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ABSTRACT

Morphine is a potent opiate analgesic which is used to treat severe and debilitating pain. Increasingly this drug is prescribed to be used outside of medical settings. The literature on effects of morphine in humans is inconsistent but it does suggest the potential for cognitive impairment. In this study animal model of morphine effects on working memory was studied. In Experiment 1 it was found that measures of working memory were affected at doses lower than doses needed to impair latency. Within session analysis showed that the effects of morphine were memory load dependent. These findings were problematic because it was discovered that in Experiment 1 the memory load effect was confounded with the number of stimuli present. Experiment 2 was designed to remove this confound. When the number of stimuli present for choice was limited to two it was found that baseline accuracy did not decrease with the increase in memory load. Measures of working memory were affected at doses lower than doses which also increased latency. These findings have potential human implications. They suggest that morphine can disrupt spatial functioning. Special caution should be taken when these drugs are prescribed outside of hospitals. Also the doses should be minimized if no potential for relapse exists.
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INTRODUCTION

Opiate drugs come from opium secreted from the opium poppy (*Papaver somniferum*), native to the Middle East, now also cultivated extensively throughout Asia and the Middle East. Evidence exists that opium was used and cultivated as far back as 6000 years ago by Sumerian and Assyrian civilizations (Maisto, Galizio, & Connors, 2011). Traditionally opium was obtained by scoring the poppy pod with a knife and draining the white, milky substance. After drying, the liquid formed a gummy brown substance which was consumed orally, smoked (Maisto et al., 2011), or administered through punctures in the skin (Gruber, Silveri, & Yurgelun-Todd, 2007). In the nineteenth century, laudanum, a liquid containing opium, was widely sold in Europe and America.

In 1803 the process of separating morphine from opium was developed. Morphine is the major chemical found in opium and it is ten times more potent than raw opium. Subsequently the hypodermic needle was developed and morphine could be injected which resulted in a large dependence problem (Maisto et al., 2011). During the Civil War morphine was widely used for treating wound pain. Many injured troops became dependent which resulted in this problem being called the “Soldier’s Disease”. In 1874 diacetylmorphine, a “non-addictive” synthetic version of morphine was developed, which was ten times more potent and was christened heroin because of its “heroic” properties. Heroin was used to treat pain and relieve cough. Later it was discovered that heroin produced dependence even more quickly than morphine. Heroin is converted to morphine in the brain; however, it crosses the blood-brain barrier much more efficiently than morphine and is therefore more potent. Today heroin is a Schedule I drug in the U.S. because of its extremely high abuse potential and no medical use. Heroin is still used in Europe as a pain reliever (Maisto et al., 2011) and as a maintenance drug in opiate dependent
patients (Dürsteler-MacFarland, Stohler, Moldovanyi, Rey, Basdekis, Gschwend, Eschmann, & Rehm, 2006).

The term “opiate” is used to describe drugs naturally found in opium exudate (i.e., morphine, codeine and thebaine). The term opioid, conversely, is used to describe exogenous drugs (non-natural or synthetic) that bind to opiate receptors and produce agonist effects. Injecting, smoking, or sniffing opiates results in intense euphoria (often referred to as “rush”), which is followed by few hours of a relaxed, content state. Physiological effects include respiratory depression, decreased blood pressure, lowered body temperature, nausea, vomiting, constricted pupils, constipation, and drying of bodily secretions such as mucus or tears (Maisto et al., 2011). The strongest analgesic effects and strongest abuse potential are produced by drugs with strong activity at the mu receptor. Repeated use often results in drug tolerance and withdrawal symptoms at cessation of use which are characteristic of dependence. Symptoms of withdrawal usually begin within hours to days after prolonged use and include “craving, anxiety, drug-seeking behavior, yawning, sweating, lacrimation, rhinorrhea, mydriasis, gooseflesh, muscle twitching, anorexia, insomnia, increased pulse, respiratory rate and blood pressure, abdominal cramps, vomiting, diarrhea, and weakness” (Gruber et al., 2007, p.301).

The level of heroin abuse has evened out in the past decade; however, the use of prescription opiates has been increasing dramatically (Compton & Volkow, 2006). Non-medical use of prescription drugs has tripled since the beginning of 1990s (Subramaniam & Stitzer, 2009). Most attention has been given to OxyContin (manufactured by Purdue Pharma); its main ingredient is the generic drug oxycodone. OxyContin was developed to treat chronic and severe pain; therefore, one tablet contains 20-160 milligrams of oxycodone in a time released capsule. Opiate abusers quickly realized that by crushing the tablet and injecting the powder, effects of a
full dose can be obtained immediately. These practices have resulted in very large numbers of abuse, dependency and lethal overdoses. Since 2001 the highest dose tablet (160 mg) has been discontinued but other doses are still on the market. Young people are most likely to abuse prescription opiates (Maisto, et al., 2011). Wu, Pilowsky, and Patkar (2008) found that near 10% U.S. adolescents between 12 and 15 years of age reported non-medical use of opiates. Compton and Volkow (2006) reported three possible reasons for the drastic increase in prescription opiate abuse. The increase in number of prescriptions written for opiates could cause increased availability of these drugs for illicit use. Recently prescription drugs have been more available for purchase via the internet which allows for obtaining them without the supervision of a physician. Also, prescriptions are more often written by primary care physicians who may have less expertise in pain management (Maisto, et al. 2011).

Acute and chronic effects of opiate drugs generally do not raise concerns about cognition. However, the populations whose opiate drug use we are mostly concerned about are those who take the drug for pain relief outside of medical settings as well as individuals in methadone maintenance therapy. Those individuals often attempt to return to their daily activities including working where unimpaired cognitive function is needed. Currently we do not know how many individuals take opioid medications in the workplace. Even though drug use is often controlled in the workplace through random drug testing, those who obtained their drugs by prescription are not targeted by these screenings. It is very important that we explore the kinds and extent of cognitive impairment these drugs may be causing to find out whether it is safe to take them outside of medical settings.

Some studies have explored the effects of chronic opiate use in methadone maintenance patients. Methadone is a long-acting synthetic full mu opioid agonist (binds to a receptor of a cell
and triggers a response of that cell) that is chemically different from morphine and heroin but also acts on opioid receptors and therefore produces similar effects. Methadone is used in opiate drug dependent patients as an alternative to those drugs. While it reduces opiate withdrawal symptoms, it is associated with quick development of profound tolerance to its sedative effects, and therefore is thought to allow patients to function without impairment (Mintzer & Stitzer, 2004). Methadone has been used as a treatment option for opioid addiction since 1960s (Pakesch, Loimer, Grünberger, Pfersmann, Linzmayer, & Mayerhofer, 1992). Patients who take methadone can reduce their drug use, avoid dangers associated with the use of street drugs and return to their productive lives.

Although methadone and other opiates are generally not thought of as producing pronounced cognitive effects, several studies suggest otherwise. Guerra, Sole, Cami, and Tobena (1987), for example, studied ninety-three heroin addicts (65 males and 28 females) admitted to a voluntary detoxification clinic and thirty-one matched control subjects (18 males and 13 females). The control subjects, who were students at a Technical Secondary School, were matched to the heroin addicts for demographic, cultural and educational factors. Heroin addicts were administered a neuropsychological test battery two to three weeks prior admission to a detoxification unit. Upon being admitted, subjects were detoxified for a period of one week (no longer than 10 days). To ease the detoxification process, they were given decreasing doses of methadone and clonidine (high blood pressure medication, often used to treat opiate withdrawal symptoms). By the end of the detoxification subjects received no drugs. After 7-14 days after admission patients were retested with the neuropsychological battery. At least two weeks were allowed between both test administrations. The neuropsychological battery included tests which measured perception and attention (Toulouse-Pieron), verbal fluency (F factor of PMA),
immediate recall measured by Digit Span (Immediate Auditory Memory), short- and long-term memory (Memory of words), and intelligence (Raven’s Progressive Matrices). Personality inventories were administered to assess any psychopathologies which could impact their performance on tests. Heroin-using subjects were found to have used street heroin for a mean of 4.9 ± 2.1 years, in the amount of 485 ± 336 mg daily before beginning of treatment. Their intelligence level was comparable to that of the subjects in the comparison group. Results on the test battery administered prior to detoxification revealed that heroin users performed significantly worse than control group participants on all measures (attention, verbal fluency, and memory). The performance improved significantly during post-detoxification such that it became similar or even better than that of control subjects on all measures except for verbal fluency which was slightly but not significantly worse. These results suggest that impairment caused by heroin lasts for only a short period of time and it disappears once a patient is drug free. The impairment observed prior to detoxification could have been caused by subjects still being intoxicated.

Pakesch, et al. (1992) studied 42 HIV-1 positive opiate abusers (13 diagnosed with opioid dependence, 29 with polysubstance dependence) in comparison to 31 HIV-1 negative opiate abusers. Dependence is defined as overwhelming preoccupation with using and obtaining a drug and a strong predisposition to return to using after a period of sobriety (American Psychiatric Association, 2000). Thirty of the HIV positive subjects were in a methadone maintenance program and received between 10 mg and 100 mg methadone daily. Fourteen patients also received other psychotropic medications, such as benzodiazepines for sleep problems. The control subjects (24 polysubstance dependent, 7 opioid dependent) were also methadone maintenance program participants and they received between 10 mg and 160 mg methadone
daily and no other psychotropic medications. The two groups were matched for age and sex as well as educational level. Both groups were additionally compared to a group of 50 HIV negative and non-drug abusing healthy volunteers matched for age, sex and educational level. All subjects were given a battery of psychological tests including assessments of fluid intelligence (Raven’s progressive matrices), verbal intelligence (a multiple-choice word test), visual retention (the Benton test), attention and concentration (an alphabetical reaction test), and short-term memory (numerical memory test). An abbreviated version of the Minnesota Multiphasic Personality Inventory – 2 was used to evaluate the subjects’ personality traits. Results of the analyses revealed that the intelligence level of seropositive subjects did not differ from the intelligence level of seronegative subjects or from the normal population. The two groups of addicts were significantly impaired in comparison with healthy control group on the number of correct answers on the Benton visual retention test, although there were no significant differences between the two addict groups. There were no significant differences between the three groups on the measure of attention (measured by the total score on the alphabetical reaction test). Concentration under time stress, which was measured as a percentage of correct answers on the alphabetical reaction test, was significantly reduced in HIV-1 positive patient group in comparison with healthy controls. Short-term memory was significantly reduced in the patient group in comparison with the healthy control group but not with the addict control group. These findings suggest that long-term drug abuse has a significant impact on cognitive functioning. However, we are unable to determine whether the impairment was caused by long term street drug abuse, use of methadone or maybe other drugs also taken by patients. If these effects are caused by the patients’ use of methadone this would disprove the belief that the drug causes no
impairment and patients can function normally while taking it. A better study is needed which differentiates between long term drug abuse as well as the use of other drugs.

The study by Curran, Kleckham, Bearn, Strang, and Wanigaratne (2001) attempted to measure acute impairment after patients received daily doses of methadone. The subjects were heroin-dependent inpatients. Patients were randomly assigned to two groups: either 50% or 100% of their daily stabilization dose, and a placebo, in a double blind, cross-over design. Episodic memory, defined by Carlson (2007) as “complex perceptual memories of sequences of events that we witness or are described to us” [p. 453] was measured here by delayed recall of prose. A single dose of methadone was found to induce episodic memory impairments in patients who had a history of heroin use averaging more than 10 years as early as three hours after administration of 100% of their daily dose of methadone. These effects could be avoided if the methadone dose was divided among two daily administrations rather than one (Curran et al. 2001). Again, this study suggests that methadone has the potential to produce cognitive impairments if administered in one daily dose. Authors suggest that dividing this dose into two daily doses could prevent the impairment. It is unknown, however, if the results would be the same with patients with shorter histories of drug abuse. Also the impairment could have been caused by patients’ overall intoxication from the high dose of methadone rather than a more selective cognitive effect caused by methadone. Better measures are needed which distinguish between profound drug impairment (i.e. intoxication) and cognitive impairment.

A study by Mintzer and Stitzer (2002) allows us to differentiate the effects of methadone from the history of drug abuse. The authors investigated cognitive impairments in methadone maintenance patients in comparison with two groups: abstinent abusers and nonusers. All three groups were matched for factors such as age, education level, socioeconomic status, etc. The
levels of drugs were controlled with urine tests prior to testing sessions. Participants were given a cognitive test battery, results of which showed no significant differences between methadone maintenance patients (MMP’s), abstinent abusers, and nonusers except for the marginally significant results showing impairment in MMP’s in comparison to abstinent abusers on two measures of recognition memory: hit rate (proportion of words presented in the initial list that were recognized by the participants as having been presented in the study list) and a signal detection measure of the subject’s ability to discriminate between words they were shown in the initial study list and words that were not presented to them. The impairment on recognition memory measures points to a complex relationship between opioid use and cognition. The performance of abstinent abusers was less impaired than that of methadone taking patients but worse than that of nonusers, which suggests that methadone in addition to the history of drug use caused impairment that was larger than that caused by the history of drug use alone. We could conclude that it is not methadone alone which causes cognitive impairment but the combination of methadone and the history of drug abuse. However, the populations in which we are interested (i.e., those who receive methadone daily) generally have histories of drug abuse; therefore, we have to take that into account when we consider their ability to function normally.

A study by Darke, Sims, McDonald, and Wickes (2000) provides another example that methadone has the potential to disrupt cognition. The authors studied 30 MMP’s enrolled in methadone program for a mean of 60 months who were compared with 30 controls matched for age, gender and education. Subjects were tested using a test battery consisting of the Wechsler Adult Intelligence Scale-III, Wechsler Memory Scale-Revised, California Verbal Learning Test, Complex Figure Test, Controlled Oral Word Association Test, and Wisconsin Card Sorting Test. The groups did not differ in premorbid functioning on the IQ measure. The MMP’s performed
significantly worse than the control group on measures of information processing, attention, short- and long-term visual memory, short- and long-term verbal memory, and problem solving. However, these findings are difficult to interpret. The MMPs differed from control subject in several aspects, such as 73% of them experienced overdosing a median of three times while none of control subjects did. In addition, sixty three percent of MMPs met the criteria for a life-time diagnosis of alcohol dependence while only 10% of control group subjects met these criteria. Also the MMPs had three times the number of head injuries that control group subjects did (67% vs. 20%). We do not know whether the cognitive deficits were caused by the use of methadone or these other health risks.

The studies described above disprove the belief that methadone does not cause cognitive impairment. Despite many methodological issues, such as subjects’ long-term drug abuse (Pakesh et al., 1992), inability to distinguish cognitive impairment from global impairment (Curran et al., 2001), and other medical history differences (Darke et al., 2000), there is apparent cognitive impairment. This impairment should be further investigated and it should be considered when methadone treatment is prescribed.

In addition to methadone maintenance treatment patients, we are also interested in the cognitive effects of opiates in individuals who take only acute doses. Sometimes the acute doses are given in addition to already present long term opiate pain management therapy. Kamboj, Tookman, Jones, and Curran (2005) investigated acute effects of immediate-release morphine on cognitive functioning in palliative care patients who were already receiving sustained-release opioids ($M = 190.7 \pm 266.6$ mg, range 30-800 mg). The study utilized a double-blind, placebo-controlled design. To assess immediate and delayed episodic memory, participants completed a prose recall test. Participants were presented with one story pre- and one story post-treatment.
They were asked to recall the stories both immediately and after a delay period (one story 20 minutes after immediate recall, and the other story 65 minutes after immediate recall). To assess attention and working memory, participants completed forward and backward Digit Span tasks in which they were required to repeat a list of digits that was read to them either in the same or reverse order. Morphine produced significant impairment of delayed prose recall. No significant effects on forward and backward Digit Span tasks were found. The authors used a relatively uncomplicated test battery, so they were unable to discriminate which specific processes of memory contributed to the observed amnesic effects. Also, the range of drug doses given to patients was very wide ($M = 21.4 \pm 25.6$ mg) so it was not possible to assess a drug dose-effect curve to find out at which doses the impairment begun and how different doses affected performance.

Hanks, O’Neill, Simpson, and Wesnes (1995) administered two doses of morphine (10 mg & 15 mg) to subjects who performed computerized cognitive tasks (simple reaction time, choice reaction time, number vigilance, memory scanning, immediate and delayed word recall, word recognition, picture recognition, critical flicker fusion threshold [CFFT] and subjective measures of alertness, calmness and contentment) 30 minutes before, and 1, 2, 4, and 6 hours after administration of the drug. The results showed few significant effects in comparison with placebo, but significant impairment was found in both doses at 1 hour in delayed word recall and picture recognition sensitivity.

Kerr, Hill, Coda, Calogero, Chapman, Hunt, Buffington, and Mackie (1991) studied 15 male volunteers who had no history of drug or alcohol abuse and were not taking any drugs at the time of the study. Each subject was seated on a hospital bed placed in a sound proof testing chamber for the duration of the testing session. Pharmacokinetic tailoring sessions were run
before the testing sessions in order to calculate the exact doses needed for each subject to achieve identical drug concentrations during the testing sessions. Participants were then tested during infusion sessions (where the drug was administered into a participant’s system) with morphine and saline on different testing days. Each testing session was separated by a minimum of 7 days and the order of testing was counterbalanced between subjects. During each testing session the preset plasma concentration was reached and maintained. At three set times the volume of infusion was incremented with additional doses so that three step increases in plasma morphine concentration could be reached (20, 40, and 80 ng/ml). Each concentration level was maintained for 60 minutes. After one of the level increases a placebo level increase was introduced. The saline session included a similar placebo increase. This session was placed randomly in between drug infusions.

An hour-long test battery measured verbal comprehension, memory, and motor performance. The tests were performed in the same order every hour. The battery was practiced 12 times during pretest sessions in order to minimize learning effects during drug testing. On each testing day the subjects practiced the battery one time before beginning of the session and then completed it four times during the testing session. To test verbal comprehension and memory, the RSVP technique was used. Single words from a passage were displayed on a computer screen and subjects tapped the keyboard space bar to advance the display to a new word. After the entire passage was presented (approx. 300) the subject was presented with a distraction task after which comprehension and memory of the passage was assessed. After each daily session the subjects were given another memory task which consisted of questions about each of the passages read during that day. In a visual perception task subjects were presented and asked to identify 10 randomly presented letters. Motor performance was assessed with tapping
and manual isometric force tasks. The tapping task was performed with the subject’s both hands and the force task was performed with their preferred hand. Performances at low, medium, and high plasma concentrations were compared with performances during saline injection sessions during corresponding hours.

The analyses revealed that plasma concentrations of morphine similar to the therapeutic range increased the time subjects took to read the words and answer questions about the passage. It was concluded that this increase was due to impaired ability to absorb and process information during drug infusion. Subjects had no impairments in immediate recall of passage; therefore their short term memory and comprehension were not impaired. The recall of information at the end of session however was significantly impaired after morphine dose in comparison with saline infusion. Morphine also affected motor performance by decreasing the subjects’ ability to maintain low levels of force.

The study by Friswell, Phillips, Holding, Morgan, Bradner, and Curran (2008) used an experimental design to examine the effects of opioid drugs in patients without history of drug abuse or significant health issues. Friswell and colleagues studied the cognitive impairments caused by morphine and oxycodone in healthy, young, non-drug using participants. The study used a double-blind design. The participants were administered standard doses of morphine and oxycodone (morphine: 10 mg; oxycodone: 5 mg) and placebo, during three separate testing sessions. Participants were administered a memory test battery which included Digit Span Forward and Digit Span Backward. The results revealed a significant impairment on the Digit Span Backwards task in both drug conditions in comparison to placebo (participants recalled one fewer digit on the backward span task following morphine comparing with placebo).
Participants in this study did not demonstrate impairment on Digit Span Forward, which suggests that the drug affected manipulation rather than maintenance of the information in working memory (in Digit Span Forward task participant recalls digits exactly as they were presented, in Digit Span Backwards participant recalls the digits starting with the last one). The fact that participants were not impaired on Digit Span Forwards suggests that their memory was intact, therefore impairment on Digit Span Backwards must have been caused by impairment in cognitive processes other than memory. It is difficult to generalize these findings to a clinical population because participants in this study were not taking the drug for pain which may itself have an effect on cognitive functioning and can impair memory. Also, even though the study used an experimental design which allowed for random assignment, its significant shortcoming is the single dose of both morphine and oxycodone. Impairments were significant only on the Digit Span Backward task; however we cannot be sure that impairment on other measures would not occur with different doses of these drugs. In order to fully understand the effects of these drugs we need to explore a full range of doses to find out whether impairment emerges and if so, what is the lowest dose where the impairment becomes significant and what dose causes impairment that goes beyond cognitive functioning and spreads to wider areas of functioning. Further research utilizing a full range of doses is necessary.

The studies discussed above suggested that morphine can disrupt delayed recall of information or impairment on the Digit Span Backwards. In the Hanks et al. (1995) study subjects were impaired on delayed word recall while in the Kerr et al. (1991) study subjects performed worse on memory for passages, but only after relatively long delays. Friswell et al. (2008) demonstrated no working memory impairment (no difference in performance on Digit Span Forwards) but there was impairment of other cognitive processes which are required to
perform the manipulation of the information in working memory (impairment on Digit Span Backwards). In these studies subjects had no history of prolonged drug use and were tested only once in each drug condition.

Studies of opioid drug effects on cognitive function and memory have been mixed, but do suggest the potential for disruption. Studies examining the long term effects of opioids show impairments of cognitive measures such as attention, verbal fluency and memory prior to detoxification however these impairments disappear after patients are detoxified (Guerra et al., 1987). Studies of the effects of methadone in patients with history of drug abuse are much more problematic. While they do show impairment such as short term memory decline, disruption on visual retention test (Pakesch, et al. (1992) or episodic memory (Curran et al., 2001) it is often unclear whether the effects are caused by the acute dose of an opioid, past abuse of other substances or other serious health problems that some of the subjects had. Studies of single opioid administrations in participants with no history of drug use show some impairment such as impaired delayed recall of information (Kerr et al., 1991) or delayed word recall and picture recognition sensitivity (Hanks et al. 1995) or Digit Span Backwards (Friswell et al., 2008). In addition some of the studies (Kerr et al., 1991; Friswell et al., 2008) examined the effects of opiates with only few doses of a drug which is not sufficient because it does not allow us to understand the effects the drug produces at different doses. Additionally because these studies employ different measures of cognitive performance and study subjects with different histories of drug use the results are inconsistent and do not allow us to draw systematic conclusions.

These concerns justify the need to conduct research using nonhuman animal models. Animal research allows for much more rigorous controls, including control of genetic makeup, housing conditions, diet, etc. By conducting research using subjects who are housed under the
same conditions we are able to reduce between subjects variability and focus on the change caused by the independent variable (i.e., drug). Also, animal research allows for much more comprehensive testing, with a wide range of doses and several determinations of a single dose and a comparison to baseline and placebo controls.

Animal models of memory often distinguish between spatial and non-spatial paradigms. Many studies suggest separate neural control that occurs within these memory processes. For example, researchers believe that special cells in hippocampus, called “place cells,” fire at a higher rate when an animal is at a specific place in the environment (O’Keefe & Dostrovsky, 1971). Spatial memory tasks require an animal to navigate using distal cues (objects in environment). Spatial memory is believed to require constant updating of animal’s “cognitive map”. Non-spatial memory tasks, on the other hand, do not require an animal to use distal cues. The animal discriminates between non spatial stimuli which do not require it to navigate within the environment.

There are few studies that have tested spatial memory and opiates in animals. Braida, Gori, and Sala (1994) used a spatial memory paradigm to test the effects of morphine. They employed an 8-arm radial arm maze to test the effects of different doses of morphine on working memory in rats. In this task rats were allowed to navigate through the maze (locations at the end of each arm were baited). Dependent variables that were measured included proportion of each correct entry into an arm, total errors, and the time taken to complete the task. Several doses of morphine were administered (2.5, 10, 25, 50, 100 mg/kg). A measure of analgesia, the tail-flick response test (caused by a high intensity beam of light fixated on the animal’s tail) and a measure of catalepsy were used to assess non-cognitive impairment. Testing sessions did not begin until both of the symptoms had disappeared. Morphine was found to significantly decrease percentage
of correct arm entries (calculated as the number of correct entries out of the first eight) in the working memory task at 25 mg/kg and 50 mg/kg doses. However, this conclusion may be called into question. Lack of catalepsy and anesthesia do not indicate there was no performance impairment. A drug may have different effects on an animal such as to decrease in motivation, motor impairment, visual impairment, or even the animal’s appetite all of which may affect accuracy without disruption of working memory. An animal may not be cataleptic and still be impaired. These measures assess only two types of physical impairment. Better impairment measures are necessary to be able to conclude that morphine produced cognitive impairment. A procedure needs to be developed which compares two similar components, one which changes on every session (acquisition) and one which has been previously learned and remains the same between sessions (performance).

Galizio, Keith, Mansfield, and Pitts (2003) employed such a control task in their study of morphine and spatial learning. They utilized the Morris Swim Task which is one of the most popular spatial tasks measuring working memory. It requires an animal to navigate in a circular pool filled with opaque water and locate a hidden escape platform. An animal must use extra-maze cues to find the platform (usually distinct set of visual objects) and only these cues are intended to help the animal navigate. Galizio and colleagues utilized this task to investigate the effects of morphine on spatial acquisition. Their procedure included two components: acquisition and performance. Each of the conditions was signaled by a distinct pattern of curtains surrounding the pool. In the performance component the location of the platform was always the same. In the acquisition component the location of the platform changed between sessions but remained the same between trials. After locating the platform on the first trial, the escape latencies shortened on consecutive trials. Morphine showed selective effects in this task, which
means that morphine impaired acquisition without causing a motor impairment. These selective effects were observed at 3.0 mg/kg and 10.0 mg/kg doses.

The above studies used a spatial paradigm to study the effects of morphine on working memory. In contrast, Pitts, Buda, Keith, Cerutti and Galizio (2006) used a task that did not require spatial navigation. This study utilized a touch screen monitor on which rats were required to nose-poke one of six locations arranged in a 2×3 stimulus array. Similarly to a previously mentioned study, there was a performance and an acquisition component. In the performance component the six locations all displayed a particular shape and color and only one location remained correct across every experimental session. In the acquisition component, all locations were lit in the same color and displayed the same shape that was different from the color and shape in the performance component. In this component the location of the correct key changed across each session, but remained the same within sessions. After finding which location was correct, the rat was required to remember that position and make the same choice on each consecutive trial. During the acquisition component the latency until the nose-poke to the correct stimulus was recorded. After the first trial (on which the rat made several responses before finding the correct key) the latencies gradually decreased. Morphine did not produce selective effects on acquisition, that is, 10.0 mg/kg significantly impaired responding in both the acquisition component and the performance component (drug disrupted overall functioning).

In summary, these studies have shown that morphine produced significant impairment in spatial learning tasks (Galizio et al., 2003; Braida et al., 1994), but not in a non-spatial learning task (Pitts et al., 2006). The dissimilar effects of morphine on cognitive functioning in these studies (selective impairment in a spatial task, but no selective impairment in non-spatial tasks) require us to ask whether they are caused by the difference in the task (i.e., spatial vs. non-
spatial) or whether it is the type of reward presented (i.e. food vs. escape). In the study by Galizio et al. (2003) which required rats to navigate using distal cues to a submerged platform, the correct response (i.e. locating the platform) was rewarded by escape from an aversive stimulus (i.e., water) while the study by Pitts et al. (2006) which required rats to poke a specified location on an array of stimuli, the correct response was rewarded with a food pellet. In the first task (spatial task, escape contingency), morphine selectively impaired acquisition. However in the second task which utilized a non-spatial task and included food as reward, morphine did not impair acquisition until doses high enough to produce more general impairment were reached. It is unknown whether the difference between the task requirements or the difference in reward may have been factors in the different effects of morphine. A study is needed which utilizes a spatial memory paradigm but does not involve escape.

The above studies are viewed as animal models of working memory. However, working memory, as it is discussed in humans, has two important characteristics not addressed clearly in those procedures: duration and capacity. The first one addresses the issue of how long an item is stored in working memory before it either becomes forgotten or stored in a long term memory. The second addresses the amount of information that an organism can hold in working memory. Unfortunately neither duration nor capacity of working memory has been studied very broadly in animals, for example, it is not clear how water maze studies assess either capacity or duration limitations. Working memory in animals is defined as “a short term memory for an object, stimulus, or location that is used within a testing session, but not typically between sessions” (Dudchenko, 2004, p. 700). While this definition does not explain what working memory in animals is, it tells us how to measure it and differentiate from other kinds of memory such as long term memory.
To assess the duration of working memory, tasks such as Delayed Match/Non-Match to Sample (DMTS/DNMTS) are used. In this type of memory tasks an animal is rewarded for either matching or non-matching to an originally presented stimulus. The duration of working memory is measured based on the delay introduced between the sample and choice trials. Only a small number of delay studies are available. Hudzik and Wenger (1993) studied the effects of morphine in squirrel monkeys using titrating delayed matching-to-sample procedure. In this study the monkeys responded on three keys. The center stimulus key was illuminated in either blue or white color (each color paired with different noise), after pressing on the center key (considered as observing behavior) the monkey was presented with a delay (3 s on first five trials, if all five responses were correct the delay value increased by 3 s, if in four out of five previous trials response was correct, the value of the delay remained the same, if in three or fewer of the previous trials the response was correct, the delay value decreased by 3 sec., to a minimum value of 3 sec.) after which two of the three keys were illuminated (one in blue, one in white, in random order) and the animal was required to make a matching response. A response to the correct key (that was illuminated in the same color as the stimulus key) was rewarded with food pellet. Administration of morphine did not cause selective impairment in memory, doses that were high enough to disrupt accuracy also decreased rates of responding (i.e. rates of responding were used here as an indicator of performance).

Although DMTS permits assessment of duration of working memory in non-humans, assessment of working memory capacity has proven more difficult. Among the most popular tasks for measuring the capacity of working memory in humans is the Digit Span task. A participant is usually read a string of digits and is subsequently asked to recall them in the exact order in which they were read. The number of digits recalled correctly and in the correct order is
scored as the span. The average span acquired by humans on this task is 7 ± 2 (Miller, 1956). Dudchenko, Wood, & Eichenbaum, (2000) developed a spatial span procedure to assess spatial working memory in rats. In this study rats discriminated between locations of cups placed in a square arena. On a first trial rats were placed in a square arena containing a single cup, in which they were required to dig in order to retrieve a food reward. On the next trial one cup was added and placed in a new location (cup from previous trial remained in its previous location). This time digging in a cup placed in a novel location was rewarded. On each subsequent trial previous cups remained in their locations and one more cup was added and placed in a novel location. During one testing session each location was used only once. On the next day the locations were reused; therefore rats were not required to remember locations from one day to another. Always choosing the cup placed in novel location was rewarded. After rats were reliably choosing cups placed in new locations, half of them underwent hippocampal lesions with injection of ibotenic acid while the other half underwent control lesions which were identical except for the injection of ibotenic acid in hippocampus. After recovering from surgery rats were returned to testing sessions until their performance stabilized. Results of this study revealed that rats which were hippocampectomized were significantly impaired on the spatial task in comparison with their control counterparts. Interestingly, rats which were trained on an olfactory (non-spatial) span task (rewarded for choosing novel odors) and which were also hippocampectomized performed at levels which were similar to their control counterparts. Thus, the findings of the study supported the theory that the hippocampus plays a crucial role in spatial working memory. Deal, Poerstel, Watterson, Jacobs, Goldstein, and Galizio (2010) utilized an olfactory span procedure similar to the one developed by Dudchenko et al. (2000) to study the effects of morphine on working memory span. This procedure consisted of an acquisition and performance components.
On each acquisition trial the rat discriminated between one novel and several previously sampled stimuli. The performance component consisted of several stimuli, only one of which was always correct while the remaining were always incorrect. After developing a stable baseline the rats were tested with several determinations of different doses of morphine (1, 3, 10, 18 mg/kg) and saline. The results of this study showed that morphine impaired acquisition accuracy in a dose-dependent fashion, but only at doses that also impaired performance. This means that the impairment was nonselective to working memory because morphine caused global impairment of responding.

Several studies mentioned above revealed that morphine selectively impairs performance in spatial tasks (e.g., Galizio et al., 2003). Dudchenko et al. (2000) demonstrated that hippocampal lesions also produce impairment in the spatial span task. In studies which utilized nonspatial procedures, morphine has generally produced only nonselective effects (e.g., Pitts et al., 2006). In the Galizio et al. (2003) study escape from aversive stimulus was used as reinforcement. In contrast, Dudchenko et al. (2000) used food as reinforcement. It remains unclear whether it is the difference between tasks type (spatial vs. nonspatial) or difference in reward type (escape vs. food) which produces differential effects of morphine on cognitive performance. A need exists to develop a memory task which requires spatial navigation utilizing distal cues, similar to those in the Morris Swim Task, but with an appetitive reinforcement contingency. Further, there is a need to explore the relationship between drug effects and the number of items to remember. The spatial span task used in the Dudchenko et al. study (2000) appears to meet this need. The present study utilized this procedure to investigate the differential effects of morphine. If morphine selectively impairs responding on the spatial span task it would
suggest that it is the spatial component of the task which accounts for the difference and not the type of reinforcement.

EXPERIMENT 1

METHOD

Subjects

Five male Harlan Sprague-Dawley (Harlan Laboratories, Indianapolis, IN) rats were used in the study. Two of these rats were excluded due to not meeting criterion in one of the training stages (A7 did not reach criteria for any of the phases and was artificially advanced to new phases and finally dropped from the study in the six-trial phase, A12 did reach criterion required to begin drug injection however stopped responding during baseline trials and was also dropped). The animals were housed individually in temperature controlled cages. The colony room was under a continuous reversed light-dark cycle (lights on at 7 p.m. and off at 7 a.m.). The rats were fed with Purina Lab Diet grain pellets, approximately one hour after a testing session and maintained at approximately 85% of their free-feeding body weight. Water was available ad libitum.

Apparatus

The apparatus used in this study was a modified version of the apparatus used by Dudchenko et al. (2000). We used a circular arena measuring 94 cm in diameter, surrounded by a 29 cm high wall (see Figure 1).
Figure 1. The apparatus used is a circular arena that is 94 cm in diameter, surrounded by 29 cm high wall. There are 18 holes that are spaced evenly (there are 12 holes on the outer ring, and 6 holes on the inner ring) that are filled with plastic cups.

There were 18 equally spaced holes, 12 of them forming an outer circle (positioned similarly to the digits on the clock, numbered in order from 1-12) and 6 forming an inner circle (numbered in order from 13-18). Plastic, 4 oz. Fabri-Kal condiment cups, filled with plain play sand, were positioned in the holes. White noise was emitted continuously thru a speaker during all testing sessions. Six distinct visual stimuli (8 inches high × 17 inches wide) were equally spaced on the wall of the arena so that the rat could get accustomed to them (see Figure 2).
Figure 2. Illustration of the alignment of visual stimuli and cup positions in relation to arena.

Preliminary training

Training sessions began at approximately 60-90 days of age and were run Monday through Friday. Rats were initially held for 15 minutes until accustomed to experimenters. Sucrose reward pellets were gradually introduced in their home cages. After the rats were gentled and consumed the sucrose pellets readily, they were introduced to the arena. During the first session they were allowed to explore the arena freely for 15 minutes. During the next session one cup filled with sand was placed in a random location and a sucrose pellet was placed on the surface of the sand. After consuming the pellet, the rat was removed from the arena and placed in a holding cage. The cup was moved to a new location and a reward pellet was placed on top of the sand. Rats were returned to the arena and allowed to find the cup and consume the pellet. This process repeated for approximately 30 minutes. This training continued on
subsequent sessions until a rat completed 12 trials in less than 30 minutes. On the next trial an opaque lid was placed next to the cup. Over next few sessions the lid was gradually moved over the cup so that at first it only touched the rim of the cup, and then it was gradually moved until it covered a larger area of the cup so that the rat had to displace the lid in order to retrieve the pellet. This process continued until the rat readily displaced the lid covering the entire cup and consumed the pellet on each of the twelve trials. The displacement of the lid with snout or front paws was used to define a response throughout the rest of the testing. In the next phase of the training the pellet was gradually pushed in to the sand and buried deeper into the sand so that after the rat displaced the lid it had to dig in the sand with its paws. After the pellet was completely buried in the sand, it was gradually pushed deeper until it was approximately 1 cm under the surface of the sand. The training continued until the rat was able to retrieve pellets on all 12 trials. Initially the experiment did not involve burying pellets. Rat W28 did not learn to non-match to positions in a timely way. To increase the response cost, we decided to begin burying the pellets under the surface of the sand. This process was started during the three-trial phase.

Non-matching to position

Three-Trial Phase

After the rat readily displaced the lids and dug in the sand for pellets, it was trained in the Three-Trial Phase of the non-match to sample span procedure. The sessions began with one cup placed in a randomly chosen location, covered with a lid and containing a sucrose pellet buried 1 cm below the surface of the sand. After the rat retrieved the pellet it was removed from the arena for a 30 second intertrial interval (ITI) and placed in a holding cage. If the animal made an incorrect response it was allowed to dig in other cups until a correct response was made. If no
response was made within 2 minutes the rat was removed from the arena and the response was scored as incorrect. The cup in which the animal had already dug was then removed and two new cups were added, one of them was placed in the previous location and one in a novel location. The animal was returned into the arena and only digging in the cup located in the novel location was reinforced. Upon retrieving the pellet, the animal was removed from the arena and the cups were removed and three new cups were placed in the arena, two in the previous locations and one in the novel location (see Figure 3). After the animal was reintroduced into the arena it was once again only rewarded for digging in the cup positioned in the novel location. When the animal made an incorrect choice it was allowed to move to a new location until it made a correct response. Only choices of novel location as the initial response were scored as correct.

Figure 3. The picture illustrates the non-match to sample memory span procedure. On the first trial marked as “Span 0” there is only one cup in the arena. The rat is reinforced for digging in that cup. On the next trial the cup in which the rat has dug before is no longer correct, this time the rat is reinforced for digging in the cup placed in the novel location. The same process continues on each consecutive trial, the novel location is always correct (from Dudchenko et al. (2000) p. 2971).

After the third trial, the process was repeated beginning with a single cup in a novel location and on subsequent trials a second and third cup location was added. This process was continued for two additional spans, so that at the end of the session the animal had been exposed to four blocks of three trials each. Each location was used only once during a given session.
After a location was used on one block, it remained empty on subsequent blocks. To aid in discrimination of the locations, the positions were chosen so that there was at least one unused position between them (since each location was numbered in order, on each block only positions which have either an even or odd number ascribed to them were used). After reaching a criterion of eighty percent correct responses on two consecutive sessions, the animal was moved to the next phase of the training. One rat was moved to the Four-trial phase after extensive training without meeting criterion in the hopes that exposure to the next phase might improve performance.

Four-Trial Phase (see Figure 3)

In this phase the animal began with digging in a single cup on the first trial. Cups were added on each trial, one at a time, until there were four cups in the arena (4 trials). This process was repeated two more times so that there were three blocks of four trials. All other conditions were identical as in *Three-Trial Phase*. This phase continued until the animal reached a criterion of three consecutive days at or above seventy five percent correct choices. In this phase another rat failed to meet criterion after extensive training and was moved to the Six-trial phase.

Six-Trial Phase (baseline)

This phase of the training was similar to previous phases but this time the sessions were divided into two blocks of six trials. This phase served as the baseline schedule to study drug effects. The animal was trained until it reached a ten day stability criterion in which the difference between the average performance on the first half of the ten day period and the average performance on second half of the ten day period was equal to or less than ten percent of the average performance on all ten days (adapted from Perone, 1991). After reaching the stability
criterion, drug administration began. In this phase one rat failed to meet a criterion and was dropped from the study.

To prevent the animals from scent marking the cups in which they have dug, all cups and lids were replaced on each trial. To ensure the rats were not scent marking their paths in the arena, the visual stimuli were moved such that the arena was physically rotated by 45 degrees without changing the location of the distal stimuli. This process was performed once during each block of trials before or after Trial 4 such that on one block the table was rotated before Trial 4 and on the other block the table was rotated after Trial 4. On the next session this sequence was reversed.

Drug administration

Drug doses were prepared daily by dissolving morphine in an isotonic sodium chloride and administered in volume of 1 ml/kg on Wednesdays and Fridays. Monday data was used to calculate baseline performance. Saline was administered on Tuesdays. Drug solutions were injected intraperitoneally. Several doses were tested (1.0, 3.0, 10, 18 and 30 mg/kg). There were two to four determinations of each drug dose.

Dependent measures

Several dependent measures were considered for analyses. Percent correct was calculated as the proportion of the number of correct responses to the overall number of trials and multiplied by 100 in order to obtain a percent value.

Span was calculated as a number of consecutive correct responses minus one (there was only one cup on the first trial therefore the animal had nothing to discriminate between). A separate span index was calculated for each block of trials and an average span on one testing session was used for analyses.
Longest run was obtained as the highest number of consecutive correct responses (after making the first incorrect response a new run began starting on the next correct response). Longest span is believed to be a better measure of the animal’s memory (sometimes an animal makes an incorrect response on a first or the second trial but continues to make correct responses throughout the block of trials; the longest span included the animal’s performance after the incorrect response).

Latency (calculated as the time it took the rat to retrieve the pellet; the timer was started when the animal was placed in the arena and stopped when it began digging in sand).

Total number of errors was scored as the number of all incorrect responses during a session. This measure was not available for one subject due to recording error.

Control measures

Several control measures were included during the testing sessions to ensure that behavior was under the control of spatial stimuli and not other local cues. Rodents are known for their remarkable sense of olfaction and they may use the scent of the reward pellet to help them navigate into the correct location. “Non-bait” trials were conducted on each baseline day on which all cups remained unbaited and the pellet was dropped into the cup after the animal made correct response. Four trials were chosen, two of them on each trial block. These trials were 2, 4, 9, and 11 or 3, 5, 8, and 10. These sequences were alternated such that the first one was always used on Mondays, and the second one on Thursdays.
RESULTS

Table 1 shows the number of trials required by each rat to reach criteria in each phase. A mean of 13.4 sessions (range = 10-20 sessions) was required for the rats to complete pretraining. The three-trial phase was completed relatively rapidly with a mean number of 22.75 sessions (range = 7-36 sessions). The four-trial phase required slightly more sessions to complete with a mean of 26.25 sessions (range = 7-45 sessions). Finally the six-trial phase required the most training with a mean of 29.33 sessions (range = 10-67). The total number of sessions required before drug administration averaged 93.33 trials (range 83-111). These calculations do not include data from the two animals that did not reach criterion on some phases.

Table 1

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pretrain</th>
<th>3-Trial</th>
<th>4-Trial</th>
<th>6-Trial</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>W2</td>
<td>10</td>
<td>36</td>
<td>30</td>
<td>10</td>
<td>86</td>
</tr>
<tr>
<td>W28</td>
<td>10</td>
<td>27</td>
<td>7</td>
<td>67</td>
<td>111</td>
</tr>
<tr>
<td>Z1</td>
<td>20</td>
<td>7</td>
<td>45</td>
<td>11</td>
<td>83</td>
</tr>
<tr>
<td>A12</td>
<td>15</td>
<td>21</td>
<td>23</td>
<td>50*</td>
<td>109*</td>
</tr>
<tr>
<td>A7</td>
<td>12</td>
<td>22*</td>
<td>12*</td>
<td>9*</td>
<td>55*</td>
</tr>
</tbody>
</table>

*subject failed to meet criterion

In order to determine whether rats may have been able to track the scent of the reward pellet, comparisons of performance on baited and non-baited trials was conducted. Performance on all non-baited trials was compared to performance on all analogous trials that were baited.
This comparison is shown in Table 2. Accuracy on baited trials was better than on non-baited trials for all three subjects. The mean percent correct on baited trials for all three subjects was 82% (range = 71.5%-88.5%) while mean percent correct on non-baited trials for all three subjects was 71.17% (range = 64.5% - 78.5%). The decrease in performance on non-baited trials may suggest that pellet detection played some role in responding. However, it is worth noting that chance performance was 40.83% and performances on non-baited trials were much higher than that in all three rats. It is possible that the animals used pellet detection on some trials.

Table 2

Comparison of baited to non-baited trials

<table>
<thead>
<tr>
<th>Subject</th>
<th>Baited</th>
<th>Non-baited</th>
</tr>
</thead>
<tbody>
<tr>
<td>W2</td>
<td>88.5</td>
<td>78.5</td>
</tr>
<tr>
<td>W28</td>
<td>71.5</td>
<td>64.25</td>
</tr>
<tr>
<td>Z1</td>
<td>86.0</td>
<td>70.75</td>
</tr>
</tbody>
</table>

For baseline sessions where table rotation was conducted, performance on trials that occurred before table rotation was compared to performance on comparable trials that occurred after table rotation. The results of this comparison are shown in Table 3. Mean percent correct on trials before table rotation was 72.44% (range = 63.16% - 87.5%). Mean percent correct on trials after table rotation was 73.02% (range = 60% - 90.63%). These comparable performances achieved on trials before and after table rotation suggest that rats did not use odor marking of the traveled path in order to navigate the apparatus.
Table 3

Comparison of performance on Trial 4 before and after a table rotation

<table>
<thead>
<tr>
<th>Subject</th>
<th>Before Rotation</th>
<th>After Rotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>W2</td>
<td>87.50</td>
<td>90.63</td>
</tr>
<tr>
<td>W28</td>
<td>66.67</td>
<td>60.00</td>
</tr>
<tr>
<td>Z1</td>
<td>63.16</td>
<td>68.42</td>
</tr>
</tbody>
</table>

Additionally, to control for experimenter bias, 20 randomly chosen videos were scored by a different rater who was blind to original scoring. This experimenter recorded the locations where all responses were made during a session. These were compared to originally recorded locations. This interrater bias check revealed 100% agreement between raters.

Figure 4 shows baseline percent correct on last ten sessions before the beginning of the drug administration phase for the three animals that met all baseline training criteria. Although performances were stable across the 10-session criterion, there was a considerable variability in the session to session performance. Percent correct values ranged between 66.67% and 91.67% for Rats W2 and W2, while Rat Z1’s percent correct varied between 50% and 83.33%, but all three rats consistently showed above chance levels of accuracy.

Figure 5 shows span and longest run values on these same sessions. As expected, longest run values (mean = 3) were higher than span values (mean = 1.81). W28 showed the most variability with a mean span of 1.5 (range = 1-6) and mean longest run of 3 (range = 1-6). W2 had a mean span of 1.83 (range = 1-2.33) and a mean longest run of 2.8 (range = 2-3). Z1 had a mean span of 2.1 (range = 0.5-4) and a mean longest run of 3.2 (range = 2-5).
Figure 4. Individual subject baselines. Graphs include percent correct values from the last ten sessions before drug administration began. Dotted line represents chance.
Figure 5. Individual subject baselines. Graphs include span and longest run values from the last ten sessions before drug administration began.
As shown in Figure 6, two out of three subjects (Z1 & W28) had somewhat better percent correct values on the first block of trials (a set of six trials that was run twice during one session) than on the second block. Subject W2 achieved higher mean percent correct on the second block but the difference was slight. Overall, performances did not differ from one span task to the other.

![Bar chart showing percent correct values for subjects Z1, W28, and W2 in 1st and 2nd blocks.](image)

Figure 6. Comparison of individual subjects performance on first and second block of trials. Vertical bars represent standard error of the mean.

Individual percent correct values obtained by subjects during morphine administration phase are shown in Figure 7. Rat W2 exhibited similar performances on baseline and saline sessions and there was little effect of 1.0 or 3.0 mg/kg morphine. Beginning with the 18.0 mg/kg dose, performance decreased as morphine dose increased. For Rat W28, percent correct values remained similar for baseline, saline, 1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg. Only 18.0 mg/kg morphine decreased percent correct. Rat Z1 had a slightly increased percent correct at 1.0 mg/kg morphine, but doses of 3.0 mg/kg and higher produced considerable decreases in accuracy.
Figure 7. Mean percent correct values are displayed for baseline, saline and each morphine dose. Vertical bars represent standard errors of the mean.
Figure 8 shows each subject’s mean span and longest run for baseline, saline and all morphine doses. Rat W2 did not exhibit any considerable decrease in either measure until a dose of 18.0 mg/kg was reached. Rat W28 had an increase in span and longest run at a dose of 3.0 mg/kg, doses of 1.0 mg/kg and 10.0 mg/kg were comparable with baseline, but a decrease at 18.0 mg/kg was observed. Rat Z1 showed a slight increase in span and longest run at dose 1.0 mg/kg and steep decreases at doses of 3.0 mg/kg and higher. Longest run remained relatively similar for doses 10.0 mg/kg, 18.0 mg/kg and 30 mg/kg, while span continued to drop at these doses.

Latency, one of the measures of performance, is shown in Figure 9. Rats W2 and W28 had similar values for baseline, saline, 1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg. Latency increases began with each higher drug dose. For Rat Z1 latencies were similar for baseline, saline, 1.0 mg/kg, and 3.0 mg/kg morphine. Increase in latencies began at dose 10.0 mg/kg and continued to increase with higher doses. The increases at the highest doses were mostly caused by the rats not responding and the latency being recorded as 120 seconds which was the maximum length of the trial. The latency increases are critical to the interpretation of the other morphine effects because once this measure of performance is affected we must consider the drug effect as a global impairment. This is because increased latency is associated with more generalized effects of morphine on areas of functioning such as motor movement or motivation, and such effects preclude interpretation in terms of selective action on working memory processes. However, effects on accuracy and longest run without corresponding latency effects occurred at 3.0 mg/kg for Rat Z1. Thus, effects of morphine at these doses cannot be attributed to gross impairment of functioning.
Errors made by subjects during testing sessions are another index of working memory which is not affected by trials scored as incorrect due to the animal’s failure to respond. Data were available for two subjects (W2 and W28) for total errors in a session at different morphine doses (Figure 10). During the time Rat Z1 was in drug injection phase, the laboratory video recording equipment was out of order and the sessions could not be recorded, thus errors were not scored. Rat W28 had a similar number of errors for all doses with only a slight increase at 10.0 mg/kg and 18.0 mg/kg while Rat W2 had a very large increase at higher morphine doses (10.0 mg/kg, and 18.0 mg/kg) with a maximum number of errors at dose 18.0 mg/kg. The fact that the increase in errors occurred for this rat at 10.0 mg/kg may mean that the effects of the drug emerged earlier and at the dose that was lower than dose which also caused the increase in latency.
Figure 8. Mean span and longest run values for baseline, saline and each morphine dose. Vertical bars represent standard error of the mean.
Figure 9. Individual subject latency for each drug condition. Vertical bars represent standard error of the mean.
In summary, all dependent variables (percent correct, span, longest run, latency, errors) were affected similarly by morphine. For Rats W2 and W28 effects began emerging at 18 mg/kg morphine with the exception of errors which increased for Rat W2 and W28 at 10 mg/kg morphine (although that increase was very minimal for subject W28). Rat Z1 appeared to be more sensitive and effects of morphine began emerging at 3.0 mg/kg for percent correct, span, and longest run and 10.0 mg/kg for latency.

To further examine the effects of morphine with respect to place remembering, we analyzed within session performance. Figures 11 and 12 illustrate percent correct plotted as a function of the number of places to remember for baseline, 1.0 mg/kg and 3.0 mg/kg (Figure 11), and 10.0 mg/kg and 18.0 mg/kg (Figure 12). There are separate panels for each subject and the lower right panel shows the mean of all three subjects.
Baseline accuracy decreased for all subjects as the number of places to remember increased. In each case accuracy was highest on Trial 1, showed slight decrease on Trial 2 and was lowest on Trial 6. Generally effects at 1.0 mg/kg and 3.0 mg/kg morphine were comparable to baseline for Rats W2 and W28; however, for Rat Z1, 3.0 mg/kg morphine showed striking effects and the function of this dose was much steeper. The accuracy of this rat was below chance levels on the last trial due to animal’s not responding. This suggests that this dose affected this animals global functioning. All doses began at 100% correct responding on Trial 1, which simply means that responses were being made, as there was only one stimulus available.

Figure 12 shows the effects of higher morphine doses and it is worth noting that Rat W2 was the only subject with perfect accuracy on the first trial for all three doses. This perfect accuracy suggests that any other decreases from baseline accuracy were not related to deficits in global functioning. If global functioning was affected, the responding on the first trial would also be affected. Other rats’ performances were less than 100% on Trial 1 at doses 10.0 mg/kg (Z1) and 18.0 mg/kg (W28). At dose 10.0 mg/kg the accuracy of Rats W2 and W28 was only slightly lower than the accuracy at baseline for most trials 1 – 4, it dropped sharply on trial 6 for Rat W2 and also decreased on trials 5 and 6 for Rat W28. The accuracy at this dose for Rat Z1 was much lower than the accuracy at baseline. The accuracy at 18.0 mg/kg for all subjects was strongly affected in comparison with baseline accuracy. Interestingly, percent correct was very similar for Trial 1 and Trial 2 at all doses which suggests that any effects at morphine doses of 1.0mg/kg, 3.0 mg/kg and 10 mg/kg (for Rats W2 and W28) were not associated with gross impairment.
Figure 11. Trial-to-trial analysis of percent correct values for baseline, 1.0 mg/kg, and 3.0 mg/kg. Vertical bars represent standard error of the mean.
Figure 12. Trial-to-trial analysis of percent correct values for baseline, 10.0 mg/kg, and 18.0 mg/kg. Vertical bars represent standard error of the mean.

The within session performance data for baseline showed a decline in performance as the number of places to remember increased and the function was altered by morphine in dose-dependent fashion. Although the inverse relation between the number of places to remember and accuracy might be interpreted as an effect of memory load, there is a problem with this explanation. The number of places to remember is confounded with the number of comparison cups present in the apparatus. In order to control for that confound Experiment 2 was designed.
EXPERIMENT 2

METHOD

Subjects

One subject (Z1) previously used in Experiment 1 was used in this experiment.

Apparatus

The apparatus was the same used in Experiment 1.

Procedures

Nine-Trial Phase

Following completion of Experiment 1, the procedure was changed such that only a single trial block of nine stimulus positions was used. As previously, cups were added on each trial, one at a time, until there were nine cups in the arena. There was only one block of trials in this phase. This training continued for 72 sessions associated with a different experiment.

Nine-Trial-2-Comparison Phase

This phase was similar to the Nine-Trial Phase with the exception that a maximum of two cups were present in the apparatus at any time. The session started with one cup in one position. On consecutive trials there was one cup in a novel position and one cup in a randomly chosen position that was used previously. This training was continued for five sessions and then the drug procedure began.

Drug Administration

Morphine doses were administered on the same days as in Experiment 1. Two determinations of each dose (1.0 mg/kg, 3.0 mg/kg, 10.0 mg/kg, & 18.0 mg/kg) were administered.
RESULTS

Figure 13 provides a comparison of percent correct values on the final five sessions of the Nine-Trial Phase (left panel) with those of first five sessions in the Nine-Trial-2-Comparison Phase. As seen on the graph, performance in the Nine-Trial-2-Comparison Phase was generally better than in the Nine-Trial Phase. The mean percent correct from the first was 73.33% (range = 44.44% - 100%) while the mean percent correct from the latter was 88.89% (range = 77.78% - 100%).

Figure 13. Z1’s percent correct values from last five sessions in Nine-Trial Phase (left panel) and last five session in Nine-Trial-2-Comparison Phase before drug administration began (right panel). Dotted lines represent chance.
Figure 14. Rat Z1’s span and longest run values from five training sessions before drug administration began.

Figure 14 shows similar comparison of span and longest run values for the last five sessions of the Nine-Trial Phase (left panel) and five sessions in the Nine-Trial-2-Comparison session before drug administration. In the Nine-Trial Phase the mean span was 4.8 (range = 2–7) and the mean longest run was 5.2 (range = 3–7) while in the Nine-Trial-2-Comparison Phase the mean span was 5 (range = 3-8) and the mean longest run was 5.2 (range = 3 – 8). Unlike in the 6-Trial phase where longest run values were always higher than span values, here span and longest run have the same values on four out of five sessions. This is because the first error usually did not occur until well after the first half of the session which also makes that span the longest run for that day.

In Figure 15 mean percent correct values for baseline, saline and each morphine dose are shown. Baseline mean percent correct was 93.33 (range = 77.78-100). Accuracy began to decrease at 3.0 mg/kg where the percent correct value was equal to percent correct for 10.0 mg/kg. A further decrease was observed at 18.0 mg/kg.
Figure 16 illustrates mean span and longest run for each drug condition. Here longest run values were higher than span values. The difference was minimal at baseline, 3.0 mg/kg, and 10.0 mg/kg. Much larger differences were observed at saline, 1.0 mg/kg, and 18.0 mg/kg.

![Graph showing percent correct values for baseline, saline and each morphine dose. Vertical bars represent standard errors of the mean.](image1)

Figure 15. Mean percent correct values are displayed for baseline, saline and each morphine dose. Vertical bars represent standard errors of the mean.

![Graph showing mean span and longest run values for baseline, saline and each morphine dose. Vertical bars represent standard errors of the mean.](image2)

Figure 16. Mean span and longest run values for baseline, saline and each morphine dose. Vertical bars represent standard errors of the mean.
Latency, as shown in Figure 17 was not affected until the 18.0 mg/kg dose. Thus the decreases in accuracy and span at 3.0 mg/kg and 10.0 mg/kg occurred at doses that did not affect overall performance as assessed by latency, and may be viewed as effects specific to cognitive processes and not to overall functioning. However, the sharp increase at the 18.0 mg/kg dose suggests that these effects extended to the overall functioning of the animal.

Figure 17. Mean latency for Rat Z1 for baseline, saline and each morphine dose. Vertical bars represent standard error of the mean.
Figure 18. Mean total errors in a session at different morphine doses. Vertical bars represent standard error of the mean.

Mean errors are shown in Figure 18. Increase in errors in comparison with baseline and saline was observed at 3.0 mg/kg, 10.0 mg/kg and 18.0 mg/kg, this further supports the finding that decreases in accuracy at doses 3.0 mg/kg and 10.0 mg/kg were not associated with gross impairment. This conclusion can be made because no increases in latency were observed at this dose.
Figure 19. Trial-to-trial comparison of mean percent correct on baseline trials in the Nine-Trial Phase (left pane) and mean percent correct on baseline trials in the Nine-Trial-2-Comparison Phase (right panel).

Figure 20. Trial-to-trial accuracy for each morphine dose. Left panel includes baseline, 1.0 mg/kg, and 3.0 mg/kg, right pane includes baseline, 10.0 mg/kg, and 18.0 mg/kg.

To examine how limiting of the number of stimulus cups to two affected the within session performance we compared the mean baseline within-session accuracies in the Nine-Trial Phase and the mean baseline within-session accuracies in the Nine-Trial-2-Comparison Phase. This comparison is shown in Figure 19. The baseline accuracies in the Nine-Trial Phase (left pane) decreased with the addition of each stimulus. The accuracy on Trial 1 was at 100% and it decreased to 36% on Trial 9. The baseline accuracies in the Nine-Trial-2-Comparison Phase
(right pane) did not decrease as the number of stimuli to remember increased. The percent correct remained between 80% and 100% and it was equal on Trial 1 and Trial 9. This comparison is important because it shows that it was not the number of stimuli to remember, but rather the number of stimulus cups present in the arena that caused the decrease in accuracy in the Nine-Trial Phase. When the number of stimuli was limited to two, there were no within-session decreases in accuracy.

Figure 20 shows a comparable analysis for baseline, 3.0 mg/kg (left panel) and 10.0 mg/kg (right panel). These doses were chosen because they were high enough to produce some effects, but not so high as to produce overall impairment of responding. The effects of both doses of morphine were more apparent when there were more places to remember which supports the claim that these effects involved processes sensitive to the memory load. The effects of morphine at 3.0 mg/kg were mostly due to the sharp accuracy decreases on Trial 8 and Trial 9 when the percent correct dropped to 0%. At 10.0 mg/kg drug effects were mostly due to the accuracy decreases on Trials 5-9.

DISCUSSION

This study demonstrated that rats can be taught a spatial span task. In Experiment 1 the rats achieved a mean baseline percent correct of 78.02%, an average span of 1.81 and an average longest run of 3. The longest runs were usually longer than the spans because rats often continued to make runs of correct responses after they made their first incorrect response. For that reason longest run provides a better index of rats’ capacity to remember places in this procedure. Baseline responding decreased in a similar fashion in Experiment 1 as the responding observed in Dudchenko et al. (2000) who trained rats on a similar spatial task in order to study
the effects of hippocampal lesions on working memory. The average of each animal’s median span was 4.56. The mean accuracy for baseline was around 57%. Unlike Dudchenko et al. (2000) who were able to train rats on 12 locations, in our study the decrease in accuracy occurred faster when the number of stimuli continued to increase. For that reason we used a maximum of six locations in Experiment 1. It is not known why the rats in Dudchenko et al. (2000) study were able to respond with high accuracies with 12 locations while our animals’ accuracies decreased more rapidly which allowed for a maximum of 6 locations. One possibility is the difference between apparati. Dudchenko et al. (2000) used a square apparatus which had low borders. It is possible that the shape of their apparatus helped the animals to discriminate between locations. Also the low border may have allowed the animals to navigate using distal cues of the room in which they were tested.

In Experiment, baseline accuracy decreased throughout the session. On the first trial accuracy was perfect but with the addition of each stimulus cup it decreased and was lowest on the last trial. Dudchenko et al. (2000) also showed a gradual decrease in baseline accuracy. Accuracy of the animals in their study was at 100% at the beginning of the session and it decreased with each consecutive trial and was around 20% on Trial 12. Dudchenko et al. (2000) interpreted their results as the effect of memory load increase on working memory. We noticed a problem in this explanation. Alternative interpretation of these results was that the increase in the number of stimulus cups caused the accuracy to decline.

In Experiment, percent correct, span and longest run were unaffected by morphine except when high doses of 18.0 mg/kg were administered in Rats W2 and W28. Latency was affected at the same dose in these rats. Rat Z1 exhibited higher sensitivity to morphine as percent
correct, span and longest run showed decreases at 3.0 mg/kg while the decrease in latency did not begin until 10.0 mg/kg.

Latency was often very elevated at higher doses due to the fact that the rats were not responding and the trials were scored as incorrect and given the maximum latency of 120 seconds. In order to find out whether the latencies were increased due to nonresponding or making many incorrect responses errors measure was scored. For Rat W2, errors increased slightly at 3.0 mg/kg and more substantially at 10.0 mg/kg which suggests that effects emerged at this dose, before span, longest run and percent correct were increased. These effects also occurred at lower doses than the increase in latency which suggests they were not caused by gross impairment of functioning. For Rat Z1, the effects on accuracy, span and longest run occurred at lower doses than the effects on latency which brings the same conclusion that the effects of morphine on cognitive functioning occurred before effects on global functioning.

The within session analyses of morphine effects showed that, similar to baseline, at 1.0 mg/kg, 3.0 mg/kg, 10.0 mg/kg (Rats W2 and W28), accuracy on Trial 1 was at 100%. For Rat Z1 accuracy on Trial 1 was at 100% for doses 1.0 mg/kg and 3.0 mg/kg. This suggests that rats were able to make responses at these doses but as the session continued this ability decreased. This type of effects was present at 3.0 mg/kg in Rat Z1 whose accuracy declined beginning with Trial 2. Also in Rat W2 similar effects were present at 10.0 mg/kg (decline on Trial 6) and 18.0 mg/kg (decline beginning with Trial 3). Rat W28 exhibited comparable results at 10.0 mg/kg (decline beginning with Trail 5) and 18.0 mg/kg (decline beginning with Trial 4) where the accuracy on Trial 1 was at 75% but it was at 100% on Trial 2. The ability to make correct responses on Trial 1 but decrease in accuracy on later trials suggests that this impairment was not caused the effects on global functioning. This analysis of within session accuracy further confirms the conclusion
that was reached previously; such that affects at doses 10.0 mg/kg in Rat W2 (increased errors) and 3.0 mg/kg in Rat Z1 (decreased span, longest run and accuracy) were not associated with impairment of global functioning.

As mentioned above, the decrease in ability to make correct choices was at first interpreted as the function of the increasing number of stimuli to remember. However, as noted earlier, there is a confound between the number of stimuli to remember and the increase in the number of stimulus cups available for choice in the Dudchenko et al study as well as in Experiment 1. This increase could cause difficulties in finding the novel stimulus cup and lead to a different interpretation of within session effects and drug interactions. One potential explanation of why the accuracy functions were sharply decreasing at higher doses was that morphine affected not the ability to correctly remember positions but to inhibit the responses at incorrect positions.

Experiment 2 was designed in order to test whether the number of stimulus cups present interfered with rats’ ability to locate novel locations. In this task the number of stimuli was limited to two but the number of places to remember incremented up to nine as the session progressed. Under baseline conditions in Experiment 2, the mean percent correct was at 93.33%, average span was 5.8 and the average longest run was 6.4—much higher than was observed in Experiment 1. Unlike the previous experiment, the difference between span and longest run was small because the subject often did not make an incorrect response until after the first half of the session so that the longest run was scored as equal to span. The within session analysis of accuracy at baseline showed that, unlike in Experiment 1, the accuracies did not decrease when the number of positions to remember increased. The accuracies on all trials ranged between 80% and 100% and they were equal on Trial 1 and Trial 9. These findings confirmed that the decrease
in within trial accuracies in Experiment 1 was caused by the increase in the number of stimulus cups and not the increase in memory load. The percent correct, span and longest run measures obtained in Experiment 1 were probably affected by processes associated with global functioning such as motor movement and vision. This finding also complicates the findings by Dudchenko et al. (2000) where the number of stimuli also increased with each trial. It is possible that the decreases in accuracy of hippocampectomized rats could have been caused by disruptions other than those limited to working memory.

In Experiment 2 percent correct, span and longest run were decreased and errors were increased by morphine at doses 3.0 mg/kg 10.0 mg/kg without increases in latency. In baseline, there was no decrease as the number of places to remember increased, but after 3 or 10 mg/kg of morphine accuracy decreased (they were highest on Trial 1 and lowest on Trial 9). This suggests that morphine effects were memory load-dependent (the effects increased with the increase in the number of stimuli to remember).

The findings of Experiment 2 suggest that morphine can affect memory for places. Further research might benefit from the inclusion of additional controls. To prove that these effects were selective to working memory a separate acquisition and performance components are needed. Our task had only one component which was designed to measure working memory. The acquisition and performance components are usually very similar with the exception that the acquisition component requires an animal to use working memory while performance component does not. A good performance component task would be one in which one location is always correct. This condition would have to be signaled by a different set of visual stimuli. Although our study did not contain a performance component it contained a performance measure (latency). Because accuracy, span, longest run and errors were affected at doses lower
than those needed to impair latency, it is predicted that if a separate performance component were added to the procedure, it would also be affected differentially.

Our results were similar to the results of experiments which tested spatial acquisition and included both acquisition and performance components. The study by Galizio et al. (2003) used a spatial task in which rats were required to locate a hidden platform. In the acquisition component the location of the platform was different on each session while in the performance component the location remained the same from session to session. Morphine was found to disrupt the ability to locate the platform in the acquisition component at doses lower than those needed to disrupt the ability to locate the platform in the performance component. This kind of differential effect is referred to as selective to acquisition. The difference between the study by Galizio et al. (2003) and our study is that while rats were required to locate the same location as in the first trial (match to sample), in our study they were required to locate a novel location (non-match to sample). Nevertheless the effects of morphine seem to be the same in both spatial tasks.

The effects of morphine were different in a nonspatial experiment done by Moerschbaecher and Thompson (1983) who trained monkeys on acquisition of conditional discriminations. The monkeys were required to press two levers in an order that changed from session to session when a certain shape was presented on a white background in an acquisition component and in always the same order on performance component (signaled by a shape presented on a color background). In this study morphine did not produce selective effects because increase in the errors (measure of acquisition) did not emerge until the rate of responding was also decreased (measure of performance). These findings were similar to the findings by Pitts et al. (2006) who used another nonspatial design where rats were required to nose-poke always the same location on a 2×3 stimulus array in a performance component and a
location that changed between sessions in an acquisition component. In this study similar to the previously described study, morphine affected acquisition at doses that also affected performance. Another study that used a non-spatial task and was not able to demonstrated selective effects of morphine was the study by Deal et al. (2010) who used an olfactory span task that was very similar to ours. In fact their rats were trained on an apparatus that was identical to the one used in our study except they were required to discriminate between scented cups.

The task in which selective effects of morphine were shown (Galizio et al., 2003) used escape as reinforcement. The studies where no selective effects were shown used food as reinforcement (Moerschbaecher & Thompson, 1983; Pitts et al., 2006; Deal et al., 2010). It was not clear whether these effects were different because of the type of the task used (spatial vs. non-spatial) or the type of reward presented (food vs. escape). Our study attempted to examine the effects of morphine in a spatial task in which food was used as a reward. It was hypothesized that the effects on percent correct, span and longest run would be present before there were effects on latency which would support the hypothesis that it is the type of the task that is responsible for the different effects of morphine and not the type of reward. This hypothesis was partially supported in Experiment 1 but it was concluded that the task was confounded with an unexpected variable (number of stimuli present). After the task was modified and the confound was removed (Experiment 2) morphine was shown to affect accuracy at doses that did not produce disruption of behavioral processes other than those related to the number of places to remember: working memory.

The design of this study included several control measures. Rodents are known to have a very sensitive sense of smell. We attempted to control for the possibility of the rats using smell in obtaining of the reward. One of the control methods involved the rotation of the apparatus.
during the session to ensure that the rats were not scent marking their path in order to aid in navigation. The comparison of the accuracy on trials before and after the rotation revealed that there were no substantial differences. To control for the possibility of rats smelling the reward and using that scent to locate the novel stimulus, we inserted nonbaited trials into the baseline sessions. The analysis of these trials revealed that the detection of the sucrose pellets played some role in responding. The accuracy on trials where there was no reward was worse than on trials where reward was present. Although this accuracy was affected, it remained above chance. We believe that the pellet detection played some role in responding but it was not the exclusive method of solving the spatial task. Future studies should use a better control to prevent the possibility of pellet detection. One option is to always present the reward after the response is made and to prevent the scent of the reward from lingering on a stimulus cup.

There were other limitations in this study. As mentioned above our experiment did not include a separate performance component which would allow to imply selective effects of morphine on working memory. A similar task (signaled by a different set of visual stimuli) in which an animal is rewarded for always choosing the same location is needed. This type of task does not tax working memory. If the animal exhibits effects of the drug in the acquisition component but continues to respond correctly in the performance component, then selective effects of the drug on working memory are present.

In Experiment 2 it was found that the animal’s accuracy did not decrease throughout the session when nine locations were used. It would be useful to assess how many locations can be remembered by rodents before their accuracies begin to decrease. Also, it would be of interest to find out if the accuracies change if the limit of stimuli is changed, for example to 3, 4 or 5
stimuli. A study should be designed which manipulates the number of stimuli available for choice as well as allows for testing of higher memory loads.

The findings of this study support the findings of studies with humans reviewed above that suggested the possibility that morphine and related opiates may impair cognitive functioning. The present findings suggest that morphine, whether taken for pain or as a replacement therapy for addiction to opiates, is likely to cause disruptions in spatial working memory. In humans, these disruptions might be manifested in different ways such as problems in remembering how to get to places, following driving directions, or finding a way back. These findings question whether doses of morphine which disrupt working memory should be prescribed outside of medical settings and for an extended period of time. The author of the current study believes that when opiate drugs are prescribed for an extended period of time, the individuals should be educated about the possible disruptions in spatial functioning. When prescribing very high doses for a period of time that is longer than a few months, patients should be monitored for possible memory disruptions. Also methadone patients should be placed in intensive substance abuse therapy and pain patients should be taught how to manage their pain without medications. If possible, these patients’ doses should be tapered gradually. This should be done with caution because of the very high relapse potential in those addicted to opiates. Hopefully future studies will examine the effects of morphine on working memory in humans and assess whether the potential for significant disruption of functioning exists and whether there are ways to minimize these disruptions.
LITERATURE CITED


Dudchenko, P., Wood, E., & Eichenbaum, H. (2000). Neurotoxic hippocampal lesions have no effect on odor span and little effect on odor recognition memory but produce significant


