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The Interaction of Pain and Morphine on Analgesia, Locomotion, and Cognitive Functioning

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The interaction of pain and morphine on analgesia, locomotion, and cognitive functioning

A Thesis

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Master of Science
in
Psychology

by

Brandon A. Baiamonte

B. S., Louisiana State University, 2005

M. A., Southeastern Louisiana University, 2007

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Abstract

Opioid medications are medicine's best weapon against severe intractable pain, but prolonged use of these medications can be complicated by side effects like tolerance and mental clouding which, themselves, can be disabling. The present study examined the independent and combined effects of inflammatory pain and opioid medication on spatial memory for a well learned task in Sprague-Dawley rats. The Hargreaves method was used to verify the pain state of the animals after complete Freund's adjuvant injection and morphine treatment. Whereas pain had little effect on spatial memory, morphine had profound detrimental effects that persisted beyond the analgesic effectiveness of the drug. However, morphine-induced cognitive deficits were absent when morphine was provided to animals in chronic pain. Also, analgesic tolerance was significantly attenuated in these animals. Taken together, these results suggest that chronic pain activates a neural mechanism that antagonizes the unwanted effects of opioids.

Keywords: radial maze, morphine, opioids, memory, pain, analgesic tolerance

Introduction

Pain has been described as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (IASP, 1979). It is useful to distinguish between acute and chronic pain. Acute pain serves a protective function by warning the organism of potential or actual injury that should be avoided or treated. Acute pain leads to a withdrawal response and protective responses that prevent continued use of the injured body part to avoid further harm to that particular region. Chronic pain, however, is longer in duration and may serve to motivate the organism to rest and attend to the injury during healing (Siegfried, Frischknecht, & De Souza, 1990; Hunt & Mantyh, 2001). When chronic pain outlasts healing, it can have a devastating impact on individual sufferers and society, leading to needless suffering, healthcare expenses, and lost productivity (Rosenblum, Marsch, Joseph, & Portenoy, 2008).

The reduction of pain and suffering is fundamental to good clinical practice. However, the clinical management of pain is not always a simple endeavor; the benefits offered by any interventions have to be weighed against the potential risks of treatment. In pharmacological pain management, the most effective pain relievers are derived from the opium poppy (papaver sativum). As a class, opioid drugs are capable of providing profound analgesic relief. The side effects of opioids include respiratory suppression, gastrointestinal slowing, addiction, and mental clouding. Although these are very real and serious concerns, there is evidence that they are more common in “recreational” or illicit opioid use than in proper pain management. An approach using basic science is needed to better inform clinical decisions about opioid use.

Pharmacological Treatment of Pain

Opioids and their receptors. Opioid medications are the best weapon against severe intractable pain. Opioid drugs, like morphine, codeine, hydrocodone, etc., act on opioid receptors in the CNS to produce their effects. Opioid receptors consist of mu (μ)-, delta (δ)-, and kappa (κ)-opioid receptors (Harrison, Kastin, & Zadina, 1998). These receptors show a distinct pattern of expression and organization throughout the somatosensory systems, the limbic system, and the extrapyramidal system. Mu and delta sites are concentrated in the more rostral areas of the CNS and appear to have complementary distributions (Sharif & Hughes, 1989). Limbic structures, such as the neocortex, show predominately μ -receptor populations, with relatively fewer δ and κ sites (McLean, Rothmann, & Herkenham, 1986). In more caudal areas, μ - and κ -receptors are more pronounced (Mansour, Khachaturian, Lewis, Akil, & Watson, 1988).

All opiate receptors to date are believed to be members of the G-protein coupled receptor superfamily (Connor & Christie, 1999). Members of this family show a conserved structure, with seven membrane spanning regions and exert their effects predominately by activating second messenger cascades (Connor & Christie, 1999). In the case of opiate receptors, the effects of agonist binding are usually associated with the inhibition of cAMP production (Collier & Roy, 1974; Sharma, Nirenberg, & Klee, 1975), the inhibition of calcium influx (Brown & Birnbaumer, 1990), or the opening of an inward rectifying potassium current (DiChiara & North, 1992), all of which are inhibitory with respect to the formation of an action potential by the postsynaptic neuron.

The role of each receptor type has been dissociated to some degree. Supraspinal analgesia has been attributed predominately to μ -receptors (Fu & Dewey, 1979). Although δ -

receptors have been shown to be involved in some supraspinal analgesia pathways, they are mainly localized in the medullary reticular formation (Jensen & Yaksh, 1986). Agonists for each of the three receptor types produce antinociception at the spinal level, however, κ -agonists are unique in their suppression of mechanical nociceptive impulses; μ and δ appear to mediate thermal nociceptive signals (Schmauss, 1987).

The presence of multiple receptor types predicts that a number of endogenous opiate peptides exist to serve as agonists at these receptors. There are four known families of endogenous opioid peptides: enkephalins, β -endorphin, dynorphins, and endomorphins (Zadina, Hackler, Ge, & Kastin, 1997; Kandel, Schwartz, & Jessell, 2000). Each of the opioid peptides has been found to have a distinct pattern of interaction with the receptor subtypes (Pasternack, 1986; Leslie & Laughlin, 1993). Beta-endorphin seems to show at least moderate affinity at these sites, but lacks high specificity (Hollt, 1986; Law, Loh, & Li, 1979). The enkephalins seem to be the natural ligand of δ -receptors (Lord, Waterfield, Hughes, & Kosterlitz, 1977) and dynorphins show marked affinity and selectivity for κ -receptors (Chavkin, James, & Goldstein, 1982; Corbett, Paterson, McKnight, Magnan, & Kosterlitz, 1982). Endomorphin-1 and endomorphin-2 display high affinity and selectivity for the μ -receptor (Zadina et al., 1997). These endogenous opioid peptides activate the opioid receptors in response to a painful stimulus, thus regulating nociceptive transmission. Morphine acts by mimicking the action of the endogenous opioids by inhibiting the firing of dorsal horn neurons responsive to nociceptive stimuli thus producing analgesia.

Resistance to the utilization of opioids. Unfortunately, a hesitancy to utilize opioid therapy has developed which has led to inadequate pain management (Zenz, 1991; Cleary &

Backonja, 1996). The number one reason physicians have become reluctant to prescribe opioid medications is fear of legal sanctions if the drugs wind up on the illicit market, a phenomenon called opiate shunting (Popenhagen, 2006). Patients are also reluctant to use opioid therapy for reasons such as fear of side effects that include analgesic tolerance, withdrawal, and addiction. Although we do not propose to directly study opiate shunting in the clinical setting, we believe that studying the effects of opiates and their side effects will help to shed light on the clinical aspects of pain reduction with opioids.

Pain's Interaction with Opioids

Pain and analgesic tolerance. Although analgesic tolerance has been demonstrated in some clinical research there is growing evidence that tolerance is not a significant threat to good pain management. It has also been shown that analgesic tolerance develops rapidly in pain-free individuals, but fails to develop in people with chronic pain (Twycross, 1988; Melzack, 1991; Foley, 1993; Portenoy, 1994, Chen & Vaccarino, 2000). Other studies have shown that the occurrence of side effects such as analgesic tolerance, mental clouding, euphoria, respiratory suppression, and physical dependence seem to be reduced (Zenz, 1991; Forbes, 2006) or eliminated (Portenoy, 1996) when the patient is in pain.

In the past two decades, animal studies have verified that pain can attenuate the development of analgesic tolerance in certain situations (Melzack, 1991; Vaccarino et al, 1997; Vaccarino, 1999). The development of tolerance to morphine analgesia has been well established in models of phasic or brief, escapable pain (Mucha, Kalant, & Linseman 1979). However, the development of tolerance in models of persistent pain is less clear (Vaccarino et al, 1997). Several models of persistent pain, e. g. subcutaneous (s. c.) formalin (Vaccarino &

Couret, 1993; Bardin, Kim, & Siegel, 2000), s. c. CFA (Chen & Vaccarino, 2000), and surgical pain (Ho, Wang, Liaw, Lee, H., & Lee, S., 1999) have been shown to attenuate tolerance development, while in other persistent pain models tolerance develops normally. The reasons for the discrepancies may be due to the type of pain model used, dose and route of morphine administration, or the presence or absence of pain during morphine injection (Cleary & Backonja, 1996).

Pain and opioid reward. Researchers have shown that pain attenuates the rewarding effect produced by morphine in both the conditioned place preference (CPP) and self administration models (Suzuki, Kishimoto, Misawa, Nagase, & Takeda, 1999; Narita, Kishimoto, Ise, Yajima, Misawa, & Suzuki, 2005). It is believed that the pain state leads to a sustained activation of the κ -opioidergic system in the nucleus accumbens resulting in the suppression of the rewarding effects. Researchers (Ozaki, Narita, Iino, Sugita, Matsumura et al, 2002; Ozaki, Narita, Mizoguchi, Suzuki, & Tseng, 2003) have also shown that the rewarding effects induced by opioids have been absent under a neuropathic-pain state of a mouse or rat. Researchers have shown that mesolimbic dopamine neurons are involved in the brain mechanisms of reward and reinforcement (Wise & Rompre, 1989). Ozaki et al (2002) believe that their findings suggest that this modification of morphine-induced place preference may result from a suppression of morphine's ability to stimulate dopamine release in the nucleus accumbens due to a reduction in the μ -opioid receptor-mediated G-protein activation in the ventral tegmental area.

Mechanisms of Pain/Opioid Interaction

Taken together, the above findings suggest that the state of pain may alter the effects of opioid medications on the body. It is important to discuss possible mechanisms for this phenomenon since the ability of pain to alter opioid effects is the basis for the hypothesis of the current study. It has been reported that chronic pain can induce anxiety among other negative effects (Von Korff & Simon, 1996). Many researchers (Sauro, Jorgensen, & Pedlow, 2003; Bomholt, Harbuz, Blackburn-Munro, G., & Blackburn-Munro, R., 2004) have concluded that stress results in a series of mechanisms that are aimed to protect the organism and restore homeostasis. Our working hypothesis is that pain's ability to activate the stress systems of the brain alters the body's response to morphine. This stress response includes both neural, (limbic and sympathetic nervous system activation) and a neuroendocrine response governed by the hypothalamus through the pituitary and adrenal glands, also called the hypothalamic pituitary adrenal (HPA) axis (Bomholt et al, 2004; Narita et al., 2006; Vierck, Acosta-Rua, Rossi, & Neubert, 2008). The stress activates parvocellular neurons in the paraventricular nucleus of the hypothalamus, which releases corticotrophin-releasing factor (CRH) (Bomholt et al, 2004). The release of CRH stimulates secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary (Jameison & Dinan, 2001).

Research has shown that disruption of the HPA axis reduces the effects of pain on tolerance and this has been shown by the ability of hypophysectomy (Holaday, Dallman, & Loh, 1979) and adrenalectomy (Wei, 1973) to reverse pain's effect on analgesic tolerance. Furthermore, Vaccarino et al (1997) demonstrated that pain failed to attenuate analgesic tolerance to morphine when adrenal steroid synthesis was inhibited by chronic metyrapone

administration because metyrapone blocks the elevation of corticosterone during stressful events without effecting basal corticosterone levels (Freo, Holloway, Kalogeras, Rapoport, & Soncrant, 1992). This finding implicated the stress induced elevation of corticosterone in the blockade of tolerance by pain. Therefore, it is believed that stress is the key factor that is present with pain that prevents tolerance to opioids.

Activation of the stress response is a likely target for the mechanism of pain's ability to modify opioid effects, since many stressful stimuli are capable of activating and modulating the endogenous opioid system upon which morphine acts (Watson & Mayer, 1981; Narita, Kaneko, Miyoshi, Nagumo, Kuzumaki, Nakajima et al., 2006). In many circumstances β -endorphin is released simultaneously with ACTH by the anterior pituitary. The simultaneous release of ACTH and β -endorphin is facilitated by the fact that they share the same pro-peptide molecule (proopiomelanocortin). β -endorphin is released into the general circulation in physiologically significant amounts, a process that has been linked with stress-induced analgesia (Rubinstein, Mogil, Japon, Chan, Allen, & Low, 1996). ACTH acts on the adrenal cortex to affect the release of glucocorticoids into the general circulation (Jameison & Dinan, 2001; Bomholt et al, 2004).

Stress Effects on Opioid Receptors

Narita et al (2006) investigated whether chronic pain could change opioidergic function in the amygdala. It has been well documented that all three opioid receptor types are associated with anxiety and stressful situations (Kiristsy-Roy, Appel, Bobbitt, & Van Loon, 1986; Broom, Jutkiewicz, Folk, Traynor, Rice, & Woods, 2002; Pfeiffer, Brantl, Herz, & Emrich, 1986). Narita et al (2006) found a decrease in the stimulatory effect of a μ - and δ -opioid receptor agonist and an increase in the G-protein activation by a κ -opioid receptor agonist following CFA

injection. Zubieta, Smith, Bueller, Xu, Kilbourn, Jewett et al (2001) has also shown, via positron emission tomography, a reduction in μ -opioid receptor availability in human subjects during sustained pain. Narita et al (2006) concluded from his findings that sustained pain increases the release of endogenous opioids interacting with μ -opioid receptors in the amygdala and this results in the internalization and recycling of μ -opioid receptors. The reduction in μ -opioid receptors as a result of release of ACTH in the HPA axis alters the effects of opioids on the organism. Therefore, the organism will be in a stressful state as a result of the pain, which will reduce the amount of μ -opioid receptors resulting in a modification of opioidergic effects. One possibility is that the deleterious effects of morphine on cognitive functioning can also be modulated by the stressful nature of pain through this particular mechanism, similar to how stress modulates opioid tolerance and opioid reward.

While Narita et al (2006) emphasize the importance of the release of ACTH in the HPA axis as a mechanism for pain's ability to modify opioids' effects, other researchers believe that the release of β -endorphin in the HPA axis is the mechanism for altering opioidergic effects. The relative analgesic versus endocytosis or "RAVE" theory emphasizes the effect of agonist activity and receptor endocytosis on receptor mediated signaling (Finn & Whistler, 2001). It is believed that agonist activity and receptor endocytosis have opposing effects on the ability of the receptor to signal. It has been shown that morphine has a high RAVE value due to its inability to promote receptor desensitization and endocytosis (Whistler, Chuang, Chu, Jan, & von Zastrow, 1999). However, endorphins and opioid drugs with lower abuse potentials induce receptor desensitization and endocytosis, resulting in a lower RAVE value (Finn & Whistler, 2001). These two observations have led researchers to suggest that drugs with a high RAVE value have a tendency to produce adverse effects due to prolonged signaling that leads to mu receptor

sensitivity. Researchers have shown that a reduction in prolonged signaling of drugs with high RAVE values helps reduce the development of side effects (Finn & Whistler, 2001). He, von Zastrow, & Whistler, J. L. (2002) have also shown that the development of tolerance was attenuated by the simultaneous injection of endogenous ligands and morphine. Therefore, another possible mechanism for pain's effect on opioid analgesia and reward is that the stress induced by the painful stimulus will activate the HPA axis causing a release of the endogenous opioid, β -endorphin. This release of β -endorphin and administration of morphine will result in a reduction of the adverse effects of prolonged signaling induced by morphine. Through this mechanism, it is possible for pain to modify the deleterious effects of morphine and possibly minimize the adverse effects of opioid medications.

Opioid Effects on Cognition

Cognitive functioning incorporates a wide variety of mental activities. Cognition has been defined as the "brain's acquisition, processing, storage and retrieval of information" (Lawlor, 2002). Domains of cognition include attention, concentration, simple recall, working memory, verbal memory, and executive function. Clinical research has shown that ingestion of opioid drugs is detrimental to cognitive function (Forbes, 2006). According to Zacney (1995), the negative effects of opioids are most pronounced in "healthy volunteers" who show delayed reaction, confusion, and a host of other specific dose-dependent deficits. In pain patients, the sedation and mental clouding experienced is limited to a few days after the initiation or escalation of opioid dosing (Forbes, 2008).

While there are a number of basic science studies demonstrating the effects of opioids on pain-free animals, there is a paucity of studies on the effects of opioids on animals in chronic

pain. Opioids have been shown to disrupt both acquisition and recall of a variety of learned responses (McGaugh, 1983). It has also been shown that acute morphine disrupts operant responding and chronic morphine delays acquisition of simple and cued operant responses (Wang, Dong, Cao, & Xu, 2006). Zheng, Li, Yang, & Sui (2002) reported that chronic morphine delays acquisition in the Morris water maze, but this finding has not been replicated (Wang et al, 2006)

Pain's Effects on Cognition

Equally clear is the fact that pain, especially severe pain, disrupts cognition. People experiencing pain show deficits in attention and reaction time (Zenz, 1991; Lorenz, Beck, & Bromm, 1997), and more complex tasks such as memory tasks (Crombez, Eccleston, Baeyens, & Eelen, 1996; Lorenz et al, 1997; Eccleston, Crombez, Aldrich, & Stannard, 1997). It has been demonstrated that formalin pain (Ceccarelli, Scaramuzzino, & Alosi, 2001), bowel pain (Millecamps, Etienne, Jourdan, Eschalier, & Ardid, 2004), and neuropathic pain (Benbouzid, Choucair-Jaafar, Yalcin, Waltisperger, Muller, Freund-Mercier et al., 2007) can disrupt approach and exploration of novel objects in experimental animals. Although some authors argue that their observations demonstrate a disruption of working and reference memory, the deficits in approach behavior may be due to pain's anxiogenic properties since some of pain's effects are reversed by administering anxiolytics and antidepressants (Benbouzid et al, 2007). Therefore, it is not clear if the deficits in performance may be due to anxiety rather than memory errors. Thus, Hermanstynne, Flake, & Gold (2007) have also shown that pain, e.g. temporal mandibular joint (TMJ) pain, can disrupt operant responding for food, however, this finding is potentially due more to the avoidance of TMJ pain rather than disruption of memory for the response. While

these models attempt to test the effects of pain on memory, they fail to provide a valid measure to adequately assess memory performance.

Hypothesis and Specific Aims

While opioid medications are the best pharmacological weapon against severe pain, the utilization of opioid medications to alleviate chronic pain has been limited due to the potential for undesirable side effects and the tendency for these drugs to surface in the illicit drug market. The possibility of analgesic tolerance and deficits in cognition that accompany opioid ingestion often hinders clinicians and patients from utilizing opioid therapy (Melzack, 1991; Ersek, Cherrier, Overman, & Irving, 2004). Fortunately, the positive and negative effects of opioids are separable to some degree, as evidenced by the ability of opioids to achieve analgesia without euphoria or significant cognitive dysfunction in some pain patients (Zenz, 1991; Forbes, 1996). Clearly the presence of pain can alter the time course of opioid reward and analgesic tolerance development. However, the interaction between the effects of opioid medications and pain on learning and memory are unclear. A better understanding of this interaction will improve the clinical management of long term pain.

This project examined the independent effects of pain and morphine as well as the interaction between pain and opioids on recall in the radial maze. The central hypothesis is that the stress response induced by pain modifies the deleterious effects of acute and chronic opioids on working and reference memory. This central hypothesis was examined in three specific ways.

First it was important to determine the effects of pain on working and reference memory errors in a dose dependent fashion. Although this was a novel attempt at directly testing the

effect of pain on memory, it was believed that pain would impair memory performance by increasing the amount of errors across all 5 days of testing. Also, it is believed that tolerance to pain's effect on memory would not develop.

The next step was to determine the effects of morphine on working and reference memory errors. Acute morphine administration was expected to increase both working and reference memory errors relative to the pain- and morphine-free control group. Chronic morphine administration was also expected to lead to an increase in working and reference memory errors.

Finally, the current study determined whether pain attenuates the deleterious effects of opioids on working and reference memory errors. It was believed that pain would activate a stress response in the HPA axis that would modify opioids' negative effects, thus reducing the amount of working and reference memory errors. Also, animals that were in pain were expected to show continued analgesia in response to morphine injection while those not in pain would not show tolerance to the analgesic effects.

In order to achieve proper clinical pain treatment, it would be beneficial to find ways to minimize the side effects that physicians and patients fear while maximizing analgesia. By achieving this goal, physicians and patients will become more confident in opioid medications, thus becoming more likely to utilize opioid therapy for pain management. This current design is will prove to be valid as a model for the study of analgesia, cognition, and other outcome measures relevant to pain management. As a result, this model will be an improvement over the typical approach of studying these variables independently and help to develop a pharmaceutical solution to increase analgesia while curtailing cognitive impairment.

Methods

In order to test the hypotheses mentioned above, a factorial design was conducted that crosses 2 levels of pain (0, 70 ml CFA) x 2 doses of morphine (0, 10 mg/kg). Table 1 outlines the entire factorial design. This design allows for comparisons of the effects of pain and morphine alone on cognition and analgesia as well as the interaction between pain and morphine on these factors.

Animals

Forty male Sprague-Dawley rats, housed in pairs, were maintained at 80-85% free feeding weight and were given free access to water (Floresco, Seamans, & Phillips, 1997; Schrott, Franklin, & Serrano, 2008). Each rat weighed between 200 and 225g at the start of training and the restricted diet was maintained until the end of the project. These animals were on a 12 hour light cycle with all the experiments occurring in the first 6 hours of the dark cycle.

Materials and Apparatus

Drugs. Morphine Sulphate (Paddock Laboratories, USA) was dissolved in physiologic saline and administered in a volume of 1ml/kg subcutaneously (s.c.) on the dorsal surface of the body. Equal volume saline was injected in control subjects. Pain was elicited by s.c. injections of complete Freund's adjuvant (CFA, Sigma, USA) 100% concentration subcutaneously (s.c.) into the plantar surface of the left hindpaw.

Radial Maze. An eight-arm radial maze constructed of ABS plastic floors and Plexiglas® walls was used to assess cognitive functioning. This maze consists of an octagon center, which is where the animal begins. There are 8 identical arms that extend from the center start box with a

Table 1. Factorial Design

	Pain Severity- Dose CFA (μl)		
		0	70
Level of Analgesia	0	No Pain/No Morphine	Pain/No Morphine
Dose of Morphine (mg/kg)	10	No Pain/Morphine	Pain/Morphine

bait cup at the end of each arm opposite the opening of the arm. The bait cup is 2cm from the floor so the food is not visible from the arm opening. The baited arms consisted of sugar-coated cereal (Kellogs, USA) as a reward.

The experiment consisted of habituation, shaping, and training of the animals (Spritzer, Gill, Weinberg, & Galea, 2008). On day 1, pairs of animals received two 5-minute habituation trials where nine small pieces of cereal are spread throughout the maze. The animals were allowed to freely roam the maze and eat the cereal. This was followed by two 5-minute shaping trials, where the individual rats must retrieve pieces of cereal placed in the food cups at the end of every arm. The training trials consisted of two 5-minute trials daily. In the first “reminder” trial, animals were allowed 5 minutes to obtain cereal bait from 4 randomly selected arms and these same 4 arms were baited for every training trial. The remaining 4 arms were blocked by guillotine barriers at their entrances. During the second trial, the same four arms were baited but access to all arms was allowed. The behavior of the animals was video recorded and analyzed using AnyMaze software. During the second trials, working memory errors are defined as re-entry to arms previously entered in the trial and reference memory errors are defined as entry into unbaited arms (Hodges, 1996; Floresco et al, 1997; Schrott et al, 2008). The total distance traveled down each arm was assessed. Animals were trained until they obtain the four rewards while making 1 or fewer incorrect arm entries. Animals who did not reach this asymptotic level of performance by 20 trials were eliminated from the study.

Hargreaves Test. Animals were placed in Plexiglas® enclosures with glass floors (20cm²) suspended 30cm from the table top and allowed to habituate for 30 minutes. The area of the animal’s hindpaw targeted by the s.c. injections was stimulated from below using a halogen heat

source. The latency to produce a nocifensive withdrawal response was used to measure analgesia. The other hindpaw was also tested as the control paw.

Procedure

The day following the establishment of criterion performance in the working and reference memory radial maze task, the animals were randomly assigned to a pain severity x morphine dose condition. Baseline thermal withdrawal latencies were measured and the animals received s.c. injections of 0 or 70 μ l CFA.

To establish the pain state of the subjects, thermal withdrawal latencies were measured 24 hours post-CFA injections (pre-morphine baseline). For the next 5 days, radial maze trails continued as before except that animals received s.c. injections of morphine (10 mg/kg) or saline 30 minutes prior to testing in the radial arm maze and testing in the maze was followed immediately by thermal withdrawal testing to assess the effects of morphine/saline treatment on pain.

Both morphine and tonic inflammatory pain can reduce locomotion and can confound the dependent measures in this study since they depend in part on locomotor ability. The dependent measures used in the current study was not based on latency to obtain reward, but rather data from arm entries which are more resistant to locomotor effects. The AnyMaze[®] software was used to generate a measure of running speed which can be compared across conditions as measures of locomotor impairment. This measurement helped to determine the relevance of locomotor effects.

Control Study. Morphine may produce a state of nausea in rats leading to a behavior termed pica (Takeda et al, 1993). Mitchell, Wells, Hoch, Lind, Woods, & Mitchell (1976)

described pica as an illness-response in rats characterized by eating non-nutritive substances, such as the cage bedding, since they are incapable of emesis. When pica is present, rats are unlikely to engage in behavior for a food reward – thus if animals receiving morphine show decreased performance in the maze, it may be due to cognitive disruption or lack of motivation. However, some researchers (Kelley, Bakshi, Haber, Steininger, Will, & Zhang, 2002) have shown that opioids selective for the μ -opioid receptor can actually increase food uptake and show improvement in performance for behaviors rewarded with highly appetitive foods especially for hedonic foods. Due to this potential issue with pica, a control study was conducted to determine whether animals that are administered morphine eat more or less food than animals without morphine. Animals were injected with either 10 mg/kg of morphine or saline. The animals were then placed in an open field and the amount of food consumed by both conditions was recorded.

Results

All animals included in the study reached criterion performance in the radial maze task in 4 - 15 days. Six animals were excluded from testing when they failed to reach criterion performance by the 15 day cut-off and were replaced with additional subjects to maintain sample size. All data were examined for statistical outliers and none were found; no data were excluded from analysis. All subsequent analyses were performed using SPSS for Windows (version 16.0) with the probability of a Type I error set at 0.05. The data from memory errors and locomotor speed were analyzed using separate 2 x 2 x 6 mixed factorial ANOVA with Pain (0, 70 μ l) and Morphine Dose (0, 10 mg/kg) serving as between subjects variables and Trials (baseline and Days 1-5) serving as a within-subjects variable; analgesia was analyzed in a 2 x 2 x 7 Pain (0, 70 μ l) X Morphine Dose (0, 10 mg/kg) X Trial (baseline, post-CFA, Days 1-5) mixed factorial ANOVA. All significant 3-way interactions were followed by planned analysis of the interaction contrast for pain x analgesia at every time point. Dunnett's test was employed to control the family-wise error rate as we compared the treatment groups to the no pain/no morphine control. Significant results discussed below indicate $p < 0.05$ for the comparison.

Analysis of Working and Reference Memory

Working and reference memory data from the radial maze test trials is summarized in Figure 1 and Figure 2 respectively. The 3-way interaction indicates that the animals in the no pain/morphine condition displayed significantly more errors than the no pain/no morphine control group [$F(6, 108) = 17.85, p < 0.05$]. Dunnett's test subsequently revealed that the no pain/ morphine group displayed significantly more errors than the no pain/no morphine control on days 2-5.

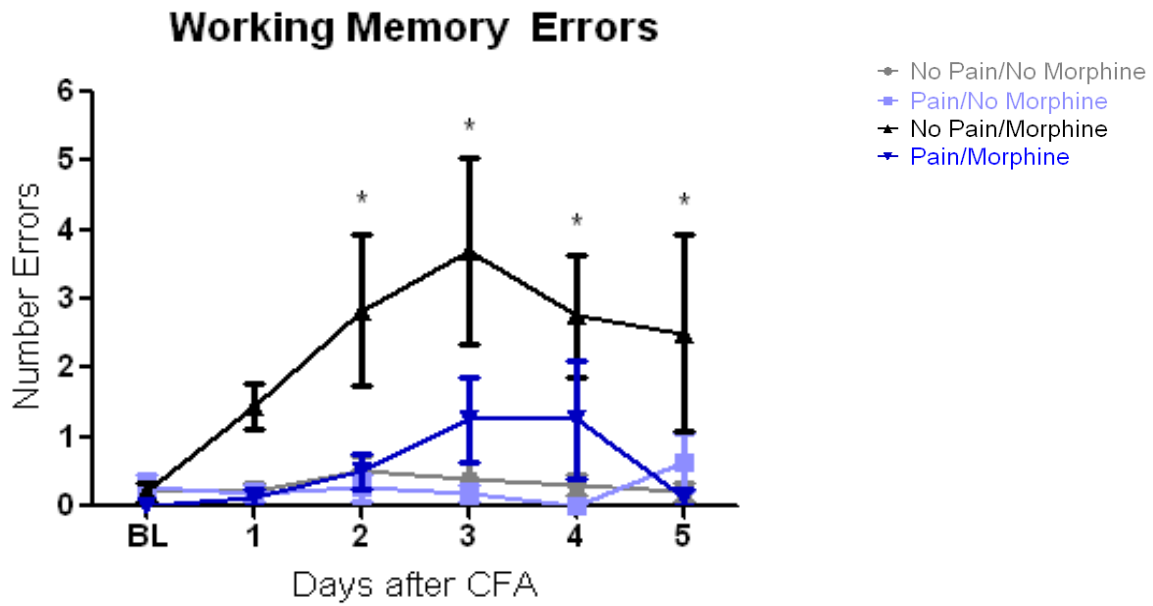


Figure 1. Mean (+/- SEM) number of arm re-entries for 5 days following induction of an inflammatory injury (pain groups) or no injury (control groups). * Indicates the No Pain/Morphine group was significantly different from the No Pain/No Morphine group ($p < 0.05$).

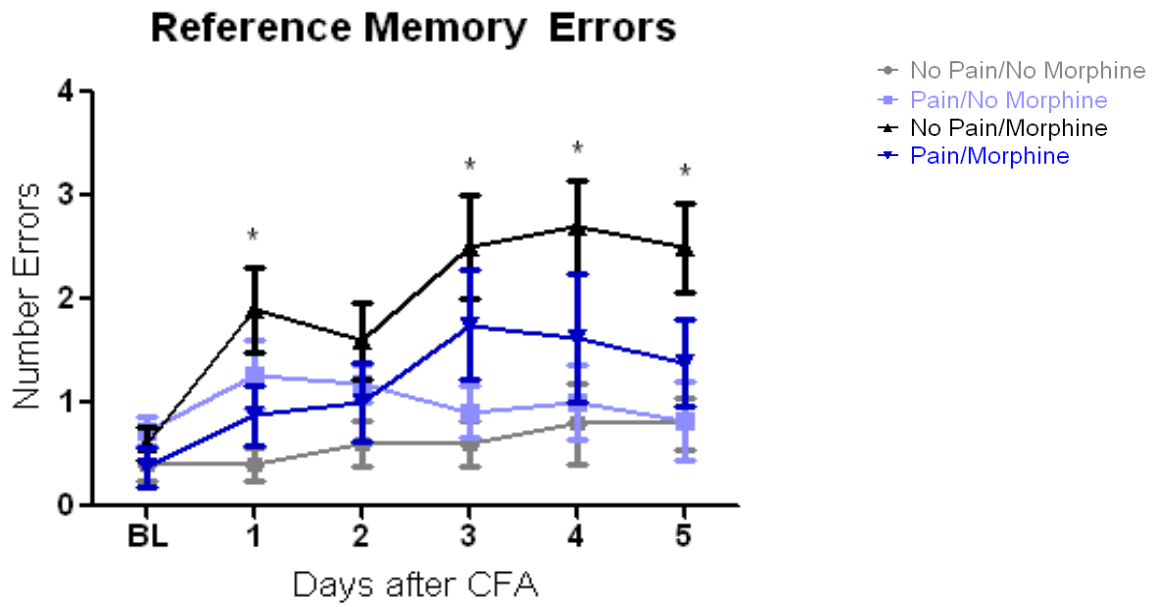


Figure 2. Mean (+/- SEM) number of incorrect arm entries for 5 days following induction of an inflammatory injury (pain groups) or no injury (control groups). * Indicates the No Pain/Morphine group was significantly different from the No Pain/No Morphine group ($p < 0.05$).

Analysis of Locomotor Slowing

Locomotor data from the radial maze experiment is presented in Figure 3. Again a 3-way interaction was conducted and revealed significantly slower navigation speeds for both groups receiving morphine injections when compared to the two groups without morphine injections. Dunnett's test was performed on all significant 2-way interactions, indicating both groups receiving morphine navigated the maze at slower speeds than the no pain/no morphine control on days 1 – 5. No other significant effects were observed.

Analysis of Analgesia

Thermal withdrawal threshold testing data are summarized in Figure 4 which indicated that CFA injection produced a pronounced thermal hyperalgesia that was acutely reversed by morphine administration. A significant 3-way interaction [$F(6, 108) = 27.46, p < 0.05$] was observed. Subsequent analysis revealed 4 significant findings: 1) no difference in baseline sensitivity was noted; 2) 24 hours after injury, both groups that received CFA were significantly hyperalgesic; the pain/no morphine group remained hyperalgesic throughout the 5 days of testing and 3) on days 1-4 both groups receiving morphine injections showed significant elevations in their withdrawal threshold, but 4) on day 5, the withdrawal threshold for the pain/morphine group was significantly elevated from the no pain/no morphine control.

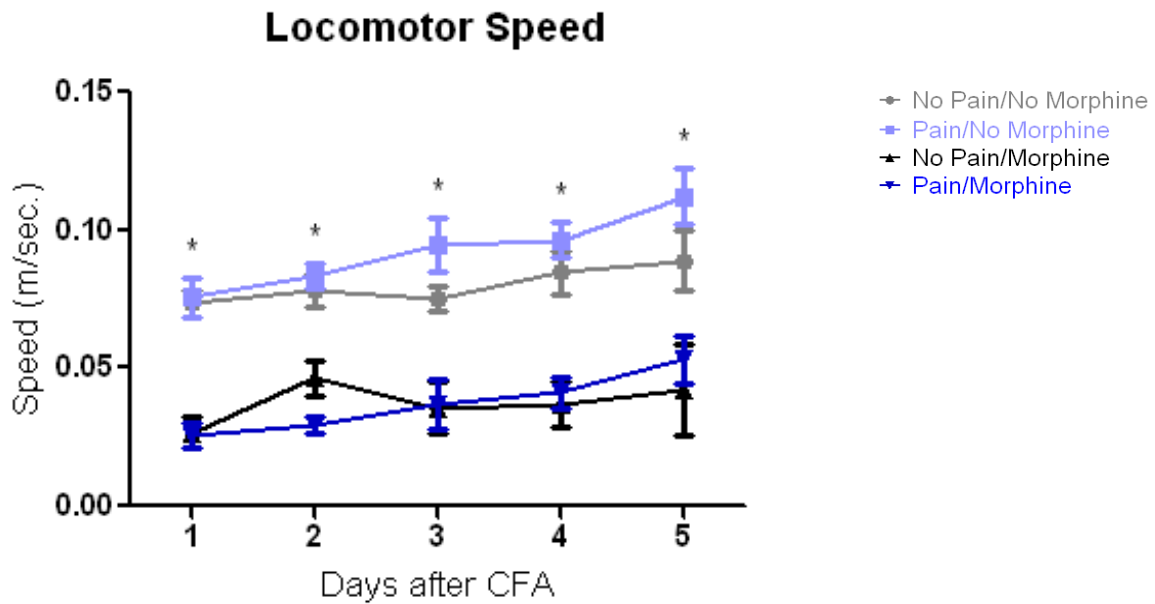


Figure 3. Mean (+/- SEM) speed in the radial maze for 5 days following induction of an inflammatory injury (pain groups) or no injury (control groups). * Indicates that the No Pain/Morphine group and Pain/Morphine group were significantly different from the No Pain/No Morphine group ($p < 0.05$).

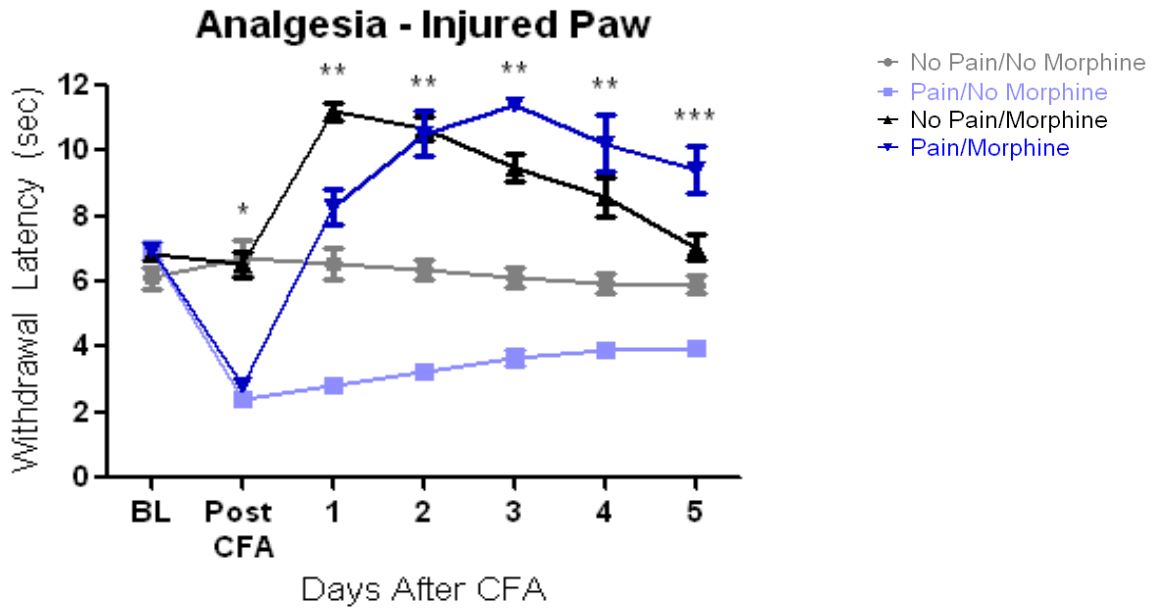


Figure 4. Mean (+/- SEM) thermal withdrawal latency. * Indicates the groups receiving CFA differed significantly from the No Pain/No Morphine group ($p < 0.05$). ** Indicates all groups differ significantly from the No Pain/No Morphine group ($p < 0.05$). *** Indicates the Pain/No Morphine and the Pain/Morphine groups differ significantly from the No Pain/No Morphine group ($p < 0.05$)

Analysis of Control Study

Data from the separate free feeding study revealed that the morphine and saline control groups consumed all the available food (4x the amount in the maze during test trials) within the 10-minute time period, resulting in the same average weight of food consumed and no variability, rendering the calculation of t unnecessary.

Discussion

Despite resulting in produced significant inflammation and thermal hyperalgesia, CFA administration at the dose employed in the current study failed to impact working and reference memory errors on an acquired radial maze task. This was surprising given reports of significant reductions in cognitive performance following rodent models of colitis (Millicamps, et al. 2004), formalin-induced pain (Ceccarelli et al., 2001), and neuropathy (Benbouzid et al., 2007). The difference in outcomes may be due to the pain model employed or the sensitivity of the different memory tasks employed to the effects of CFA-pain.

Not surprisingly, morphine administered to pain-free animals resulted in increased memory errors on our task. This wholly expected finding is in keeping with numerous reports that acute (Ragozzino & Gold, 1995; Li, Wu, Pei, & Xu, 2001) and chronic (Spain and Newsom, 1989; Miladi-Gorji, Rashidy-Pour, & Fathollahi, 2008; Wang et al., 2006) opioid administration, as well as opioid withdrawal (Ma, Chen, He, Zeng, & Wang, 2007) impair performance in a variety of rodent models of learning/memory. Importantly, when the same dose of morphine that lead to a significant increase in memory errors was administered in the presence of CFA-induced inflammatory pain, there were no disruptions of memory observed. The relationship between pain and morphine has not been well studied. In the only other examination of the interaction between the cognitive effects of pain and morphine identified for this review, similar results were obtained (Millecamps, et al., 2004). The researchers induced colitis in rats and visual non-selective, non-sustained attention was assessed. Chronic inflammatory pain produced deficits in the task, which were attenuated by effective analgesic treatment.

Explanations for Memory Impairment

The increase in working and memory errors observed in pain-free animals may be due to a variety of factors, including locomotor slowing (Grecksch, Bartzsch, Widera, Becker, Holtt, & Koch, 2006) and alterations in appetite that have been observed following opioid administration (Takeda, Hasegawa, Morita, & Matsunaga, 1993). The reversal of opioid-induced cognitive effects and the attenuation of analgesic tolerance contrasts with the inability of pain to reverse morphine-induced locomotor slowing. This is consistent with other reports of locomotor slowing in the presence of opioids across several experimental designs (Timar, Gyarmati and Furst, 2005). The observation that pain interferes with some opioid effects leaving others in tact is intriguing because it suggests that different neural mechanisms exist for each of these outcomes. We suggest that locomotor slowing does not account for the increase in errors in pain-free animals because slowing was also observed in CFA + morphine treated animals, yet this group made few errors.

Likewise, decreased appetite is an unlikely explanation for the mistakes made by morphine-only animals because morphine did not alter food intake relative to saline when separate groups of animals were presented with the food stimulus used in the maze. There was no difference between the morphine group and saline group in terms of food consumed. Therefore, one can assume that morphine at the doses used in this study does not cause an avoidance behavior towards food and the poor performance was due to cognitive deficits rather than some other confounding factor.

Biological Underpinnings. Alternatively, morphine treatment could influence radial maze errors through direct or indirect effects on attention, spatial memory, or response selection circuitry. Two possible sites of action are the caudate nucleus (CN) and locus coeruleus (LC).

The CN has been found to mediate a hippocampal-independent learning circuit that is essential in reference memory performance (Packard & White, 1990). For example, Packard & White (1990) trained animals in a version of the radial maze task similar to the current task where they baited the same 4 arms on each trial. Packard & White (1990) demonstrated that rats with CN lesions were impaired on radial maze tasks that assess reference memory, while working memory performance was unaffected by CN lesions in the task. Other researchers were able to demonstrate opposite findings in radial maze tasks that assess working memory with lesions in the hippocampus disrupting working memory performance and CN lesions having no effect on working memory (Packard, Hirsh, & White, 1989). As one of the three subdivisions that make up the striatum (Kandel, 2001), the CN receives afferent fibers directly from all areas of the neocortex (Hokfelt and Ungerstedt 1969), the thalamus, raphe nuclei, and amygdala (Villablanca, 2010). The CN projects to the frontal cortex (via the thalamus) and, downstream, it projects to the globus pallidus, thalamus, and substantia nigra pars reticulata (Villablanca, 2010). The striatum as a whole has been implicated by many researchers as a neuroanatomical structure that mediates the addictive and dependence-producing properties of morphine (Glick, Cox, & Crane, 1975). Many behavioral findings have supported the idea that morphine inhibits dopaminergic activity in the striatum in general (Lal, O'Brien, & Puri, 1971; Puri & Lal, 1973; Gianutsos, Drawbaugh, Hynes, & Lal, 1974; Kuschinsky & Hornykiewicz, 1974), and the CN in particular (Datta, Thal, & Wajda, 1971). For instance, the caudate nucleus is believed to be responsible for coordinating motor activity in rats (Elliot, 1963). However, after a large dose of morphine, rats have been shown to become immobile or catatonic due to the inhibition of the caudate nucleus (Datta et al., 1971). Also, Lal et al. (1971) used Haloperidol to block dopamine receptors and found that this drug exacerbated withdrawal symptoms in rats and humans. Based

on these research findings, evidence has been provided for the inhibition of the striatum as a result of morphine administration.

While CN inhibition resulting from opioid administration can explain reference memory impairment in pain-free animals, suppression of LC activity can be a more global explanation of both working and reference memory impairment following opioid use. The LC contains noradrenergic neurons and is responsible for maintaining vigilance and responsiveness to unexpected stimuli (Kandel, 2001). Researchers have determined that the LC has an excitatory influence on both the striatal and limbic dopamine systems (Lategan, Marien, & Colpaert, 1990). Despite the LC excitatory influence on striatal and limbic systems, research has shown that morphine administration decreases LC activity (Korf, Bunney, & Aghajanian, 1974, Millan, 2002), resulting in a dose-dependent range of effects from inattention, sedation and catatonia (Aston-Jones, Gonzalez, & Doran, 2005). It is important to note that the LC has two modes of activity which are called phasic and tonic (Aston-Jones & Cohen, 2005). Aston-Jones & Cohen (2005) have shown support for the phasic LC activation as a facilitator of task performance, while tonic activity results in a disengagement from the current task or poor performance. Aston-Jones & Cohen (2005) have described the relationship between the task performance and LC activity as a Yerkes-Dodson relationship. They believe that performance is poor at very low levels of tonic activation due to drowsiness and lack of arousal. Performance is best when there is moderate tonic activation and sufficient phasic activation. However, high levels of tonic activation with low phasic activity will result in poor performance as well. Based on this theory, morphine administration may decrease phasic activation and tonic activation of the LC, resulting in a disengagement from the task and lack of arousal that leads to poor performance in the radial maze. This decrease in LC activity is due to mu-receptor gated potassium currents which

decrease the firing rates of noradrenergic cells in the LC (DiChiara & North, 1992). This decrease in LC activity results in a decrease in striatal activity leading to the exacerbated withdrawal symptoms and catatonia associated with morphine administration. Also, the decrease in LC activation results in reduced stimulation of cortical circuits leading to inattention and other cognitive deficits. This inhibition of the LC results in a decrease in striatal activity in conjunction with inattention as a result of understimulated cortical circuits offers a potential explanation for the observed increase in errors in our animals.

Pain, on the other hand, increases the activity of the LC (Jones, 1991; Millan, 2002; Pertovaara, 2006). This increase in activity can lead to the possible activation of striatal dopamine system (caudate nucleus) and cortical circuits resulting in the attenuation of side effects seen with the interaction of pain and morphine administration. This theory could possibly explain how pain can modify morphine-induced analgesia and cognitive impairments. The CFA pain stimulates the LC, which increases the activity of the striatal dopamine system and cortical circuits, resulting in a reversal of cognitive impairment and a delay in the development of analgesic tolerance. This increase in LC activity could be an increase in both phasic and tonic activation of the LC, which leads to the optimal performance that was described earlier in relation to the Yerkes-Dobson relationship of Aston-Jones & Cohen (2005). The pain stimulus could possibly result in an adequate increase in both the phasic and tonic stages of the LC to increase performance and reverse the poor performance in the radial maze that was observed with the morphine-only animals in the current study.

Replication of Analgesia Studies

Importantly, pain not only interacts with morphine-induced cognitive effects, but also with analgesia. Acutely, morphine produced analgesia in both pain-free and CFA injected

animals. By the fifth day of chronic morphine administration, analgesic tolerance was evident in pain-free animals, but absent in animals suffering from chronic pain. This replicates previous findings that the presence of formalin (Vaccarino et al., 1997), surgical pain (Ho et al., 1999) and CFA pain (Chen & Vaccarino, 2000) attenuates the development of analgesic tolerance.

Conclusions

Taken together, these findings suggest that pain activates neural mechanisms which antagonize opiate effects. This interaction is not surprising given that pain activates a global stress response, which includes activation of endogenous mechanisms of pain control. The endogenous opiates released in response to stress participate in multiple feedback circuits regulating the stress response.

Furthermore, these data have relevance to the clinical management of pain. A general reluctance to prescribe opioids on the part of clinicians has been discussed in the literature (Zenz, 1991; Cleary & Backonja, 1996; Popenhagen, 2006). Reasons for this reluctance include fear of the development of tolerance, dependence, and the occurrence of side effects like mental clouding. Attitudes about opioid pain management are beginning to change; however, there is a need for better basic science to inform clinical practice. Here we present data that demonstrate the dangers of tolerance and cognitive disruption that exist in addictive behavior do not always apply to clinical pain management.

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Appendix

Institutional Animal Care and Use Committee

UNIVERSITY OF NEW ORLEANS

DATE: December 19, 2008

TO: Dr. Rodney Soignier

FROM: Steven G. Johnson, Ph.D.
Chairman

RE: *IACUC Protocol # UNO-08-004*
*Entitled: **Interaction between pain and morphine on pain sensitivity and cognitive function***

Your application for the use of animals in research (referenced above) has been approved for a three-year period beginning December 19, 2008 and expiring December 19, 2011.

Vita

Brandon Baiamonte was born in Chalmette, Louisiana and earned his B.S. in Psychology and a minor in Sociology from Louisiana State University in 2005. He went on to earn his M.A. in General Psychology from Southeastern Louisiana University in 2007. In 2008, Brandon enrolled into the University of New Orleans to earn his M.S. and PhD. in Applied Biopsychology. Upon completion of the current document, he has earned his M.S. and is on track to complete his PhD. in 2012.