard trials are open to alternative interpretations. One interpretation may be that while there were cocaine-related differences in MWM performance on standard trials, the differences were not necessarily caused by failures of memory systems. However, when dwell ratio data are considered it is difficult to support this interpretation. Under cued trial conditions there was no bias towards the previous day's platform quadrant in any treatment condition, indicating that animals were motivated to escape the water.

Dwell ratios

Measures of perseverative responding provided evidence that all animals, regardless of treatment condition, were capable of successfully using reference memory to search for the hidden platform. In all treatment conditions, and in

both SF and CF groups, animals tended to spend more time on first trial of each day searching for the hidden platform in the quadrant that contained the platform on the previous day. Since this indicates that animals are capable of retaining information over at least one day, it is clear that cocaine does not eliminate the ability to encode and retrieve information regarding the spatial location of the platform. The significance of this is that even though cocaine does compromise memory to some extent, as indicated by escape latency and path distance data, the memory disruptions can be overcome and are not necessarily catastrophic in terms of adaptive behavior.

Further, it is evident that increased dwell ratios are the result of memory-induced search strategies rather than other factors because no preference for the previous day's escape quadrant was revealed under any conditions in either group during cued trials. This indicates that cocaine did not interfere with the ability to see the platform, or to locomote to the platform-containing quadrant. Cued trials performance may, however, indicate some disruption in motor coordination. Furthermore, it is indicative of the fact that the animals are indeed motivated to escape the water.

Path distance data indicate increased path distance in cocaine-treated animals in cued trials, but dwell ratios indicate no spatial bias. It may be that animals were attempting to locomote directly to the escape platform, but were simply unable to do so. However, it should be noted that there were no obvious motor deficits in cocaine-treated animals either in terms of their ability to swim or their behavior in home cages.

Potential explanations

There are several explanations that may explain the impairments in MWM performance of animals in the cocaine groups. Because of the variety of effects cocaine has been shown to have on the hippocampus (Onaivi, Bishop-Robinson, Motley, Chakrabarti, & Chirwa, 1996; Smith, et al., 1993), perhaps the most compelling argument would be that cocaine disrupts the hippocampal processes which are necessary for normal performance on spatial memory tasks (Morris, 1983; Morris, Hagan, & Rawlings, 1986; M'Harzi, Palacios, Monmaur, Willig, Houcine, & Delacour, 1987). If cocaine inhibits normal hippocampal activity, then performance deficits may be caused by this inhibition. Smith et al., (1993) and Onaivi et al. (1996) reported that cocaine inhibits in vitro hLTP in rats. In the study by Onaivi et al., it was also shown that the same rats that had decreased in vitro hLTP also had deficits on a spatial learning plus-maze task. The fact that hLTP deficits and spatial learning task deficits were shown in the same animals provides strong evidence that hLTP is necessary for the animals to perform optimally on such tasks. While this evidence is correlational, it is nevertheless compelling.

The effects of cocaine on hLTP probably play a role in the memory deficits seen in the MWM. If this is true what are the underlying mechanisms that cause this decrease in hLTP? Smith et al. (1993) have shown that the effect of cocaine on hLTP is not due to its local anesthetic properties as hLTP can be induced when voltage-gated Na^+ channels are blocked by tetrodotoxin. Tetrodotoxin and cocaine both block voltage-gated Na^+ channels. Because hLTP can be induced

when tetrodotoxin is applied but not when cocaine is applied the local anesthetic effect of cocaine can not be the mechanism by which it inhibits hLTP.

Smith, et al. (1993) have also indicated that disruption of hLTP is not due to the effects of cocaine on NMDA glutamate receptors as is the case with AP-5. When AP-5 is administered to an animal it produces deficits in MWM performance and hLPT similar to those produced by cocaine. These deficits occur because AP-5 inhibits NMDA receptors. NMDA receptors comprise a $Ca^{++}/Na^{+}/K^{+}$ channel that, when closed, reduces positive inward current and therefore the probability of cellular depolarization. As cocaine does not block NMDA receptors Smith, et. al (1993) suggested that cocaine may block hLTP by inhibiting the phosphorylation of Synapsin I by Ca^{++}/calmodulin-dependent protein kinase II (CAM kinase II). Phosphorylation of Synapsin I by CAM kinase II has been correlated with induction of hLTP (Reyman, Brodeman, Kas, & Matthies, 1988). The inhibition of this system would be expected to interfere with hLTP-mediated behaviors such as swimming in the MWM.

Another possible mechanism by which cocaine could interfere with MWM performance is through its effects on motivational systems that are modulated by catecholamine neurotransmitters. Cocaine has been shown to block presynaptic uptake of biogenic amine neurotransmitters (i.e. dopamine, norepinepherine and serotonin). Variations in these neurotransmitter systems can have significant effects on behavior and have been implicated in such diseases as schizophrenia, Parkinson's disease and depression. The ability of cocaine to serve as a reinforcer has been attributed to its modulatory effects on the mesocorticolimbic

dopamine system. Perhaps changes in the dopaminergic system are partially responsible for the cocaine-induced deficits in MWM performance. It is possible that prolonged exposure to cocaine results in a situation in which escape in the MWM fails to become a reinforcing event. This is supported by the fact that animals in the cocaine groups failed to swim a direct path to the escape platform in cued trials. This lack of reinforcing efficacy could be due to a down-regulation of dopamine receptors in the nucleus accumbens due to long-term over-exposure to increased extracellular dopamine, or to some other compensatory mechanism designed to deal with the increase in dopamine resulting from cocaine exposure. Mechanisms such as decreased tyrosine hydroxylase production or activity would maintain that capacity.

Another explanation for the putative amnestic effects of cocaine may be that cocaine causes the expression of certain proteins that affect dopamine activity in the mesocorticolimbic system. However, the actions of these proteins have yet to be characterized. McKinze and Couceyro (1995) have reported that a novel mRNA found in rat striatum is transcriptionally regulated by cocaine and amphetamine. Through the isolation and characterization of the corresponding cDNA clones it was predicted that the mRNA translation products would be a 129 (or alternatively spliced 116) amino acid protein. Both putative isoforms of the polypeptides include a hydrophobic leader at the amino terminus. This indicates that these proteins would likely be destined for the secretory pathway. As this psychostimulant-regulated mRNA is found in the striatum it is quite possible that the proteins are involved with degrading, or otherwise inhibiting, the excessive

excitation of dopaminergic systems. The deactivation of dopamine may cause decreases in reinforcing properties.

Another explanation for the increases in escape latency and path distance of cocaine-treated animals that may also be related to lack of motivation, but not necessarily to central dopamine modulation concerns the peripheral effects of cocaine. Cocaine is a potent sympathomimetic and causes intense vasoconstriction in the periphery. The vasoconstriction of blood vessels in the skin, combined with an increase in heart rate, may result in an increase in core temperature and decreased conductive heat loss. Thus, it is possible that placement in cold water was not as aversive for animals injected with cocaine as it was for control animals. While this does not address specific variations in path distance or latency, it may have been a contributing factor.

Conclusions

No systematic studies of the effects of daily cocaine injections on MWM performance in adult rats have been reported. The present research was performed to use the MWM as a tool to elucidate those potential effects and determine the types of deficits that may be produced. Measures of reference and working memory were recorded in an effort to determine which processes are most affected by cocaine exposure. Several important effects of cocaine were observed. First, the effects of daily administration of cocaine on MWM performance vary depending on the history of the subject. Animals familiar with the task performed better than naïve animals, but the naïve animals appeared to

be more resistant to dose-dependent effects of the drug, although scale attenuation may have contributed to this effect.

All animals, regardless of treatment condition, were capable of learning the task. Although, in most cases cocaine had an effect on how quickly the animals mastered the task. Animals in all groups eventually learned to find the hidden platform over the course of 24 cued and 24 standard runs. Thus, there may be deficits in reference memory, but not deficits that result in the to learn this MWM spatial memory task.

It can be concluded from measures of working memory measures that cocaine impairs the ability of rats to use recent experience to guide behavior. In trial-to-trial and run-to-run comparisons, there was a consistent decrease in path distance and escape latency in all rats. However, there was also a consistent difference between the cocaine-treated and control animals. Control animals consistently performed better than animals injected with cocaine on all measures of working memory.

Since differences between the C20 and C40 groups in working memory were not observed on all measures, it remains to be determined whether the effects of cocaine were nominal, all-or-none effects, or if the low dose was sufficient to elicit maximum effects. In any event, further study with a wider range of cocaine doses would help to clarify this issue.

Further analyses regarding the neurophysiological events affecting MWM performance would also provide additional information regarding the effects of cocaine on MWM performance. In vivo measurement of the ability of animals to

acquire hLTP subsequent to training in the MWM could help elucidate the necessary physiological substrates involved with MWM performance. It would be of interest to determine whether cocaine caused an increased resistance to hLTP.

While it is outside the sphere of experimental psychology to investigate the molecular biological components of hLTP it is likely that the activity of cocaine on the neurobiology of the hippocampus will shed light on how spatial memory is affected by cocaine. This research demonstrates the importance of multidisciplinary approaches. If the effects of drugs of abuse on behavior are to be fully understood concerted efforts on the part of researchers in many disciplines from psychology and pharmacology to neurobiology and chemistry are mandated.

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