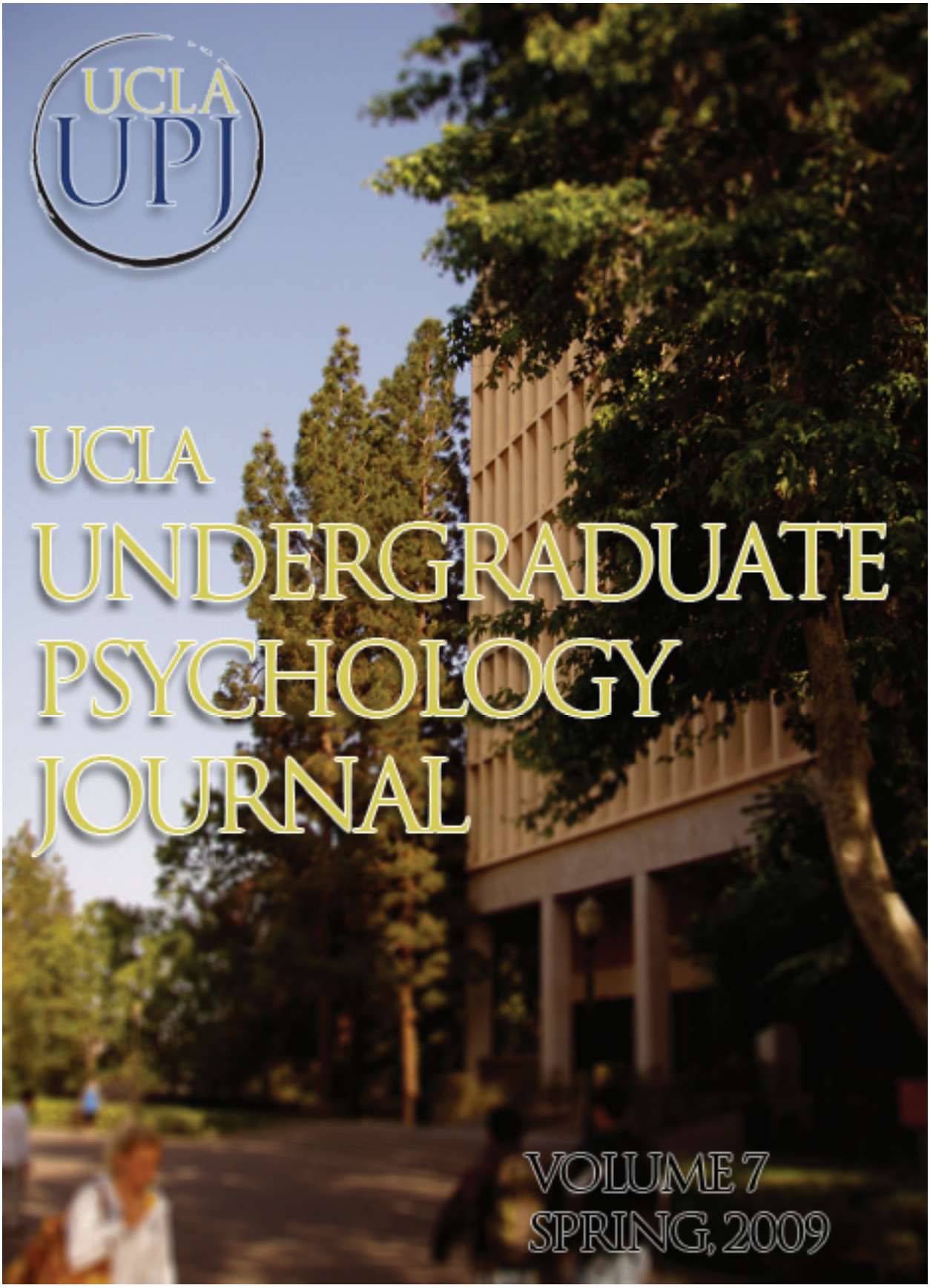
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UCLA Undergraduate Psychology Journal 2009

Greetings,

We would like to welcome you to the second consecutive edition of The Undergraduate Psychology Journal (UPJ). Over the past year, our team members, faculty advisers, and editors have worked diligently to produce this issue of the journal. UPJ reviewers have worked fervently to examine each manuscript, and much time and deliberation has been invested in each submission. As a result, we have selected our journal articles based on the most rigorous criteria, which has enabled us to publish the highest quality undergraduate research studies.

Psychology is a broad and challenging field as it touches upon nearly every aspect of the human mind. Many undergraduate students from around the nation have taken up the challenge of searching for explanations to many problems in psychology that have yet to be solved. Considering the hard work and dedication that goes into each study, UPJ hopes to provide some of those bright young researchers with the opportunity to share their hard work with faculty and their peers. We encourage our readers to continue their support by reading about the exciting research conducted by undergraduates around the nation.

Sincerely,

R. Israel Gonzales,
Editor-In-Chief

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Multiple Memory Systems:

A Comparative Analysis and Current Perspective

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Abstract

Evidence from experimental animal studies as well as human neuroimaging studies has led researchers to agree that memory is not unitary in nature, but is actually subserved by multiple memory systems localized in different regions of the brain. Further research of the neurobiological basis of memory supports a clear distinction between the declarative (hippocampal dependent) and nondeclarative (striatal dependent) memory systems. Moreover, evidence indicates that the two memory systems may, in actuality, interact with one another during learning; however, the level at which this interaction occurs, either during acquisition or response, remains unknown. Investigation of the multiple memory systems and the mechanisms of interaction is not only important for what it reveals about the evolution and adaptive function of the brain, but for its clinical applications as well.

Introduction

Converging evidence from animal experiments and human neuroimaging studies has led to the agreement that memory is not unitary in nature, but better explained by multiple systems that subserve different types of memory (Poldrack, 2003). Extensive research of the neurobiological basis of memory further supports the distinction between the two types of long-term memory systems: declarative and nondeclarative. While declarative knowledge is characterized as flexible memory for past events and facts, nondeclarative memory is characterized by relatively inflexible knowledge for habitual and procedural behaviors (Cohen and Squire, 1980; Figure 1). The key distinction between the two memory systems lies in the differential capacity to recall stored information. While declarative memory is subject to conscious recollection, nondeclarative memory is expressed through performance and is not accessible through conscious faculties (Squire and Zola, 1996). Additionally, multiple studies reveal a specialization between the two memory systems in that the declarative system appears to be responsible for rapid learning of information about individual trials, whereas the nondeclarative learning system seems to be involved in gradual learning across many trials (Poldrack et al., 2001).

Neurobiological studies have identified multiple brain systems that differ in terms of the types of memory they subserve: declarative memory relies on the hippocampus of the medial temporal lobe whereas nondeclarative memory relies on the striatum of the basal ganglia (Squire, 1992; Figure 2, 3). The hypothesis that multiple brain systems mediate different types of memory was originally derived from the results of several studies that examined spared learning abilities after hippocampal damage. Cohen and Squire (1980) report that preserved learning skills in amnesiacs are broader than reported in previous studies as amnesiacs seem to have impaired declarative knowledge, but spared nondeclarative or procedural abilities. From the synthesis of evidence from experimental animal and human brain studies, a biological perspective emerges further supporting the dissociation between declarative and nondeclarative memory and proposes that the different functions rest on the distinct anatomical organization of the memory systems (Squire, 1992; Figure 3). Additionally, several dual-memory theories examining the characteristics of declarative (hippocampal dependent) and nondeclarative (striatal dependent)

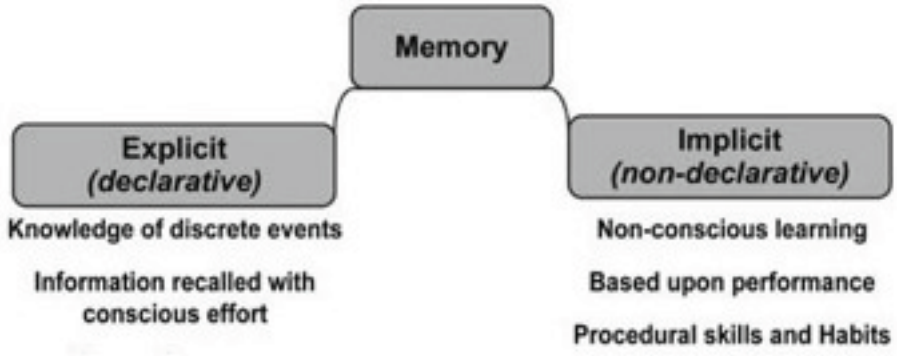

Fig. 1 Functions of Declarative and Non-declarative Long-Term Memory. Declarative or explicit memory is knowledge of facts and events, usually recalled with a conscious effort. Examples include one's knowledge of the capital of France, the date of a historical event, or a sibling's date of birth. Nondeclarative or implicit memory is procedural learning, usually recalled through performance without conscious effort. Examples include one's ability to ride a bicycle or drive a manual transmission automobile. Typically, non-declarative memory is not accessible through verbal description.

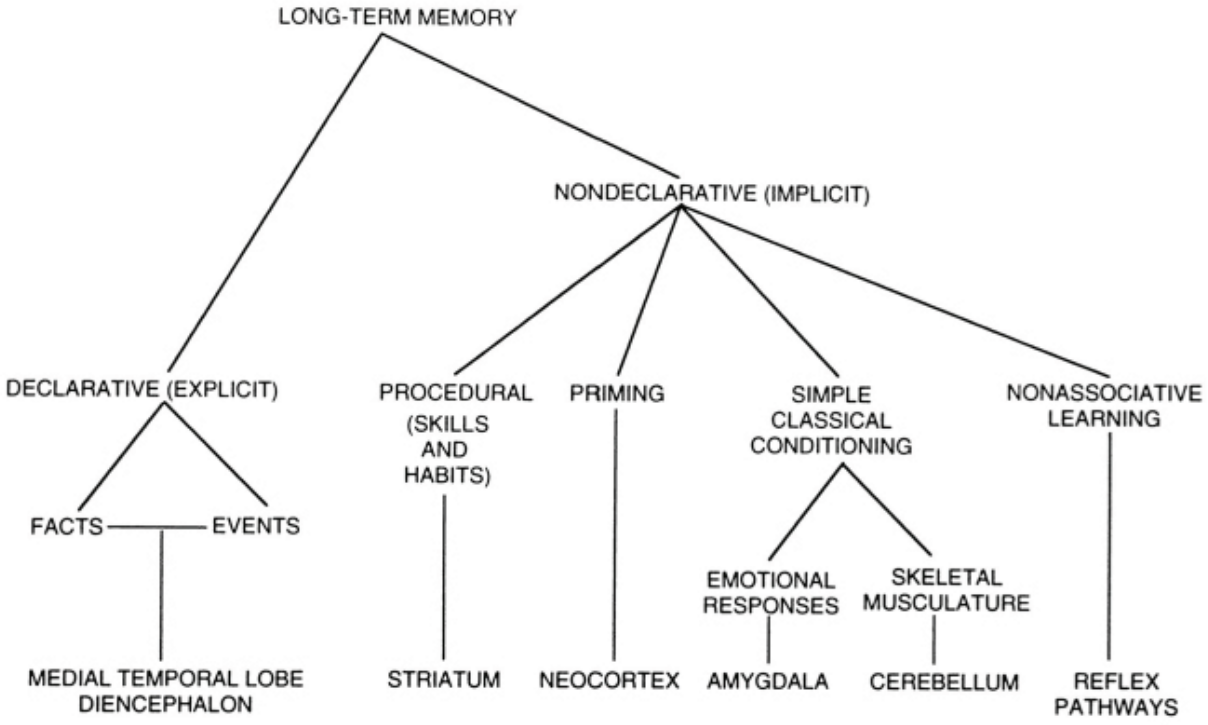

Fig. 2 Long-Term Memory Systems. Categorization of declarative and nondeclarative long-term memory systems and the specific brain structures subserving each process. While declarative memory is subserved by the hippocampus or medial temporal lobe, nondeclarative or procedural learning is subserved by the striatum (adapted from Squire and Zola, 1996).

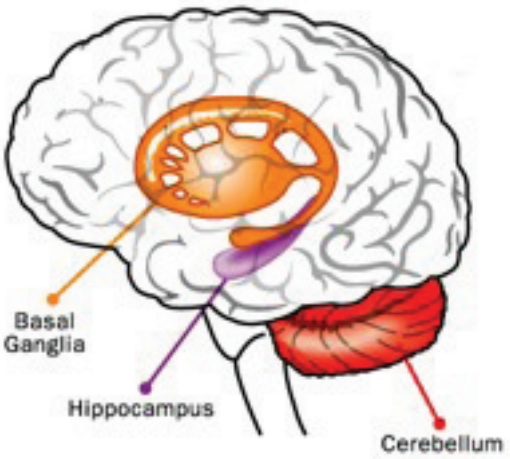

Fig. 3 Anatomical localization of the structures subserving the two memory systems. The striatum (labeled here as the basal ganglia) subserves nondeclarative or procedural memory while the hippocampus subserves declarative memory.

memory have been proposed. One such theory suggests that the effects of a dual-task (a task which includes both declarative and nondeclarative components) on the measures of nondeclarative sequence learning may be partly due to the intrusion and disruption of declarative knowledge (Jimenez and Vasquez, 2005). As evidenced by studies of both animals and humans, current research demonstrates the existence of multiple memory systems and more specifically, the fundamental differences between declarative and nondeclarative memory systems.

While evidence for a single dissociation within the hippocampal-dependent declarative memory system indicates the existence of multiple memory systems, double dissociation studies more clearly support the functional independence between the declarative and nondeclarative systems. Double dissociation studies, performed by creating irreversible and reversible lesions of the hippocampus and striatum in experimental animals, provide the most powerful evidence for the necessity of multiple memory systems. Packard et al. (1989) examined the differential effects of lesions to the hippocampus and caudate nucleus of the striatum in experimental rats while performing two radial maze tasks in order to behaviorally demonstrate a double dissociation of the mnemonic functions of the two memory systems. Additionally, Knowlton et al. (1996) utilized a probabilistic classification task, designed to separate the function of the two memory systems by relying on procedural skills, during neuroimaging of patients with amnesia (due to hippocampal damage) and patients with Parkinson's disease (due to striatal damage), further demonstrating a double dissociation within the long-term memory systems. Amnesic patients exhibited normal learning of the task, but had no declarative knowledge of the training event, whereas Parkinson's disease patients exhibited no procedural learning, but had intact, declarative memory for the training episode. Additionally, Tranel and colleagues (1994) found that while patients with lesions to the hippocampus exhibited impaired declarative memory, they did not exhibit impaired nondeclarative motor skill learning. Moreover, Heindel et al. (1989) found that those with damage to the striatal system showed impaired nondeclarative skill learning, but intact declarative memory. Multiple studies of animals and humans provide converging evidence for the double dissociation of the two memory systems, further supporting the differentiation of

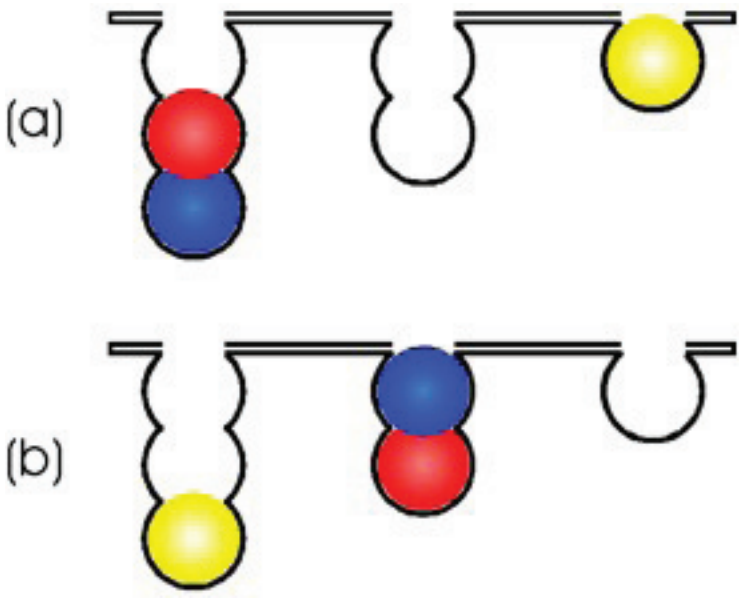

Fig 4 Tower of London Task. The Tower of London paradigm assesses executive functions such as planning through problem-solving tasks. Typically, the set-up consists of three pouches, which can hold one, two and three balls, respectively. The task is to move the differently colored balls, one at a time in a minimum number of moves, between the pouches in the lower panel (b) so that the resulting pattern matches that of the upper panel (a).

declarative and nondeclarative memory systems as they act independent of one another to support learning.

Despite the apparent independence of the declarative and nondeclarative memory systems, many studies indicate that multiple memory systems in the brain may, in actuality, interact with one another (Poldrack, 2003). These studies indicate that the interaction is cooperative in that more than one system can provide the solution in a learning situation as subjects can use both their declarative and nondeclarative memory. As one might hypothesize, using both systems in a cooperative manner may optimize learning and performance; however, in light of our evolutionary existence, another theory suggests a more competitive interaction between the memory systems in that when one system is unable to provide an adequate solution, the other system is recruited to provide the answer (Sherry and Schacter, 1987). Converging evidence across species supports the competitive nature of the two memory systems: non-human animal studies reveal that damage to a given memory system actually results in enhanced learning and, more recently, human neuroimaging studies provide further evidence supporting the competitive nature of the systems as well (Poldrack and Packard, 2003). The most recent findings favor this theory as it more completely explains the interaction of the two memory systems, while challenging the previously held hypothesis of memory systems only working independently of one another.

This review serves to outline converging evidence from both experimental animal studies and from more recent human neuroimaging studies, which indicate that memory mechanisms in the hippocampus and striatum competitively interact with one another during learning. Multiple studies provide evidence that the competition occurs between the hippocampal and striatal memory systems at the level of acquisition. However, the most current findings indicate that the competition occurs not at the level of acquisition, but at the response level after learning has already occurred.

COMPETITIVE INTERACTION OCCURRING AT THE LEVEL OF ACQUISITION

Experimental animal studies

Experimental studies within animal models report a clear dissociation between the hippocampal and strExperimental studies within animal models report a clear dissociation between the hippocampal and striatal memory systems, proving that they are functionally separate. Many animal studies have shown that the hippocampus and the striatum acquire different types of information during learning; specifically, flexible, relational knowledge by the hippocampus and inflexible stimulus-response associations by the striatum (Squire and Zola, 1996). Selectively inactivating the hippocampal or striatal system with lidocaine was used to examine the involvement of the two memory systems during a place and response learning task that utilized a cross-maze. The rats were categorized according to their response as either “place” learners (i.e., animals going to the place where the food was located during training) or “response” learners (i.e., animals making the same turning response as during training) (Packard and McGaugh, 1996). Packard and McGaugh found that hippocampal and striatal learning occurred simultaneously when rats were studied in this cross-maze experimental setup. Initially, the rat was taught to run to a particular arm of the maze from a given starting point and was rewarded. The rat was then immediately positioned in a different starting location and was tested on its ability to find the previously rewarded location.

Packard and McGaugh (1996) reported that the hippocampus was activated during place learning, in which the rat was challenged to find the previously rewarded location starting from a new position; and, that the striatum was activated during response learning to aid in making the previously rewarded motor response from the new starting location. Under certain conditions, the two systems could interfere

with one another and lead to different behavioral responses. Healthy control rats exhibited a more gradual transition as they initially relied upon hippocampal-dependent place learning strategies and then later upon striatal-dependent response learning strategies. Pharmacological suppression of a given memory system resulted in facilitation of the other system, suggesting that these structures are involved in competition at the level of place and response learning.

Such findings support the theory of competitive interaction between the hippocampal and striatal memory systems and favor competition occurring at the level of learning (Packard and McGaugh, 1996). The present findings can be interpreted to suggest that the hippocampal lesion induced experimentally reduces the cognitive interference between the two memory systems and improves the function of the striatal system to learn and perform in certain situations. According to this interpretation, when a lesion is experimentally induced and one memory system is impaired, the spared memory system is able to take over and mediate learning and task performance, leading to a different behavioral response. Moreover, when one system is impaired, it is less able to interfere in the actions of the other system, resulting in an enhanced behavioral response as well.

Human neuroimaging studies

Recent studies have provided evidence for altered patterns of brain activity in healthy subjects and patients with Parkinson's and Huntington's disease. A recent positron emission tomography (PET) study using the Tower of London task, which assesses executive functions such as planning through problem-solving, reported that mildly affected Parkinson's disease patients performed as well as the control group, but showed a different pattern of neuronal activity (Dagher et al., 2001; Figure 4). Parkinson's disease patients showed enhanced activation of the right hippocampus as compared to healthy control subjects. The data suggest that normal frontal lobe activation can occur in Parkinson's disease patients despite abnormal striatal processing, as this task directly relies on this the frontal lobe, and that right hippocampal activity is enhanced in these patients while suppressed in normal control subjects.

Dagher et al. (2001) suggest that a shift to dependence on declarative memory structures during the Tower of London task may be a result of abnormal striatal processing and an insufficient working memory capacity within the frontal lobe in Parkinson's disease patients. Moreover, the authors suggest that the enhanced activation of the right hippocampus and failed activation of the right caudate nucleus during the Tower of London task represents recruitment of the hippocampus to overcome the striatal defect within Parkinson's disease patients. As a result of striatal dysfunction, the hippocampus must be recruited to perform the frontostriatal task and to partially overcome the striatal deficits. Dagher and colleagues interpret the recruitment of the spared hippocampus within Parkinson's disease patients as evidence to support competition at the level of acquisition or learning.

Additionally, striatal and hippocampal lesions can impair performance on certain spatial memory tasks as investigated in the PET study of abnormal basal ganglia function in Parkinson's disease patients (Owen et al., 1998). Owen et al. examined the effects of striatal dopamine depletion on cortical and subcortical blood flow changes during a frontostriatal-dependent task. While performing three variations of a Tower of London planning task ((1) an easy and difficult version, (2) a variant task requiring short-term retention, and (3) a control condition involving identical visual stimuli and motor response), regional cerebral blood flow was measured in six patients with moderate Parkinson's disease and six control subjects. An inverse relationship of regional cerebral blood flow was observed between control subjects and Parkinson's disease patients, in that there was an increase in blood flow in the right striatum in control subjects and a decrease in the same region in Parkinson's disease patients. Only when the mnemonic components were increased was the hippocampal system recruited to mediate task performance. These

findings are consistent with the previous animal studies in that hippocampal recruitment in Parkinson's disease patients could be the result of striatal dysfunction. The current findings are interpreted to support competition at the level of learning in that the two competing memory systems act independently of one another and only mediate task performance if one of the other systems is unable.

COMPETITIVE INTERACTION OCCURRING AT THE LEVEL OF RESPONSE

Experimental Animal Studies

Previous research reveals that pre-training lesions of a given system can result in enhanced acquisition of a task, relative to brain-intact animals performing the same task, and this is mostly due to the reduction of competitive interference between the two memory systems (Poldrack and Packard, 2003). In a study examining the acquisition of striatal-dependent active avoidance behavior, the enhanced behavioral response following a hippocampal lesion was suggested to be the result of cognitive interference reduction (O'Keefe and Nadel, 1978). Similarly, striatal system lesions have been reported to facilitate learning of a spatial discrimination task dependent upon the declarative memory system by disrupting the ability of a potentially interfering nondeclarative strategy (Mitchell and Hall, 1988).

Additionally, Packard and McGaugh (1996) showed that the two systems can compete with one another on certain tasks and that the performance of a striatal-dependent task can be enhanced if the hippocampal system is lesioned or temporarily inactivated. Conversely, inactivation of the striatal memory system allowed for a response strategy that depended on the hippocampus, proving that the knowledge was still accessible even when it was not the dominant pathway applied. This study proved that even though one system governed the performance of the animals in the cross-maze task, multiple representations still exist within both memory systems.

Furthermore, recent findings indicate that post-training reversible inactivation of the hippocampal system can further enhance striatal-dependent response learning, suggesting that multiple memory systems compete during the memory reformation or reconsolidation period as well (Schroeder et al., 2002). According to this interpretation of the competitive interaction, pre-training lesions act to eliminate competition among multiple memory systems by removing the ability of a given memory system to process, learn, and mediate a behavior during task performance at the response level.

Human neuroimaging studies

Consistent with the idea that there is an interaction between the hippocampal and striatal memory systems, numerous studies have investigated this interaction when one system is compromised by a neurological disorder such as Parkinson's or Huntington's disease. Using functional magnetic resonance imaging (fMRI), one study investigated the neural activity associated with performing a habit-learning task in healthy control subjects and Parkinson's disease patients (Moody et al., 2004). Parkinson's disease patients exhibited less activation in the striatal memory system and greater activation in the prefrontal cortex, which had been previously determined to be associated with declarative memory retrieval. Moreover, Parkinson's disease patients showed altered activation in the probabilistic classification task. The pattern of fMRI signal during performance indicated that there was greater activation of the hippocampal memory system, usually involved in declarative memory processing, and less activation in the basal ganglia, as is expected from the disease.

The present findings suggest that patients with Parkinson's disease may rely on their declarative memory system for tasks that are usually learned implicitly using the striatal circuitry (Moody et al., 2004). In this task, declarative memory for individual trials, while less beneficial than information gained

across trials, was used by Parkinson's disease patients to make decisions regarding the cue-outcome associations. The increased activation in the hippocampal system and prefrontal cortex reflects the ability of the Parkinson's disease patients to recollect previous trials in order to make their responses. When the nondeclarative circuitry is unable to mediate learning and task performance in Parkinson's disease patients, the declarative circuitry is recruited and is able to mediate task performance. These findings reveal that in normal subjects, competition is occurring at the level of response. Both systems are able to acquire redundant information and it is only through the competitive interaction between both systems that one system is able to mediate performance. Moreover, these findings reveal that in control subjects with intact memory systems, the striatal circuitry serves to inhibit the hippocampus during this task, as it recognizes its probabilistic nature. However, when the striatal system is impaired as in Parkinson's disease patients, only the spared system is able to mediate task performance and the competitive interaction is broken down.

A similar fMRI study of healthy subjects investigated the extent to which these two memory systems are engaged to optimize learning and behavior (Foerde et al., 2006). This study involved a distracting secondary task, which was used to investigate the degree to which a subject solves a problem using either declarative or nondeclarative memory. These results demonstrated a fundamental difference in the two memory systems in that they greatly differ in their sensitivity to concurrent distraction during dual-task conditions. While the dual-task condition did not reduce accuracy, it did reduce the amount of declarative learning about the task. Moreover, performance was correlated with striatal activity under the dual-task condition, whereas task performance and declarative knowledge was correlated with hippocampal activity under the single-task condition. While either system could support learning and performance in these healthy subjects, the nature of what was learned by each system differed in terms of their sensitivity to distraction. This was further evidenced by the fact that the striatum may have been activated during the task, but did not influence the behavioral response when declarative knowledge was readily available.

While the current findings are consistent with the competing memory systems hypothesis, they extend the current theory and prove that concurrent distraction can bias the competition (Foerde et al., 2006). Thus, the current findings reveal that separate memory systems acquire redundant information and competitively interact not at the level of learning, but when the knowledge is applied at the level of response.

LOCUS OF THE COMPETITIVE INTERACTION BETWEEN MEMORY SYSTEMS

While it is difficult to interpret the level at which the interaction occurs between the hippocampal and striatal memory systems, the previously mentioned studies provide distinct interpretations and biologically plausible explanations for both loci. While many studies interpret such findings as evidence for competition at the level of learning, more recent research suggests that separate memory systems acquire redundant information and it is only when a potential competitor is eliminated that a given system is allowed to freely mediate what was previously dominated by the other memory system.

Recently, Foerde et al. (2006) demonstrated that equivalent levels of learning on the probabilistic classification task can be mediated by both the hippocampus and the striatum and that concurrent distraction by a secondary task modulated the engagement of such memory systems. These systems differ not only in the locus of competitive interaction, but also in their ability to be modulated by external factors such as distraction. This suggests that the locus of competition, be it at the level of acquisition or response, can be subject to influence by external factors as well. Through evolution and natural selection, species evolved to have multiple memory systems and neural substrates mediating different learning abilities, but fully capable of subserving multiple behaviors should one system fail. Not only does this appear to be consistent across species, but it makes sense in light of our evolutionary existence and biological function.

Costs of the competitive interaction between memory systems

Because redundant information can be acquired and mediated by the declarative memory system, the costs associated with impaired nondeclarative function are challenged (Moody et al., 2004). However, upon closer examination, the costs associated with Parkinson's and Huntington's disease become very clear. Nondeclarative habitual memory, subserved by the striatal system, is characterized as automatic responses to cued stimuli. In the previously mentioned studies, the findings proved that healthy control subjects were able to automatically perform such tasks using their nondeclarative knowledge whereas Parkinson's disease patients were able to use their spared, declarative knowledge. Using their declarative retrieval processes to perform habitual tasks may account for the decreased availability of cognitive resources for other processes, therefore explaining the cognitive slowing exhibited by Parkinson's disease patients. Even though the declarative memory system can mediate such behaviors, the loss of the cognitive ability to perform automatic, habitual activities could certainly be considered an impact in the lives of Parkinson's disease patients.

ADAPTIVE FUNCTION OF INTERACTING MEMORY SYSTEMS

Experimental evidence suggests that the hippocampal and striatal memory systems work independently, together, or interfere with one another in different situations. While extensive evidence indicates that the multiple memory systems can function independently in some learning paradigms, the findings reviewed here delineate the conditions by which an interaction occurs between the two memory systems and reveal the competitive nature of such an interaction. With the relatively recent development of fMRI in conjunction with earlier evidence provided by experimental animal models, investigation into the interaction between the two memory systems has revealed more detail regarding the competitive nature of the two systems.

The competition between memory systems reflects an adaptive mechanism for optimizing behavior depending upon the learning situation (Poldrack and Packard, 2003). Learning involves competition between hippocampal-dependent and striatal-dependent memory systems in the animal and human brain. The competitive nature of such an interaction may serve to arbitrate between the two fundamentally different requirements of learning, specifically the need for flexibly accessible knowledge and the need for automatic responses.

FUTURE DIRECTION

Many questions remain regarding the nature of the interaction between the hippocampal and striatal memory systems. The current findings suggest that multiple memory systems acquire redundant information and apply such information at the level of response when a pre-training lesion is experimentally induced. Further research should be conducted in order to delineate the experimental conditions that determine the locus of competition. While multiple studies suggest that the competition occurs at the level of response rather than at the level of acquisition, further research should address this discrepancy, as it will provide a better understanding of the two competing memory systems and their interaction.

With regard to human neuroimaging studies, one central question centers on the nature of the neurophysiological deactivations that have been observed in nondeclarative learning situations engaging the striatum (Poldrack et al., 2001). There is ongoing debate concerning increased fMRI signal and it is unknown whether both excitatory and inhibitory synaptic activity results in this increased activation.

Further investigation is necessary in order to fully understand the synaptic correlates of these signals and to better understand the deactivation associated with implicit learning situations and the striatum.

While it is clear that multiple memory systems exist and interact within the mammalian brain, little research has been conducted as to the neurobiological connections mediating such an interaction. One central question regarding animal studies centers on the neurobiological and neuropharmacological bases of the interactions observed. Possible mechanisms may include direct anatomical projections or indirect neuromodulatory influence of the brain structures within these systems (Poldrack and Packard, 2003). There has been some research of the direct anatomical projections between the hippocampus and the striatum. Using electrophysiological techniques in rats, stimulation of the entorhinal cortex of the hippocampus resulted in responses in the striatum of the basal ganglia (Finch et al., 1995). Moreover, Finch and colleagues found that the majority of the striatal responses to stimulation were inhibitory. The data are consistent with neuropsychological and behavioral findings in that a negative influence between these two structures is observed. Additionally, one indirect influence between the two systems could be that of neuromodulatory control. It is possible that neuromodulatory influence from structures other than the hippocampus and striatum is mediating the activity of these two memory systems. Further investigation into the direct anatomical projections and indirect neuromodulatory influence between the two systems may enhance our understanding of the hippocampus and striatum and provide a better understanding of the observed behavioral responses.

However, attributing the interaction between these two memory systems to direct anatomical or indirect neuromodulatory control may not provide full understanding of the interaction (Poldrack and Packard, 2003). It is quite possible that the interaction between the hippocampal and striatal memory systems is guided by top-down modulation (from cortical to subcortical structures) through connections from structures that are involved in response selection (Alexander et al., 1986). Extensive evidence for top-down attentional modulation effects can be seen in animals using electrophysiological techniques and in humans using neuroimaging. According to this theory, a higher order system could exert its effects on the hippocampal and striatal memory systems and modulate the output. This higher order modulatory system could be that of the frontostriatal circuit, previously demonstrated to be involved in other cognitive processes.

In order to examine the effects of declarative memory systems serving nondeclarative functions within Parkinson's disease patients, further investigation should be conducted into the behavioral manifestations and costs associated with a shift in dependence. Because nondeclarative, implicit learning is responsible for the automaticity of daily activities, research of the behavioral consequences associated with implicit memory loss should be conducted in order to better understand this shift in dependence and the competitive interaction associated with it (Moody et al., 2004). Understanding the shift in dependence between memory systems and the associated behavioral consequences may provide insight into the loss of cognitive functions within Parkinson's disease patients.

Finally, current research is limited in that it does not explain how various social, environmental, and physiological parameters influence the interaction between the two competing memory systems (Poldrack and Packard, 2003). Addressing these parameters will provide a better understanding of the factors underlying learning and memory and enhance our understanding of the interaction among multiple memory systems within the mammalian brain. Developmental factors have proven to have a great influence over the interaction of systems within the brain and investigation of these effects should be investigated in order to more fully understand the multiple memory systems that subserve our everyday behavior.

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Effect of Number of Items Retrieved and Number of Retrieval Attempts on Impairment in Retrieval-Induced Forgetting

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Abstract

The act of retrieving items has been shown to not only benefit the item being retrieved but also impair the retrieval of related items. This phenomenon, termed retrieval-induced forgetting (Anderson, Bjork, & Bjork, 1994), has been attributed to interference as well as suppression/inhibition mechanisms. By manipulating the number of items practiced, the number of practices, and type of practice (retrieval vs. presentation), we looked to find the underlying mechanisms of retrieval-induced forgetting. Whereas both accounts predict that practicing more items and having more practices will create more impairment, the inhibitory account predicts this to only occur in the retrieval practice condition. We found results consistent with this prediction, indicating that inhibition is, at least, an underlying part of retrieval-induced forgetting.

Introduction

Forgetting is often seen as a failure or frailty of the human memory system. As such, forgetting is traditionally thought of as a bad occurrence or a nuisance. Forgetting a person's name or an important piece of information can be a very frustrating event. But, despite this obvious frustration, forgetting can also be quite helpful. For example, if a man is asked for his phone number, he would want to recall his most current one. But if the man remembered all of his old phone numbers in addition to his current number, there would be a massive amount of interference, making it hard for the man to answer. By forgetting his old phone numbers, the man would be better able to recall his current phone number. So forgetting old, unused information, such as old phone numbers, in favor of newer, more recently practiced information can be quite beneficial. A similar mechanism that favors recently practiced information and impairs the recall of unpracticed information has been found to exist. This mechanism is called retrieval-induced forgetting (Anderson, Bjork, & Bjork, 1994).

As the name suggests, by retrieving an item in memory, recall of another item, semantically related to the item retrieved, is impaired, making the item harder to remember or forgotten (Anderson et al., 1994; Anderson, Bjork, & Bjork, 2000; Anderson & Spellman, 2005). This effect comes about using the retrieval-practice paradigm, introduced by Anderson et al. (1994). The paradigm typically consists of three distinct phases: learning, retrieval practice, and final test. The learning phase typically consists of participants learning a list of exemplars from a set of categories. For example, in the Anderson et al. (1994) paper, participants learned eight categories with six items from each category making a total of 48 items learned. Each item is presented individually with its category (e.g. Fruit: apple). After the learning phase, participants do retrieval practice for some of the items from some of the categories. In the original paradigm half the items from half the categories were given category plus stem-cued recall (e.g. Fruit: ap_____) three times each. Items from the practiced categories that received retrieval practice are termed Rp+ items. The remaining items from the practiced categories that received no retrieval practice are called Rp- items and the items from the unpracticed categories that received no retrieval practice, whatsoever, are termed Nrp items. After all of the Rp+ items had been retrieved three times each, the participants do a short distracter to try to mitigate all short-term memory effects. Finally, each participant takes a final recall test for all the items from all the lists. This can take the form of category-cued free recall (Anderson et al., 1994, Exp. 1; Anderson & Spellman, 1995) or category plus stem-cued recall to control for output order (Anderson et al., 1994, Exp. 2-3; Anderson, Bjork et al., 2000). It should come as no surprise that Rp+ items are recalled better at final test when compared to Nrp items because they were retrieved during retrieval practice (e.g. Bjork, 1975; Roediger & Karpicke, 2006). What may be surprising is the finding that Rp- item recall is impaired on the final test when compared to Nrp items, despite Rp- items and Nrp items both receiving no practice.

The retrieval-induced forgetting effect has been shown to be a relatively reliable effect. It has been shown in the context of visual stimuli (Ciranni & Shimamura, 1999), social cognition (Dunn and Spellman, 2003; Macrae & MacLeod, 1999; Storm, Bjork, & Bjork, 2005), as well as autobiographical memories (Barnier, Hung, & Conway, 2004). But, whereas this effect has been shown in a variety of contexts, it is not quite understood how, exactly, this effect comes about. Some have suggested that retrieval-induced forgetting is a byproduct of inhibitory mechanisms meant to aide retrieval of target items (Anderson et al., 2004, Anderson & Spellman, 2005; Anderson, Green, & McCulloch, 2000; Levy & Anderson, 2002), whereas others have suggested that retrieval-induced forgetting is just an effect of interference between the practiced items and the unpracticed items (Camp, Pecher, & Schmidt, 2007; Perfect, Moulin, Conway, & Perry, 2002; Perfect, Stark, Tree, Moulin, Ahmed, & Hutter, 2004). The purpose of the current study was to further explore the underlying mechanisms of this complex effect by manipulating the retrieval-induced forgetting paradigm itself to see what causes the greatest impairment of Rp- items.

The Inhibitory Account of Retrieval-Induced Forgetting

The most studied of the two major arguments states that the retrieval-induced forgetting effect is caused by inhibitory mechanisms (Anderson et al., 1994; Anderson et al., 2000; Anderson et al. 2003; Levy & Anderson, 2002). This theory claims that, when an item is to be retrieved, there is competition between the desired item and other items in the category. In order to retrieve the desired item, the undesired competitors are inhibited, allowing the desired item to be retrieved and strengthened. Because of this inhibition, Rp- items are harder to retrieve at final test creating the retrieval disparity. The important part of the inhibitory theory is that the inhibition comes about due to competition during retrieval practice. This is supported by the necessity of retrieval practice of competing items in order for the effect to come about (Anderson, Bjork, et al., 2000). The inhibitory theory is also supported by the finding that stronger competitors are inhibited more than weaker competitors (Anderson et al., 1994, Exp 3). This was shown

by Anderson et al. (1994) with items that were more closely associated with the category cue being more inhibited by retrieval practice of items within that category than items that were more weakly associated with the category cue.

Further evidence for the inhibition theory comes from Anderson and Spellman's (1995) cue-independence design. Anderson and Spellman argued that if the mechanism behind retrieval-induced forgetting is truly inhibition of an item, a competitor to a desired item should be impaired regardless of the cue. By constructing certain Nrp lists to be semantically related to Rp+ items (Called Nrp-Similar), they hoped to show inhibition of the Nrp-similar items even though they were part of a different category from the Rp+ items. Not only did Anderson and Spellman find that there was cross-category inhibition but that there was inhibition for items which were similar to the Rp- items as well. They argued that the interference cannot account for this impairment as the strengthened items were not in the same category as the items that were impaired.

Storm, Bjork, Bjork, and Nestojko (2006) strengthened the inhibitory standpoint with the introduction of impossible retrieval during the retrieval phase. In their design, Storm et al. had participants recall either Rp+ items just like the original paradigm or they gave participants impossible cues that cannot be completed (e.g. Fruit: te_____). Storm et al. argued that, if retrieval inhibition is caused by inhibition of non-target competitors during retrieval, there should still be inhibition when no item is recalled. They further argued that interference accounts only work if there is an item being retrieved and strengthened. Without an item being retrieved there should be nothing to block or interfere with the Rp- items. The results confirmed their hypothesis that, despite retrieving nothing during retrieval practice, inhibition of un-retrieved items still occurred. Even upon further analysis of the data, it was found that those who did retrieve something during impossible retrieval practice actually showed marginally less impairment than those who could not retrieve anything at all. This showed that there is no need to even recall an item, a central tenet of the interference argument, to create the impairment at the final test.

The Non-Inhibitory Account of Retrieval-Induced Forgetting

The other argument for how the Rp- items are impaired comes from those who feel that retrieval-induced forgetting is due entirely to interference (Camp et al., 2007; Perfect, Moulin, Conway, & Perry 2002; Perfect et al., 2004). This account claims that the impairment is due to strengthening of the Rp+ items such that, at final recall, the Rp+ items are so strong that they block the retrieval of Rp- items (for a review of inhibitory theory see Anderson et al., 1994). An important distinction is that the interference account claims that the interference comes about regardless of the type of strengthening (as opposed the inhibitory account's claim that recalling items is necessary to generate the effect). This view is supported by work by Perfect et al. (2004) and their demonstration that truly independent cues cannot create impairment of Rp- items. Using the same argument as Anderson and Spellman (1995), Perfect et al. argued that, if the impairment is due to inhibition, impairment should be completely independent of what cue is given at final test. In a series of experiments, they paired pictures of faces with items as well as the normal category cue for items during study. For Experiment 1 only the category was used as a cue for retrieval practice but in Experiment 2 either the category only or the category and face was used for retrieval practice. They found that the only time there was any impairment of Rp- items when items were elicited using a face cue was when the face cue was paired with the category cue at final test and both the category cue and face cue were used during retrieval practice. Their results were replicated in Experiment 3 when unrelated words were used as independent cues instead of faces. Perfect et al. argue that this lack of impairment to an independent cue contradicts the inhibitory argument.

Camp et al. (2007) also did their own variant on independent cues by introducing an independent cue for each individual item on final test. They argue that it does not matter what cue is used at final test if an item is inhibited. So, they used a different cue during the final test that was related to that item but was never previously paired with the item (e.g. for the item Elephant, participants were shown Animal: Elephant during learning, retrieved Animal: El_____ during retrieval practice, but were given a final test of Zoo: E_____). Camp et al. found no impairment of Rp- items for any of the independently cued categories at final test. They found this effect even with both free recall and cued recall for final test. Coupled with Perfect et al.'s (2004) independent cue design, Camp et al. argued that an inhibitory account of retrieval induced forgetting could not fully explain any of their results.

The Current Experiment

Whereas inhibition and interference could both create the retrieval-induced forgetting effect, the important difference is how each explains how the impairment of the Rp- items comes about. By looking at those different explanations, one could feasibly tease out which account creates the effect. One instance in which both accounts would explain an effect differently is if the number of retrievals and number of items retrieved were varied. With the same amount of total retrievals across both groups, the inhibitory account would claim that many items retrieved once should create more impairment than a few items retrieved multiple times due to the competition aspect of inhibition. As was shown with Anderson et al. (1994) and Anderson, Green et al. (2000), the items that compete more with the target item to be retrieved are impaired the most by inhibition. If this is the case, after the items are retrieved the first time in the traditional paradigm, the Rp- items should be inhibited and the Rp+ items should be strengthened. But, because the Rp+ items are strengthened and the Rp- items are weakened, there should be less competition for the next retrieval of each Rp+ item. The smaller competition should create less inhibition for the Rp- items and this rift should only get larger in the final retrieval of each Rp+ item. With multiple items retrieved just once each retrieval should have a great deal of competition, creating a lot of impairment for the Rp- items. After the first item is retrieved, the rest of the items (including the Rp+ items) should be impaired. Each subsequently retrieved item should be at approximately the same level as the Rp- items when it is retrieved. This makes it so that the Rp- items compete maximally at every retrieval. If no Rp+ item is retrieved a second time, then there is no rift between the Rp+ items and the Rp- items. This works for maximum competition leading to maximum inhibition.

The interference account also predicts that more items practiced only once should have more impairment but because multiple less strengthened items have been shown to create more interference than a few highly strengthened items. As of yet, there has been no research comparing multiple items versus multiple retrievals within the retrieval-induced forgetting paradigm, but the part-set cuing paradigm (Slameka, 1969) offers some predictions in terms of the interference account. Part-set cueing is a paradigm very similar to the retrieval-induced forgetting paradigm in that participants study and are given a free recall final test. But instead of doing retrieval practice or representing items as a separate phase during the experiment, a subset of the words is presented as a cue for the final test. Instead of helping facilitate retrieval of other items, it has been shown that the presentation of words at test has actually impaired the retrieval of the other words from the list (Basden, Baseden, & Galloway, 1977; Roediger, 1974; Roediger, Stellon, & Tulving, 1977; Slameka, 1969). So, in much the same way that the Rp+ items are strengthened during retrieval practice, the part of the list that is shown is strengthened by being re-presented. And since the interference argument claims that the retrieval-induced forgetting effect is due to strengthening an item, regardless of how the item is strengthened, the part-set cuing paradigm can be considered as nearly equivalent in how the recall of the non-listed items (in the part-set cuing paradigm) and the Rp- items is impaired. In the part-set cuing paradigm, a model by Rundus (1973) creates a ratio

rule in that the greater the amount of items that are strengthened, the more the interference. In this model, Rundus claims that one searches for items from a category and will only stop searching for items when no new items from that category can be recalled in a sufficient amount of time. This model also states that stronger items are more likely to be recalled than weaker items. So, if the same strong items are continuously recalled, the participant will stop recalling items altogether, creating the part-set cuing effect. To show his effect, Rundus showed that more items shown as cues during final test create more interference. So based on this model, strengthening multiple items once creates more interference than strengthening a few items many times. And since the same interference is present during both part-set cuing and retrieval-induced forgetting, the interference account would predict that a few items retrieved once should create more interference for the Rp- items than a few items retrieved many times.

In an effort to find the source of retrieval-induced forgetting, we chose to manipulate the retrieval practice phase of the original paradigm to see the effect of multiple retrieval practices versus multiple items. But since both accounts predict the same result, we also decided to manipulate the type of practice each participant received. With half of the participants we had them retrieve the words like the original paradigm. The other half of the participants were shown the items again in a type of presentation practice. The two accounts have different predictions with the inclusion of the presentation condition. The inhibition account would predict that, due to the need for competition during retrieval, there should be no impairment of the Rp- items in the presentation practice condition (Anderson et al., 2000). The interference account, however, states that the blocking is due to strengthening of the items regardless of practice type (Anderson et al., 1994). So the interference account would predict that there would still be the same amount of interference in both presentation and retrieval practice conditions. This means that any additional impairment due to retrieval practice over presentation practice would be evidence of inhibitory mechanisms.

By looking at the effect of different numbers of items and different numbers of items with two types of retrievals, we hoped to find the underlying mechanism of retrieval-induced forgetting. It is still debated as to what causes this unintuitive effect, and showing that either inhibition is absent or that inhibitory mechanisms in memory are present could have serious implications for further research. Currently it is not established that inhibitory mechanisms are part of declarative memory. The establishment of inhibitory mechanisms could help to provide a link between cognitive psychology and neurophysiology (in which inhibition has been known for decades). And finding out how multiple items and multiple retrievals affect the recall of other items in memory can be very helpful. In addition to this research never having been done before, finding out how many questions to ask as well as how many times to ask a type of question could help anyone who needs others to remember information. Knowing how to structure questions so that the information that needs to be rehearsed is rehearsed and so that the non-practiced information is not impaired (or at least impaired less) could be beneficial to teachers, trainers, and even writers (in terms of repeating items throughout so a person remembers what is written).

Ultimately both views predict that more retrievals will create more the impairment for the Rp- items at final test. For the inhibitory account, the more the retrievals of an item the more the Rp- items are inhibited. And for the interference account, the more an individual item is strengthened, the more it blocks other items. Both accounts also predict that more items retrieved will create greater impairment of the Rp- items. The inhibition account claims that retrieving multiple items should create more inhibition than retrieving a few items whereas practicing more items in the interference account would also create greater interference than practicing a few items. And both accounts predict that many items retrieved once would create more impairment than a few items retrieved many times. The difference between the two models would be the predictions for the presentation practice. The interference account predicts no changes between retrieval practice and presentation practice conditions. The inhibitory account, however, claims that there should be impairment in the retrieval practice condition but there should be no impairment in the

presentation practice condition.

Based on these predictions from both accounts we predicted along the lines of both accounts in that we expect greater impairment for Rp- items with more items and more retrievals. We also predicted that the many items retrieved once should create greater impairment than a few items retrieved many times. But we predicted in line with the inhibitory account in that there would be greater impairment with the retrieval practice condition than the presentation practice condition.

Method

Participants

Seventy-two undergraduate students (22 men and 50 women, mean age = 19.99) from the University of California, Los Angeles received course credit for participating in this study.

Design

Four factors (retrieval practice status, amount of practice, number of items practiced, and type of practice) were manipulated. Retrieval practice status, amount of practice, and number of items practiced were within-subjects variables whereas type of practice was manipulated between subjects. Retrieval practice status had two different levels: Rp+ and Rp-. Rp+ items were the items that received practice during the practice phase. Rp- items are the items that did not receive any practice and were items in the same category as the Rp+ items.

Amount of practice was varied such that participants either practiced a Rp+ item once or three times during the practice phase. Number of items practiced had two levels, two items or six items. For the two items condition, two items from a single category were practiced during the practice phase. In the six items condition, six items from a single category were practiced during the practice phase. This creates four types of categories: two items from the category practiced once (2/1), two items from the category practiced three times each (2/3), six items from the category practiced once (6/1), and six items from the category practiced three times each (6/3). The critical comparison is between the 2/3 and the 6/1 categories in that they both have the same number of total retrievals.

Type of practice had two different levels such that half the participants performed retrieval practice and half the participants performed presentation practice during the practice phase. For retrieval practice, participants were asked to complete a two-letter stem of the word shown along with the category cue on the screen (For example: Fruit: Ap_____). For the presentation practice, the entire word was shown with its category and the participant was just asked to write down the word (For example: Fruit: Apple). We measured the percentage of items correctly recalled at final test, that consisted of participants completing individually presented, one-letter stem shown with its category cue for all Rp+, Rp-, and filler items.

Materials

The words used in this experiment consisted of eight categories of nine items each (72 items total). For every participant there was a total of four experimental categories (a 2/1 category, a 2/3 category, a 6/1 category, and a 6/3 category) and four filler categories. The categories and six items from every category were taken from the original study done by Anderson et al. (1994). The remaining three words for every category were chosen by equating rank order across categories (M= 11.06) using category norms from Battig and Montague (1969). The words were chosen so that no two words from any category started with the same first letter and no one word could belong in more than one experimental category.

For every category three items were chosen as critical Rp- items. These critical Rp- items were

never practiced in any condition. Items were chosen such that each category's Rp- items were equally recallable (M=.707) based off of baseline (Nrp) retrieval percentages from Storm et al. (2006). This was done so that the number of Rp- items would not be confounded across number of items practiced (for the three item level there would be six Rp- items but for the six item level there would be three Rp- items) and so each category's Rp- items would be at the same level of recall before the practice phase.

All items were presented via Microsoft PowerPoint on Apple computers. The items were presented in the center of the screen in 44 pt. Arial font. Each participant was given a three-page response packet. On the first page were blanks for participant and condition number as well as blanks for demographic information. The following two pages consisted of two columns of lines on which the participants were to write their answers for the practice phase and final test, respectively.

Procedure

Before the experiment, all participants were seated at their own computer and given their response packets. Participants were told that all instructions were given via the PowerPoint presentation and were asked if they had any questions before beginning the experiment. Once all the questions were answered the participant started the experiment that consisted of three phases: the learning phase, the practice phase, and the final test.

The learning phase. Participants were presented with one of two learning lists. Each word was presented, individually, with its corresponding category (in the form "Category: Exemplar"). Each category and word were presented for 5 seconds each before moving on to the next category and word. Each list was block randomized using nine blocks of eight words such that, in each block, there would be one word from every category. Each learning list started and ended with two filler items to control for recency and primacy effects and no two words from any category were presented successively. Once all 72 items were presented once, the participants moved on to the practice phase.

The practice phase. For the practice phase participants either retrieved or practiced the Rp+ items. Each participant was either asked to complete a two-letter stem presented on the screen for the retrieval level of practice type (For example: if the participant saw Fruit: Ap_____, he or she would write down Apple in the response packet) or copy down the word presented on the screen for the presentation practice level of practice type (For example: if the participant saw Fruit: Orange, he or she would write down Orange in the response packet). All of the category and stem cues were presented individually and each participant was given 5 seconds to write down the answer. The items were block randomized with two filler items, the three Rp+ items from the 6/3 category, two of the Rp+ items from the 6/1 category, the two Rp+ items from the 2/3 category in each of three blocks. In two of the three blocks a 2/1 Rp+ item was also present. All of these items were randomized such that no two items from any category were shown successively and the practice phase started and ended with two filler items. The categories were counterbalanced such that every category was 2/1, 2/3, 6/1, or 6/3 and that every word within the category except for the critical Rp- items was one of the two Rp+ items in the 2/1 and 2/3 categories between subjects. After all the items had been practiced the participants did a distracter for 10 minutes. The distracter consisted of a color matching game.

The final test. Following the distracter, the participants were asked to retrieve all of the Rp+ items, critical Rp- items, and half of the filler items. For every item the participant was shown the category cue and then a one letter stem that they must complete (e.g. a participant is shown Fruit: A_____, and is supposed to write down Apple). Each item's cue was presented individually onscreen for 5 seconds at which time the participant wrote down the answer before the next cue was given. For the final test the

Rp- items (determined by the practice phase) were tested first, followed by the Rp+ items to control for output interference. The Rp- items were randomized for every condition, but no two words from the same category were tested consecutively. The Rp+ items were also randomized with the same criteria. Every test started with two filler items to control for recency effects.

Results

As there were different number of Rp+ items practiced and no Nrp items, we only looked at the final recall of the 12 critical Rp- items at final test. We examined the data using a 2(2 vs. 6 items practiced) x 2(1 vs. 3 times practiced) x 2(retrieval vs. presentation) mixed design, Analysis of Variance with type of practice being analyzed as a between-subjects variable. Contrary to both accounts we found no effect of amount of practice, $F(1,70) = .037$, $MSE = .003$, $p > .05$, and no effect of number of items practiced, $F(1,70) = 1.916$, $MSE = .087$, $p > .05$, with no interaction between the two variables, $F(1,70) = .482$, $MSE = .047$, $p > .05$. We did, however, find a marginally significant effect of type of practice such that the retrieval practice created more impairment of critical items than presentation practice, $F(1,70) = 3.116$, $MSE = .420$, $p = .08$. This result is in line with the inhibitory argument. However, in line with the interference account, there was no interaction between number of items practiced and type of practice, $F(1,70) = .482$, $MSE = .047$, $p > .05$, and no interaction between amount of practice and type of practice, $F(1,70) = 1.030$, $MSE = .047$, $p > .05$. And, as predicted by both accounts there was no overall interaction between all of the variables, $F(1, 70) = .322$, $MSE = .031$, $p > .05$.

Due to the marginally significant main effect of type of practice, we also decided to look at the individual differences between the two types of practice across all of the conditions. The rational behind this is that, as can be seen in Table 1, the final recall of critical Rp- items is constantly lower across all of the conditions in the retrieval practice group as compared to the presentation practice group and there is a marginally significant effect of type of practice. However, we only found a significant difference in the 6/3 condition between the retrieval practice ($M = .37$, $SD = .32$) and presentation practice ($M = .51$, $SD = .27$) conditions, $t(70) = 2.003$, $p < .05$. None of the other three conditions were significantly different.

Table 1
Average Recall of Critical Rp- items as a Function of Number of Items Practiced, Number of Practices Per Item, and Practice Type

	Practice Type	
Number of Items / Number of Retrievals	Retrieval	Presentation
2/1	0.45	0.51
2/3	0.43	0.47
6/1	0.39	0.45
6/3	0.37	0.51

Discussion

Despite not finding a significant effect of type of retrieval and any effect of different number of practices and different number of items practiced on final recall of critical Rp- items, we were able to find a significant difference between the retrieval and presentation practice in the 6/3 condition and a general trend of retrieval creating greater impairment of Rp- items at final test. This trend and, especially, the difference between recall and presentation in the 6/3 condition are in line with previous research showing that the act of retrieval is necessary for retrieval-induced forgetting (Anderson et al., 1998). The necessity of a recall mechanism in this experiment is also in support of the general inhibitory argument (Anderson et al., 1994; Anderson & Spellman, 1995; Anderson et al., 1998). We predicted that the data should fall in line with the inhibitory account and the nature of the trend, with the retrieval practice creating greater impairment, falls right inline with this prediction. This trend ultimately leads to a significant difference between the 6/3 retrieval condition and the 6/3 presentation practice condition. This significant difference indicates that there might be a difference between retrieval and presentation practice conditions. The interference account cannot account for the difference between these two conditions, so these results support the inhibitory account.

Further evidence for the inhibitory account comes from the general trends in the data. As more retrievals and more items retrieved are added there was numerically greater impairment in the recall condition. As can be seen in Table 1, as more retrievals and more items are added there is a systematic decline in the final recall of the critical Rp- items. This is very evident when comparing the 2/1 to the 6/3 condition, although not statistically significant. Although, in the presentation case, there is essentially no change between the 2/1 and the 6/3 conditions. This is in line with the general prediction of the inhibitory account. The inhibitory account would predict that, as both number of practices per item and number of items practiced increases, there should be greater impairment in the retrieval practice condition. As well as predict that impairment of the Rp- items in the presentation practice condition should not decrease or should decrease at a much lower rate (Anderson et al., 1998). But it is important to note that we were unable to find a significant effect of number of items practiced and amount of practice. This goes against previous research in both retrieval-induced forgetting (Shivde & Anderson, 2001) as well as in a think-no think paradigm that is thought to use the same mechanisms (Anderson & Green, 2001) so one should be very cautious when interpreting these results.

However, the interactions between number of items and type of practice and between the number of practices per item and the type of practice were not significant. This indicates that the both the presentation and the retrieval practices vary in the same way. The lack of interaction is support for the interference account that believes that both types of practice should create the same pattern of results (Anderson et al., 1994; Perfect et al., 2004). And further doubt is cast on the support for the inhibitory account by looking at the means for each condition. For the first three categories (2/1, 2/3, 6/1) the data for both types of practice follow the same decline. But then the 6/3 presentation condition Rp- recall actually improves from the recall of the 6/1 condition while the 6/3 retrieval condition recall is further impaired from the 6/1 condition. The sudden jump up from the 6/1 condition in the presentation practice group could just be considered noise and it may just be that the entire category has the same amount of recall.. But when looking at the data it becomes clear that it is the difference in the 6/3 category that drives the marginally significant difference between the types of practices and creates the bulk of the evidence for the inhibitory account.

One possible explanation that has been offered for this sudden jump can be attributed to the design

of the experiment. Due to the manipulation, for every person there was one category that was practiced 18 times as compared to 6 times each for two other categories and filler items and 2 times for another category. With such a discrepancy it is very likely that a person would have surmised that this category is important due to the high amount of practices. So the participant may have done covert retrievals of all items from the 6/3 category whenever an item from the 6/3 condition was presented. These covert retrievals would have not only strengthened the Rp+ items but also the Rp- items in the 6/3 condition leading to the jump in final recall of Rp- items from the 6/1 condition to the 6/3 condition. However, this leads to another question as to why it is only the presentation case that exhibits this increase in recallablity when both 6/3 conditions practiced the items the same amount of items. One explanation could be the difference in amount of time it takes to recall an item and write it down as opposed to simply writing the item down. It may be that in the retrieval practice condition the participant took all 5 seconds recalling and writing down the answer and had much less time to do covert retrievals, if any. Presentation practice participants, though, did not have to recall the items but just had to write down what was on screen. This may have given the participant in the presentation conditions time to consider the other items from the 6/3 category using the word pair on screen as a cue creating the better memory for the 6/3 Rp- items. If this is true then that would mean that the differences between the presentation practice and retrieval conditions is not the type of practice itself, but rather the amount of time participants had following writing down the item that created the effects.

However, the argument that the effects were caused by difference in time to retrieve and write down an item and just writing down an item is not entirely plausible. This explanation requires participants to notice that one category is being retrieved more than the others. In order for the participant to notice this difference the participant must have gone through at least one full cycle of practicing the 6/3items (if not more) to notice that items from one category are being recalled more than the others and the difference is not due to items from other categories not being shown yet. By this time the participants in both retrieval type conditions will have already practiced all of the 6/3 items. So for the retrieval practice condition, participants will have already recalled most of the words the first time through the 6/3 items within the allotted time and should be much faster the second time through, when the participants could first become aware of the number of items retrieved discrepancy. This means that by the time participants in both conditions had a chance to realize that more items from the 6/3 category were being practiced and misconstrue that the 6/3 category was somehow more important because of this, both groups would have time for covert retrieval. So if covert retrieval is the reason for an increase in 6/3 Rp- recallability in the presentation practice condition as opposed to the retrieval practice condition, that would be because there was some impairment in the ability to covertly recall the Rp- items in the retrieval practice condition. Ultimately this explanation still leads back to the inhibitory argument. But, since this is all conjecture, one possibility for future research would be to measure reaction times of each response to see if, in fact, the presentation practice conditions had more time following writing down the answer.

A large limitation to interpreting these data is the inability to assess whether or not retrieval-induced forgetting took place. By looking at the base rate recall of Nrp items it could become possible to look at whether or not each set of data has impairment and to what degree. If both sets of data were firmly below the base rate recall it would become clear that both retrieval and presentation created impairment and that both accounts were correct. This would indicate that retrieval-induced forgetting is just a matter of a mixture of both inhibition and interference. And if both sets of data had recall levels significantly higher than the Nrp data, then it would be clear that there is something wrong with the materials or methods pursued or we might have found a possible boundary condition. But, unfortunately, Nrp items were not included in our design. Many of the filler categories could technically be classified as Nrp categories as they were categories in which items were initially studied but were not practiced. But the filler items were not counterbalanced in such a way that they could be compared to the rest of the data. One possible route for future research would be to just run this experiment again with a proper Nrp group.

Another possibility for future research is doubling the amount of lists to be learned. This would help addresses the problem of the participants focusing on the 6/3 condition. With two 6/3 categories, the discrepancy between individual categories will become less noticeable as it is not just one category receiving most of the practice. Perhaps by doubling the number of lists the upswing for the presentation 6/3 condition will disappear and the trends can be reliably looked at. So, this manipulation should lessen the obvious differences between presentation numbers between categories, creating a clearer picture as to which mechanism(s) are taking place to create the impairment of Rp- items at final test.

Even though these results do not unequivocally differentiate between the different mechanisms thought to be present in retrieval-induced forgetting, they do have some important implications in terms of education. These data show that, for short-term recall, it is better to do presentation practice rather than retrieval practice. This supports previous work done by Roediger and Karpicke (2006) looking at the testing effect. In their study, Roediger and Karpicke found that having more study sessions improved recall of the information at immediate testing but that testing instead of studying improved the students' ability to retain the information after a week. Conversely, the present study does not argue that presentation practice is a better study event for short-term recall for the items studied but rather argues that re-study is good due to lack of forgetting of related information that may not have been studied. Often times when a student studies right before a test, she will go over as much information as many times as she can in order to "cram" as much information as possible for the test. However, the student may not study every piece of information that is on the test. So, for short-term recall, it would be desirable to remember not only what is studied immediately before the test but also information that might not have been studied right before the test. This means that the optimum way to study material for short-term memory retrieval is to simply restudy the items and not retrieve them. That way the items that are studied are remembered and the items that are not studied are not forgotten.

Finally, this experiment also adds further evidence for inhibition in declarative memory. This experiment has shown that there is a difference between the effects of retrieval practice on Rp- items and the effects of presentation practice on Rp- items. Even when the difference between the two conditions may be due to amount of time to covertly retrieve, the explanation points to an inhibitory account. Despite the lack of a NRP baseline and statistically significant evidence from our manipulation, it is clear that there is a difference between the impairment of Rp- items when Rp+ items are retrieved as opposed to merely studied.

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Intermittent Availability of Alcohol Does Not Induce Relapse Drinking: Some Micro-RNAs Seem to Hold the Key

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Abstract

Alcoholism is a chronic disease characterized by impaired control over drinking, preoccupation with the drug alcohol, use of alcohol despite adverse consequences, and distortions in thinking. [DSM-IV]. The present experiment attempts to evaluate the behavioral and genetic causes of a particular symptom of alcoholism known as the relapse drinking. It has been shown that Pavlovian conditioning procedures induce high alcohol consumption amongst mice, but there is no prior data relating it to relapse. To test whether or not such a correlation exists with the relapse effect, twelve mice were randomly assigned to differing alcohol intake procedures – six on the intermittent, with high opportunity for conditioning, and six on the continuous, serving as the controls. For measuring the alcohol deprivation effect, the mice were placed on the procedure for 5 consecutive days, followed by a 2-day deprivation from alcohol. The amount of consumption immediately on the next session after this deprivation served as an indicator of relapse drinking. During the length of the study, the entire cycle was repeated twice and the relapse drinking average was taken for each mouse. It was demonstrated that, though inducing higher alcohol consumption, Pavlovian conditioning was not significantly correlated with the amount of relapse drinking. Instead, linear regression studies showed the expression of certain genetic miRNAs might have accounted for these high averages. In light of these results, it can be implied that alcohol addiction does not necessarily occur because of an individual's conscious actions, but rather stems from a genetic predisposition.

Introduction

Alcoholism is incontestably a growing problem in today's society. Despite a lot of literature on the subject, the complexity of the molecular mechanisms and behavioral phenotypes makes the disease difficult to define or diagnose. The Medline Plus Dictionary, published by the US Library of Medicine and National Institutes of Health, has established four distinct criterions for its classification: a strong desire to consume alcohol (craving), an inability to consciously refrain (loss of control), exhibition of strong withdrawal

symptoms (physical dependence), and, over time, a need for higher and higher intake to feel the same 'high' effect (tolerance). Prior studies on animal and human subjects have demonstrated individual differences in the tendency to develop alcohol addiction and other drug-taking behaviors (Cloninger et al., 1988; Piazza et al., 1989; Bisaga and Kostowski, 1993). This study attempts to uncover some of the factors contributing to such a variation.

1.1: Role of Conditioning on Alcohol Consumption

Precise causes responsible for higher susceptibility of some subjects towards addiction remains largely unknown. However, there is considerable evidence that strong pavlovian-type conditioning (intermittent presentations of sipper delivering alcohol) induces higher than normal voluntary alcohol intake in laboratory mice. Repeated time-dependent pairings of an irrelevant stimulus, such as a metal sipper containing alcohol, with an unconditioned stimulus that generates an intrinsic reward, such as food, initiates a pavlovian-like association of the irrelevant stimulus to the reward. It has been shown that such conditioning generates compulsive behavior towards the sipper, as if in anticipation for the upcoming reward, and induces greater alcohol consumption (Tomie et al., 2001). This hypothesis was sincerely called into question by further experimentation.

Experiments conducted to analyze the details of this mechanism have shown that food reinforced groups performed equally well to controls not given any food access during the training session (Tomie et al., 2006). Thus, the previous reward-oriented hypothesis is generally not accepted today. Rather, it is believed that Pavlovian conditioning occurs between the insertion of the sipper and alcohol access; an association which might be the cause of compulsive drinking behaviors.

1.2: Neurobiological Factors Related to Alcohol Addiction

Neurobiologically, pavlovian conditioning works by way of strengthening weak synapses, or synapses that are by themselves incapable of generating a depolarization of the post-synaptic membrane. The neuron exhibiting such a weak synapse is usually responding to a neutral stimulus that has no intrinsic meaning or response associated with it – in the experiment, this would be the neuron firing when the metal sipper is present. Alone, the presence of the sipper is not of any interest to the mouse and, thus, does not elicit any response. However, located on the same post-synaptic neuron, at a different dendritic site, is the presence of a strong synapse, or a synapse that alone is able to generate a post-synaptic potential. The presence of alcohol is a substantially strong stimulus with the capability of completely depolarizing the neuron responding to its presence, thus inducing an action potential. When both pre-synaptic neurons are fired simultaneously, the strength of the weak synapse increases dramatically – leading to an association between the two. After a critical point of synapse strengthening, the weak neuron is, by itself, able to depolarize the post-synaptic membrane and the subject's mind is said to be conditioned.

Specifically for this experiment, the weak neuron responds to the presence of the sipper (or cocktail glass or shot glass etc – any object used specifically for the consumption of alcohol) and the strong neuron responds to the presence of alcohol. When these are repeatedly presented to the subject simultaneously, the subject begins to identify the object as signaling the presence of alcohol. The result of this conditioning is clear – an increase in alcohol-related behavioral responses simply due to the presence of the object of consumption (even if alcohol is not present itself). For a mouse, this means sucking or biting the stainless-steel sipper. For a human, this means a strong desire to obtain and consume alcohol.

1.3: Molecular Basis for Alcohol Addiction

Molecularly, it is hypothesized that NMDA receptors at the site of the weak synapse are stimulated because the glutamate release is paired with the membrane depolarization caused by the activation of the strong synapse. These NMDA receptors trigger various enzymatic reactions to increase the density of AMPA

receptors at the weak synapse, leading to a higher response capability. Repeated temporally dependent pairings result in enough AMPA receptors to allow the weak synapse, by itself, to induce a post-synaptic potential – the neurobiological basis for conditioning.

The insertion of the sipper, a previously neutral stimulus, is now able to trigger exactly the same mechanisms that are normally triggered with the availability of alcohol. Since stimulation occurs with such specificity, the subject feels a strong psychological craving for the alcohol each time the sipper is inserted. Referring to the previous section, the strength of this signal behaviorally gets translated into an urge for drinking at the first sight of a shot-glass.

1.4: A Case for Intermittent Presentations

Association is much more effective if there are intermittent insertions of the sipper, as opposed to one continuous presentation. An intermittent insertion allows for alcohol access only at times when the sipper is inserted, which facilitates conditioning and results in greater alcohol intake (Tomie et al., 2005). In other words, a person is more liable to become an addict if the same shot-glass is used and if it is only used to drink alcohol. The control group, similar to a person who uses the shot-glass to drink alcohol as well as other types of beverages, like water and orange juice, shows less association between the two; leading to lower alcohol intake averages. In this experiment, a previously designed apparatus of retractable ethanol-containing sipper tubes allowed the experimenter to exert time-dependent control over the sipper insertions (Samson et al., 1998; Czachowski and Samson, 1999; Files et al., 2000; Tomie et al., 2002, 2003, 2004, 2005).

1.5: Goals of the Study

Though the study addresses alcohol addiction as a whole, its primary focus is to study the effects of conditioning on a withdrawal symptom known as relapse drinking. It has been hypothesized that the presence of alcohol for a period of time causes habituation of the central nervous system, leading to reliance upon alcohol as a means for neural communication. When alcohol intake suddenly decreases, as in a withdrawal period, the CNS remains hyperactive and causes the person to exhibit serious withdrawal symptoms (Saitz, 1998). At the next available opportunity to consume alcohol, the person immediately drinks in excessive quantities – a phenomenon known as relapse. This high amount of relapse drinking after a period of withdrawal from alcohol is defined as the Alcohol Deprivation Effect [ADE] (Rodd et al., 2004). Causes, both behavioral and molecular, are still only vaguely known.

This study attempts to question the connection between conditioning procedures, shown to induce more alcohol consumption, and the alcohol deprivation effect. To perform such an investigation, mice are put through the intermittent procedure where it is expected that the association between the insertion of the sipper and the availability of alcohol would be very effective. Controls are put through the continuous procedure where the association is expected to be less prevalent. Both groups are then put through a relapse-drinking model where alcohol is taken away for a set amount of time but reinstated immediately after this deprivation period (Spanagel, 2005). It is hypothesized that such a combination of procedures would successfully induce the alcohol deprivation effect in mice while simultaneously allowing for an analysis of the influence of conditioning on the phenomenon.

At the cellular level, there has been an attempt to uncover some of the genes contributing to this alcohol deprivation effect. The prevalence of a genetic basis for alcoholism has been well established and though the interaction pathways are complex, and most not yet known, more than a dozen genes have already been discovered as being related to alcoholism (Nurnberger, 2007). Recent literature shows evidence of microRNAs being distinctly correlated with some aspects of addiction, such as alcohol

tolerance (Science Daily, 2008). Further research is well warranted for understanding addiction at the cellular level. The study attempts to contribute by, at least statistically, discerning the possible microRNAs specifically responsible for the alcohol deprivation effect.

To conclude, there are four major goals to this study: confirm previous experiments correlating intermittent availability with effective conditioning and higher alcohol intake; determine experimental conditions to induce an alcohol deprivation effect in laboratory mice; investigate if conditioning serves as the rationale behind relapse drinking; and, finally, identify the genetic basis for the alcohol deprivation effect. Investigations like this one would allow us to further understand one of the most fundamental questions in alcoholism and other addictions – why it is so hard to quit. It would provide more insight into the numerous case studies of people who, despite a strong conscious will to refrain, end up resorting back to alcohol and other addicting substances.

Methods

2.1 Animals

Twenty four CD-1 outbred white mice were used in this study. The animals were housed individually in a shoebox cage under 12 hour automated light/dark conditions. Food and water were available ad libitum on top of the homecage. All procedures were conducted in accordance with guidelines of the Institutional Care and Use Committee of National Institute of Drug Abuse, National Institutes of Health and approved by the IACUC at Rutgers University.

2.2 Training Apparatus

Six training chambers were obtained from Med-Associates with the dimensions 30.5 cm x 24.1 cm x 21.0 cm: Model ENV-008 (length x width x height) made up of a stainless steel front wall intelligence panel, a stainless steel rear wall, and a stainless steel grid floor (Model ENV-005). The ceiling, right and left walls were clear polycarbonate; the right wall was hinged to the rear wall and secured with a latch to serve as a door. A stainless steel sipper tube was inserted into the chamber through a hole in the front of intelligence panel located 3.0 cm from rear wall and 3.5 cm above the grid floor. There were four additional holes in the front and rear walls, which were identical to the hole for the sipper but were not related to the mechanical function of the drinking chamber. The purpose of these holes was to ensure that the subjects were not spending more time near the sipper hole because of nose poking and thus be more likely to drink from the sipper simply because of their close proximity to it. A retractable tube with an inserted rubber stopper was used to hold Alcohol solution. The protraction mechanism for the sipper (Model ENV-252) moved the sipper 3.8 cm from its retracted position to extend into the chamber. In the retracted position, the sipper was 3.2 cm away from the chamber. Each chamber was individually enclosed in a sound-attenuating chamber, which contained a fan for ventilation and was illuminated by a house light on the ceiling. Wearing perfume and generating excessive amounts of noise were explicitly prohibited in the training room to minimize the effects of stress on the subjects.

2.3 Alcohol

Industry grade 95% ethanol was diluted 3% using distilled water. More than enough ethanol (5ml) was placed into the sipper tubes before the start of each training session.

2.4 Procedures

All procedures and timings were computerized and run through Med-PC software. Each trial lasted for 1 minute with a total of 50 trials – 50 minutes per training session. The 24 mice were randomly divided into 2 equal groups and placed on varying procedures.

2.4.1 Intermittent procedure. During the one minute in each trial, the alcohol-containing sipper was inserted for a total duration of 10 seconds. For the rest of the 50 second time period, the sipper was out of reach for the mice and, inadvertently, access to alcohol was prohibited. This period between the retraction of the sipper and the insertion of it in the next trial was known as the Inter-Trial Interval [ITI]. The exact 10 second block when the sipper was to enter was randomized per trial to provide a random ITI. Therefore, this group received a little more than 8 minutes of alcohol access per training session.

2.4.2 Continuous Procedure (or Control Procedure). In this procedure, the sipper was inserted for the entire 60 seconds per trial. This procedure allowed a total of 50 minutes of alcohol access per training session. Since there was never any sipper retraction between trials, the ITI for this group was 0.

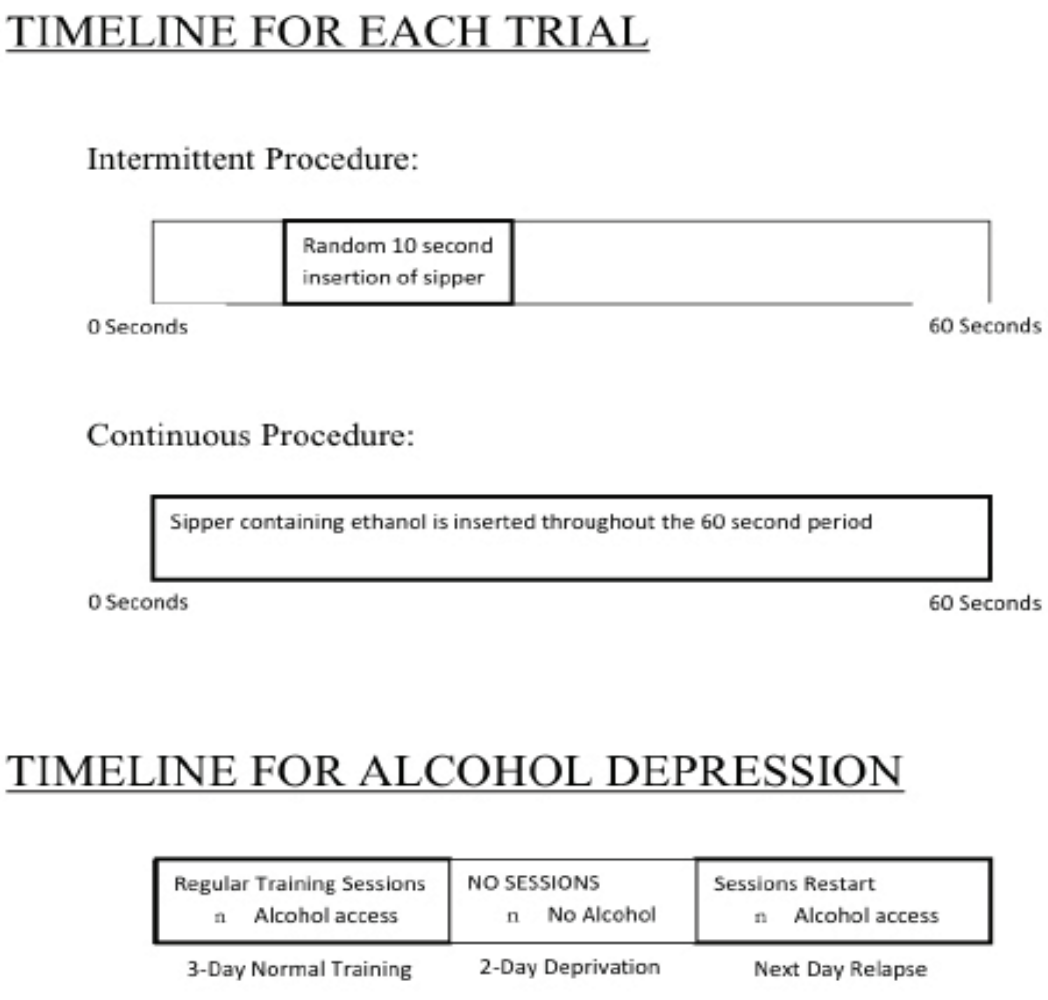

FIGURE 2.1: Procedure Timeline

(Top) A graphical representation of time with and without alcohol access for two different procedures in a given trial. (Bottom) A weekly schedule for calculating the Alcohol Deprivation Effect.

Training runs were scheduled in a way to permit one training session for each mouse per day. Four different runs, consisting of six mice each, were conducted at different times during the day. To correct for any potential bias, a run always consisted of 6 mice simultaneously undergoing trials in the six identical

training chambers – 3 on the intermittent procedure and 3 on continuous. It was maintained that there were approximately 23 hours between consecutive training sessions for each mouse. The entire study was conducted over a 19 day period.

2.5 Relapse Drinking Model for ADE

All mice, regardless of which group they had been randomly assigned to, underwent the relapse drinking procedures in accordance with the methods shown in Figure 2.1. After 5 days of respective intermittent or continuous sessions, the mice were deprived of training for 2 days. For these two days, mice were kept in their homecage with no access to the training chamber or alcohol. Neither food nor water was deprived during this alcohol deprivation period. The very next day mice resumed their regular training schedules with respective time-defined access to alcohol. Alcohol intake on this day signified the amount of relapse drinking. There were a total of two alcohol deprivation periods within the 19 day study. An average of the two was used during analysis.

2.6 Collecting data

Each mouse was weighed before being run in a training session. At the start of each training session, 5ml of ethanol was put into the sipper tube. Difference in amount of ethanol after the session served as a predictor of the ethanol consumption of the animal. To correct for differences in weight and other factors, ethanol intake, not ethanol consumption, was analyzed. Ethanol intake was calculated using this formula:

Ethanol intake [ml/kg] = ethanol concentration [3%] * ethanol consumption for that session [ml] * specific gravity of ethanol [0.7986] / weight of the mouse on that day [kg]

With respect to the alcohol deprivation effect, two critical periods were compared. An average of the alcohol intake 3 sessions before the deprivation was interpreted as providing the expectancy for the amount of drinking for the particular mouse. The next day after the alcohol deprivation period provided the amount of relapse drinking. The alcohol deprivation effect [ADE] was calculated using this formula:

ADE = relapse / (relapse + expectancy)

where relapse signified the amount of drinking on the day after the withdrawal period and expectancy signified the average baseline amount from three days before the withdrawal period.

2.7 Analysis

2.7.1 Intermittent vs. continuous. 3 mice with the most ethanol intake and 3 mice with the least ethanol intake were isolated from each group of 12 mice. The three highest drinkers from the intermittent procedure were categorized as being habituated to that procedure. The three highest drinkers from the continuous procedure were similarly categorized. The three lowest intermittent drinkers did not habituate to their procedure, nor did the three lowest continuous drinkers. Thus, these six mice were combined to provide an experimental negative control group. In such a manner, three groups were formed, as displayed in Figure 2.2 – mice habituated to the intermittent procedure (n=3), mice habituated to the continuous procedure (n=3) and negative controls (n=6). A total of 12 mice were analyzed. The rest of the mice served as subjects for another study attempting to determine the effect of increasing the concentration of ethanol. 2.7.2 Alcohol Deprivation Effect (ADE). With the ADE formula mentioned above, a value of 0.5 signified that relapse drinking was equal to expectancy [no effect]. A value < 0.5 signified that the amount of relapse drinking was lower than normal drinking expectancy [Negative ADE]. Lastly, a value > 0.5 signified that the amount of relapse drinking was higher than normal drinking expectancy [Positive ADE]. Thus, two categorizations were created with respect to the alcohol deprivation effect.

2.8 MicroRNAs

2.8.1 Data collection. All 12 mice were sacrificed the second day after the end of the study. The

animals underwent a cervical dislocation and the hypothalamus, hippocampus, caudate/putamen and cerebellum regions were dissected. The brain tissue was sent to Shanghai where an Aligent miRNA chip array was conducted. The researchers conducting this array were completely blind to the study and categorization of subjects. Data retrieved from this array consisted of miRNA signals and flags for each individual mouse.

Figure 2.2: Organizational Chart

The above chart explains the division of mice into different procedural groups that were later cross-examined for differences in miRNA expression.

2.8.2 Analysis. miRNAs were sorted, according to the phenotype of mice, in one of the three groups from the intermittent vs. continuous analysis. Any miRNA expressed in the majority of mice in a particular group (2 out of 3) was taken to be significant in the behavioral characteristics of that group. miRNAs absent from all groups were discarded. All miRNA expression levels left after this filter were re-scrambled along with their respective subjects. A detailed clarification can be seen in Figure 2.3. As a quality control for this filter, a separate analysis was conducted by another researcher completely blind to the study. All 647 miRNAs in the mouse genome were initially kept with no filters being used.

A mass Correlation Z-Test was performed between each miRNA and the ADE behavioral data in a program known as Statview. miRNAs exhibiting a strong correlation in this gross test ($p<0.05$) underwent individual linear regression. The correlation coefficient r^2 and p-value were compared from the mass and individualized tests as a quality control.

These miRNA expression levels in each mouse were grouped, in Figure 2.3, according to its phenotype in the alcohol deprivation effect procedure – Negative ADE or Positive ADE. An F-test was conducted to comment on the variances between the two groups followed by the appropriate two-group t-Test for significance.

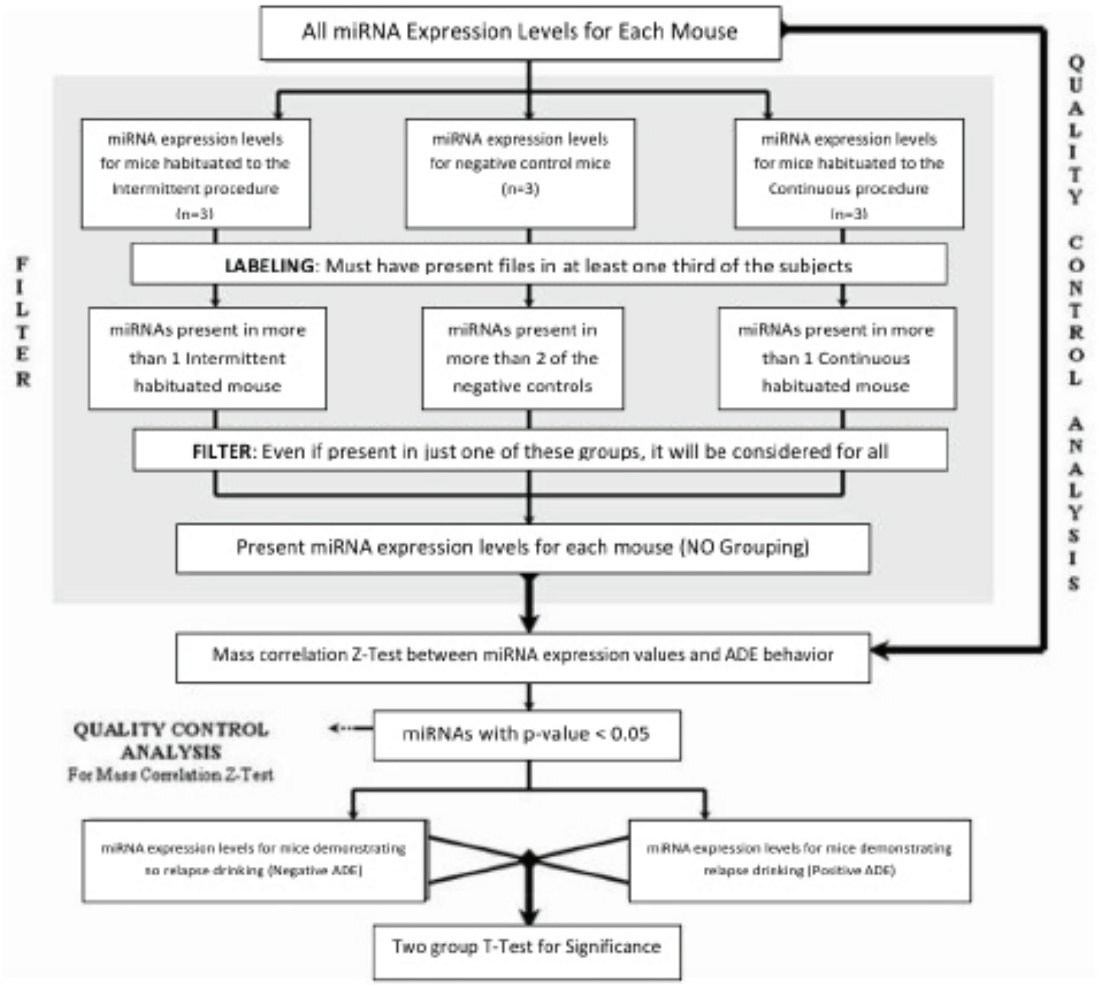

Figure 2.3: miRNA Methods
Starting with the miRNA expression array data, this figure displays the various filters used and analysis conducted with respect to the alcohol deprivation effect.

Results

3.1 Intermittent vs. Continuous

3.1.1 Negative controls. The three lowest drinkers in the intermittent procedure and the three lowest drinkers in the continuous procedure were compared to validate grouping them together as negative controls. An F-Test for variances was followed by a t-Test for significance. Results are as follows:
An F-Test indicated that the variances between the two groups were not significantly different ($F = 1.649$, $p = 0.378$). The mean ethanol intake of the low intermittent mice ($M = 0.026$, $SD = 0.002$, $N = 3$) was not significantly different than that of the low continuous mice ($M = 0.024$, $SD = 0.001$, $N = 3$) using the t-Test assuming equal variances ($t(4) = 1.989$, $p = 0.118$). In short, as is demonstrated in Figure 3.1, there was not a significant amount of difference between mice that were not habituated to their respective procedures, as was expected.

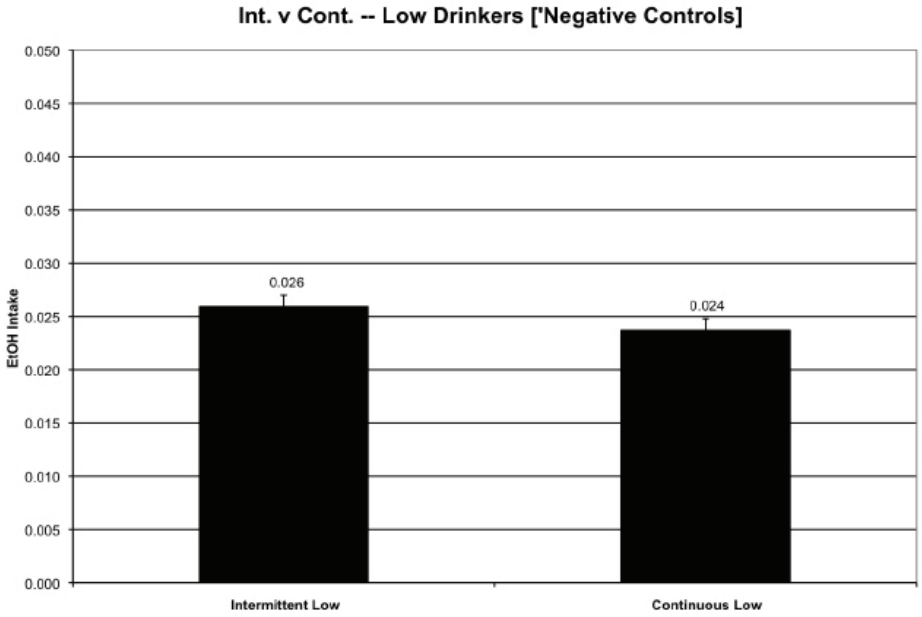

Figure 3.1: Negative Controls. The histogram of the six mice not habituated to any procedure shows no significant difference. This is further supported using a two-group T-test [t(4)=1.99, p=0.01].

3.1.2 Negative controls vs. habituated mice. The six high drinkers from both groups were grouped together and compared against the negative controls in an effort to prove that habituation to any procedure would lead to higher ethanol consumption. Results, summarized in Figure 3.2, are as follows:

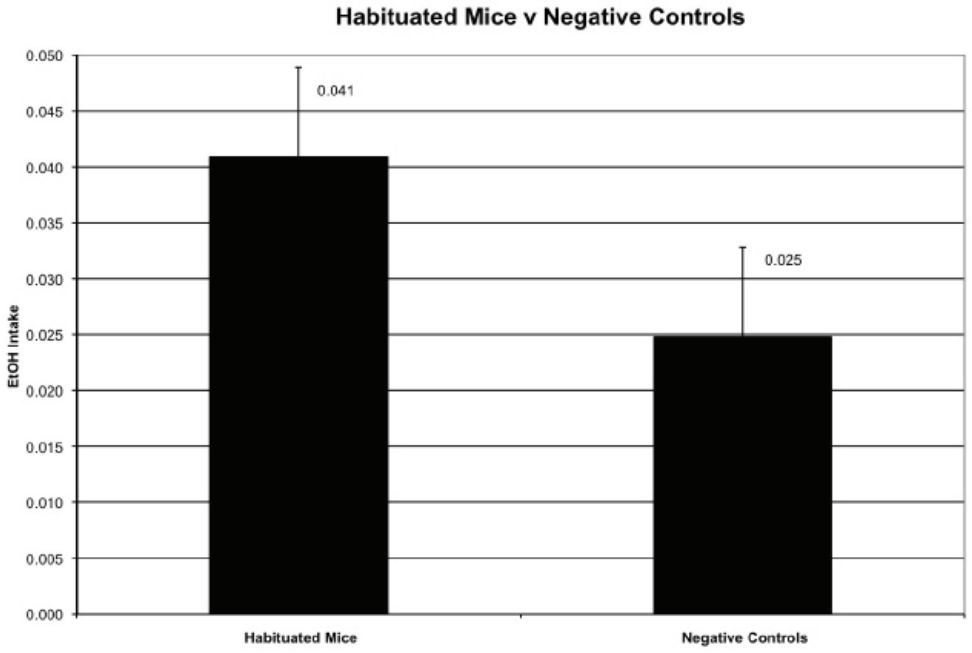

Figure 3.2: Habituated Mice v Negative Controls. Mice habituated to either of the procedures show a significantly higher ethanol intake average than negative controls. This is further supported using a two-group T-test [t(7)=8.52, p=6.1e-5].

An F-Test indicated that the variances between the two groups were significantly different (F = 6.322, p = 0.032). The mean ethanol intake of the habituated mice (M = 0.0409, SD = 0.0043, N = 6) was significantly different than that of the low continuous mice (M = 0.0248, SD = 0.0017, N = 6) using the t-Test assuming equal variances (t(7) = 8.523, p = 6.1e-5). Habituation to either of the two procedures induced higher EtOH intake. Both the continuous and the intermittent procedure both induce some level of conditioning. Thus, this result is well explained.

3.1.3 Habituated mice in differing procedures. Mice habituated to the intermittent procedure are shown in prior studies to have greater ethanol intake. The validity of this result was again retested by conducting an analysis similar to above. The results are as follows:

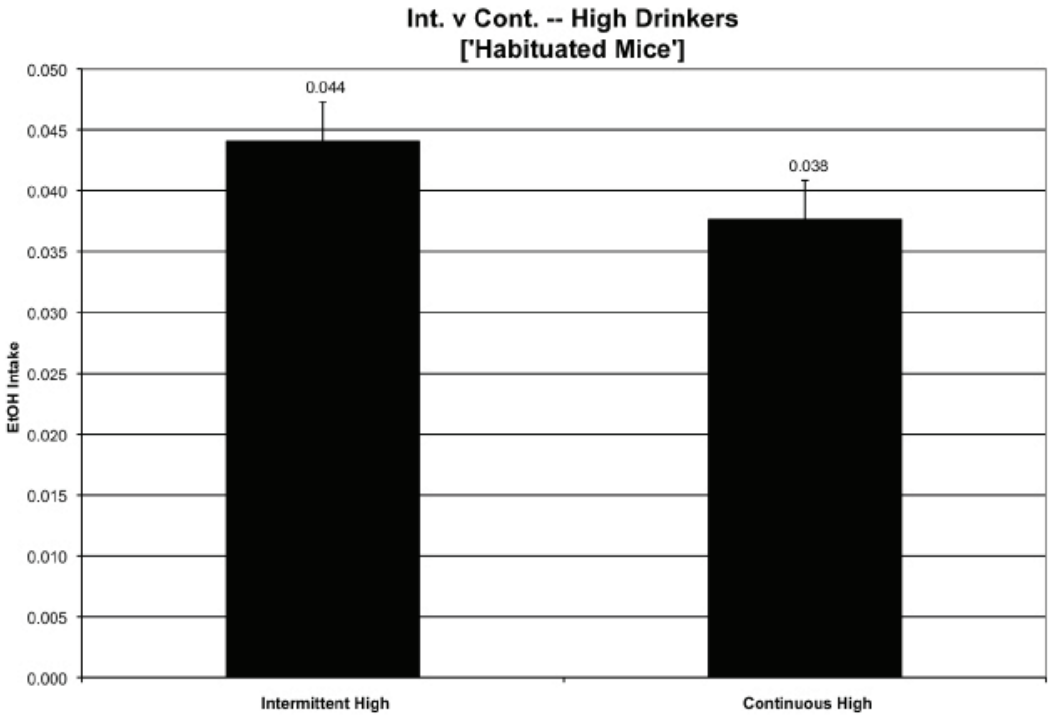

Figure 3.3: Habituated Mice. Mice habituated to the intermittent procedure show a significantly higher ethanol intake average than those habituated to the continuous procedure. This is further supported using a two-group T-test t(4)=2.88, p=0.04].

An F-test indicated that the variances within the two groups were not significantly different (F = 0.136, p = 0.119). The mean ethanol intake score of mice habituated to the intermittent procedure (M = 0.044, SD = 0.001, N = 3) was significantly higher as compared to those habituated to the continuous procedure (M = 0.038, SD = 0.004, N = 3) using the t-Test assuming equal variances (t(4) = 2.881, p = 0.04).

In otherwise identical mice, habituation to the intermittent procedure did indeed induce more ethanol intake than habituation to the continuous procedure – proving that the intermittent procedure was indeed more effective in conditioning. A graphical proof can be seen in Figure 3.3.

3.2 Alcohol Deprivation Effect

5 subjects exhibited ADE scores < 0.5 – expectancy higher than relapse – and were categorized into the Negative ADE group. 7 subjects exhibited ADE scores > 0.5 – relapse higher than expectancy – and were categorized into the Positive ADE group. The histogram in Figure 3.4 shows this differentiation. Results of an F-test and t-Test are as follows:

An F-test conducted indicated that the variances within the two groups were not significantly different ($F = 1.247$, $p = 0.384$). The mean score of the Negative ADE group ($M = 0.158$, $SD = 0.021$, $N = 5$) was significantly different than that of the Positive ADE group ($M = 0.707$, $SD = 0.017$, $N = 7$) using the t-Test assuming equal variances ($t(10) = -6.87$, $p = 4.3e\text{-}5$).

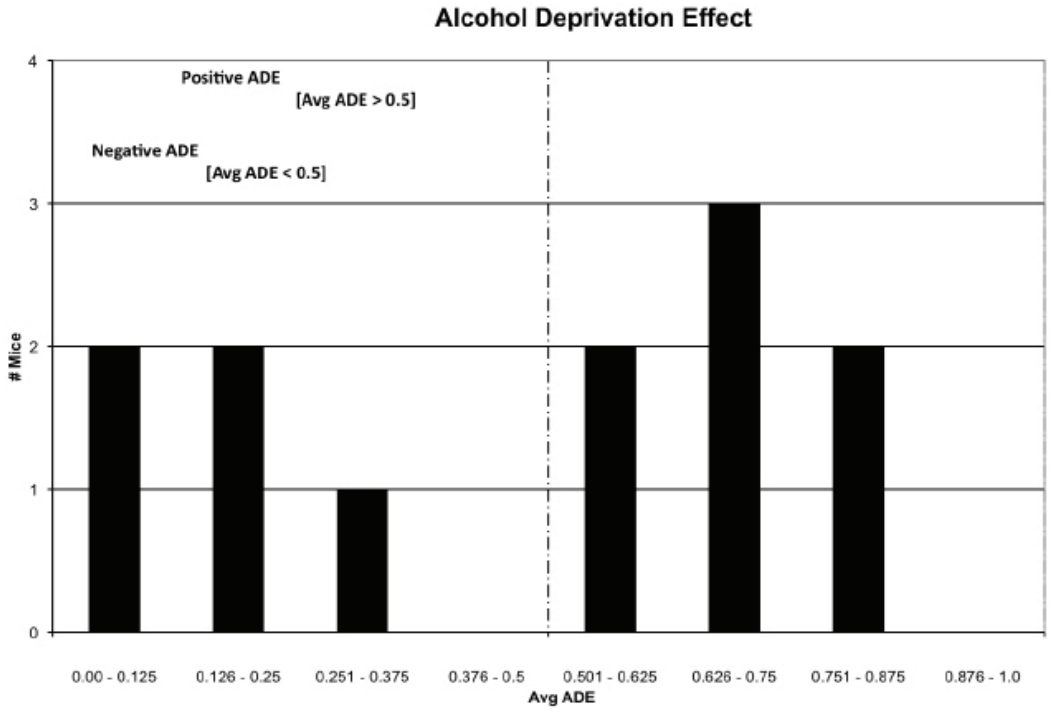

Figure 3.4: ADE Histogram. The histogram shows a stark differentiation between mice without relapse drinking (Negative ADE) and those with relapse drinking (Positive ADE). This is further supported by a two-group T-test [$t(10)=6.87$, $p=4.3e\text{-}5$].

The two groups significantly differed in their ability to demonstrate a relapse in ethanol consumption after a brief withdrawal period. The relapse drinking model was successful, at least in some mice, in inducing and quantifiably measuring ADE.

3.3 Influence of Intermittent Availability on the Alcohol Deprivation Effect

It was hypothesized that since intermittent availability induced higher drinking, it might also be the key in inducing the alcohol deprivation effect. A chi square test was conducted to comment on the preference of mice habituated to the intermittent procedure towards either of the ADE phenotypes. Results are as follows:

Negative controls showed no preference of exhibiting Positive ADE vs. Negative ADE using a chi-square test ($X^2(1) = 0.67$, $p = 0.414$). Mice habituated to the intermittent procedure showed no preference of exhibiting either of the two ADE phenotypes using a chi-square test ($X^2(1) = 0.33$, $p = 0.564$).

There was no preference of the negative controls, proving that there was no external bias variable causing the effect. However, contrary to the hypothesis, the intermittent procedure also had no influence on the alcohol deprivation effect.

3.4 MicroRNAs

Out of the 647 miRNAs in the mouse genome, 393 were found to be prevalent in at least one of the three groups – intermittent, continuous or negative controls. A mass Correlation Z-Test produced a list of 24 miRNAs that were significantly correlated to the ADE behavioral data ($p<0.05$). Each of these 24 miRNAs underwent linear regression in Excel with the same r^2 and p-value results within 2 decimal places. MicroRNAs with significant values from this regression test were interpreted as being directly correlated with the intensity of the ADE. Identically similar results were received from the quality control analysis of all 647 miRNAs with no filters. Thus, the filtering was appropriate and properly conducted.

Micro RNA Results

miRNA	Linear Regression		Grouping					F-Test for Variances		Two Group t-Test			
	Correlation R^2	p-Value	Negative ADE Mean	Negative ADE Std. Dev	Positive ADE Mean	Positive ADE Std. Dev	Higher Expression in:	F-Value	p(one-tail)	Variance	t Stat	df	p(two-tail)
mmu-miR-666-3p	0.489	0.008	8.56	0.41	10.28	1.09	Positive ADE	0.141	0.04	Unequal	-3.824	8	0.005
mmu-miR-705	0.439	0.011	12.91	1.21	14.93	1.25	Positive ADE	0.925	0.49	Equal	-2.800	10	0.019
mmu-miR-17	0.426	0.013	13.04	1.60	15.43	1.87	Positive ADE	0.737	0.40	Equal	-2.316	10	0.043
mmu-miR-425*	0.419	0.014	2.00	0.81	3.74	0.70	Positive ADE	1.336	0.36	Equal	-3.959	10	0.003
mmu-miR-680	0.401	0.016	3.78	1.92	6.40	1.21	Positive ADE	2.524	0.15	Equal	-2.925	10	0.015
mmu-miR-455	0.382	0.019	22.65	2.32	26.32	1.20	Positive ADE	3.765	0.07	Equal	-3.612	10	0.005
mmu-miR-540-3p	0.349	0.025	4.65	1.55	7.13	0.69	Positive ADE	5.081	0.04	Unequal	-3.354	5	0.020
mmu-miR-106a	0.347	0.026	9.24	2.25	12.26	1.08	Positive ADE	4.298	0.06	Equal	-3.130	10	0.011
mmu-miR-17*	0.335	0.028	13.68	1.23	15.80	1.42	Positive ADE	0.751	0.41	Equal	-2.682	10	0.023
mmu-miR-320	0.298	0.039	50.26	4.70	58.46	4.64	Positive ADE	1.026	0.46	Equal	-3.001	10	0.013
mmu-miR-671-5p	0.288	0.042	11.75	2.94	15.47	0.97	Positive ADE	9.106	0.01	Unequal	-2.725	5	0.042
mmu-miR-672	0.288	0.042	80.15	3.63	88.89	7.27	Positive ADE	0.249	0.10	Equal	-2.455	10	0.034
mmu-miR-223	0.283	0.044	116.44	10.96	89.40	22.41	Negative ADE	0.239	0.09	Equal	2.470	10	0.033
mmu-miR-338-5p	0.274	0.047	22.59	2.35	26.73	3.11	Positive ADE	0.571	0.31	Equal	-2.496	10	0.032

The expression level of each miRNA in the Positive ADE group was analyzed against that in Negative ADE group for possible significance. An F-test and t-Test were conducted to identify miRNAs that might have a strong influence on the alcohol deprivation effect. MicroRNAs with significant values from this t-test were interpreted as being influential in determining whether or not ADE would occur. Results are summarized in Table 1 and an explanation is as follows: A higher correlation coefficient (R^2) in the first column 'Linear Regression' means that higher regulation of a miRNA would lead to the phenotype with a higher mean expression for it – as given by the 'Grouping' column. The t-stat from the 'Two Group t-Test' column in accordance with the degrees of freedom provides insight of how influential the miRNA is in terms of actually displaying a particular phenotype. A negative t-stat score means that the subject with high expression of this miRNA is likely to display a Positive ADE while a positive t-stat subject is likely to display a Negative ADE.

The fourteen miRNAs listed are interesting candidates because they are significant ($p<0.05$) in both, determining whether a mouse would display relapse drinking as well as the intensity with which it will be displayed. miRNA 'mmu-miR-666-3p' is particularly interesting because it is extremely significant in both types of analysis ($p<0.01$). miRNA 'mmu-miR-223' is particularly interesting because it is the only miRNA, out of all those significant, that has higher expression levels for the Negative ADE group.

Discussion

4.1 Influence of Effective Conditioning on Higher Alcohol Consumption

Mice not habituated to any procedure, intermittent or continuous, showed no difference amongst them and, on average, demonstrated a significantly lower ethanol intake than habituated mice. This provides evidence that there are no innate factors, such as a penchant for the taste of alcohol, that cause mice to exhibit the high drinker vs. low drinker phenotypes. The degree of habituation to the behavioral procedures administered, or rather the degree to which the conditioned stimulus, lever, serves as an expectancy signal for the unconditioned stimulus, alcohol, is most influential in determining the amount of relapse drinking.

Amongst the two procedures, the insertion of the sipper only and exactly at the precise instant of time when alcohol access was permitted, as in the intermittent procedure, induced significantly higher ethanol intake within just 8 minutes of alcohol access as compared to the continuous controls with a total of 50 minutes of alcohol access per trial. Thus, the intermittent sipper procedure is proven to induce more effective conditioning and is highly influential in facilitating the pairing between the conditioned stimulus, lever, and the unconditioned stimulus, alcohol. A more effective conditioning results in stronger craving for alcohol, which leads to higher voluntary consumption.

Overall, this data further supports prior studies of addiction as a conditioned behavior and reestablishes the importance of the association between the neutral and the unconditionally response-evoking stimulus with respect to influencing higher alcohol consumption. In lay terms, it provides further evidence for the hypothesis that the amount of alcohol a person drinks is directly linked to the urge he/she feels upon viewing the shot-glass, or any other object of consumption. A normal person is not very affected by its sight and even ignores it at times. However, to an addict, the simple presence of a shot-glass reminds him/her of previous encounters with alcohol and triggers certain physiological changes that lead to an incessant urge to drink.

4.2 Alcohol Deprivation Effect

4.2.1 Relapse drinking model. Alcohol Deprivation Effect is defined as greater than normal consumption of alcohol after a brief withdrawal period, due to relapse. Approximately seven out of the twelve mice illustrated this phenomenon with 5 of them drinking twice the normal amount [ADE > 0.66]. Thus, the relapse drinking model, allowing alcohol access for 5 days following a 2-day deprivation period, successfully induced the alcohol deprivation effect in the experimental mice.

4.2.2 Behavioral procedures and the Alcohol Deprivation Effect (ADE). Though slightly unexpected, the twelve mice showed a stark distinction with respect to those that displayed relapse drinking as opposed to those that did not ($p = 4.3e\text{-}5$). Such a distinction, easily seen in Figure 3.4, provides compelling evidence of one or more factors that significantly contribute to the ADE phenotype.

Mice not habituated to any procedure did not show any preference for either displaying or not displaying relapse drinking. Further, mice habituated to the intermittent procedure, though showing higher ethanol intake levels, also did not seem to show any preference. Combination of this data proves that the demonstration of an ADE phenotype is not significantly influenced by any behavioral or environmental variable, but rather is intrinsic. Thus, effective conditioning, though influencing higher alcohol consumption, is not influential in the demonstration of the alcohol deprivation effect.

This proves that even though an alcoholic is more inclined to drink at the sight of the shot-glass, he/she is not tempted to do so under all conditions. If there has been some time in between that the person has stayed away from alcohol, this urge diminishes to a point where he/she no longer displays a physiological reaction to the shot-glass. Yet the phenomenon of binge drinking is not only prevalent but a major problem amongst alcoholics. Thus, there must be an alternative explanation of relapse drinking that does not fit this conventional wisdom of Pavlovian conditioning.
Some, yet to be determined variable, caused mice, irrespective to whether they were habituated to any procedure or not, to drink higher after a brief withdrawal period. Since all other conditions were kept constant to the best of our ability, there is a strong probability that the variable might be due to a genetic difference in the out-bred CD-1 strand of mice. Similarly, it might be the individual's genome, not external factors, that make him/her more susceptible to becoming addicted. A case for this hypothesis is presented in the miRNA discussion that follows.

4.2.3 MicroRNAs and the Alcohol Deprivation Effect(ADE). After an analysis of the miRNA expression array, some miRNAs were determined to be directly correlated with the intensity of the ADE and some of them to be influential in whether an ADE phenotype would occur. The list of 14 miRNAs showing significance in both aspects serves as a critical step in understanding addiction at a molecular level. We take full acknowledgement that these results are simply statistical hopefuls, but strongly believe future experimentation on them is well warranted. Especially since the study has led to the conclusion that a person's genetic makeup, regardless of the circumstances, induce addiction; the findings of these miRNAs is well supported. Furthermore, it is far easier to target and alter miRNAs than it is to alter behaviors; making an effective cure for alcohol addicts all the more likely.

4.3 Correlation to Humans

This study reinforces prior hypotheses that people drinking only at social gatherings or weekends tend to show higher consumption than people drinking on a regular basis. It proposes that such a counter-intuitive difference exists because the simultaneous pairing is more effective in such cases and leads to a facilitation of conditioning to the alcohol stimulus. Whether the conditioning is with friends, events or simply the 'high', any naturally (unconditioned) pleasurable situations during the time of alcohol consumption can rapidly become paired with the alcohol itself. Next time the person is placed into a similar situation; there is a strong craving for alcohol.

All too often it has been shown that many people are incapable of quitting and, even after a long commitment towards withdrawal, will binge drink at the next available opportunity to consume alcohol. This is not due to any deficit in willpower, but rather a demonstration of the strength of neurological connections formed during conditioning. The result is a great deal of alcohol consumption – a relapse.

Contrary to the popular misconception, this neurologically automated relapse was disproved as stemming from the amount of alcohol drank prior to attempting withdrawal. Though intermittent drinking would cause a person to consume lot more alcohol, it does not serve as the determining factor for exhibiting relapse.

Instead, this study provides compelling evidence that such addiction might be related to particular miRNAs. Future experimentation is necessary before any conclusions can be put forth.

4.4 Limitations to the Study

The study has had numerous limitations and, thus, future experimentation is advised throughout the paper before taking any of the statements conclusively. One of the major limitations is the use of mice as subjects. Though the Relapse Model was overly successful in mice, humans represent a completely different species and might react differently under similar conditions – though it is highly unlikely due to the similarities in our genomes and those of a mouse. Secondly, the 14 miRNAs listed are simply derived from statistical testing without any in vitro or in vivo backing. None of the miRNAs, or any of the genes they control, have been individually tested, using knockouts or other procedures, to determine their specific consequences on the phenomenon of addiction. Though these limitations might seem to make the conclusions less believable, any hypothesis in the paper is proposed only after meticulous and repetitive examination and genuinely reflects the validity of the data obtained from the study.

4.5 Future

From prior experiments and this study, it has been clearly shown that intermittent availability of alcohol induces higher consumption. However, it has been proven that this has no significant influence on relapse drinking and the alcohol deprivation effect. Since, even in otherwise identical conditions, a stark distinction was obtained, a genetically centered hypothesis of addiction seems to be reasonable. Further experimentation will be conducted on some of the genes controlled by the 14 miRNAs in an attempt to identify specific candidates contributing to this relapse drinking phenomenon.

An approach that the authors of the paper want to attempt is the creation of knockouts. By creating a knockout mouse, it becomes possible to switch off one particular miRNA and analyze its effect on behavior. There are two major advantages due to such specificity. First, it increases the confidence in the hypothesis that genetic factors, rather than the environment, contribute to addictive behavior. Second, it details the mechanisms of each miRNA; leading to a greater understanding of the phenomenon as well as an ability to reduce its effects via gene targeting. It is hoped that targeted up/down regulation of candidate genes or their proteins might serve as a possible cure for the phenomenon of addiction as a whole.

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Autonomic Nervous System Function Compared in Women with Irritable Bowel Syndrome, Healthy Controls, and Major Depressive Disorder

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Abstract

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain or discomfort combined with alteration in bowel function. IBS symptoms are thought to be explained by altered brain-gut interactions and dysfunction of the autonomic nervous system (ANS). Other clinical populations, such as those with unipolar major depression (UMD), exhibit similar ANS dysfunction. The common comorbidity of anxiety/depression in IBS prompted us to examine parallels between the two populations. We compared ANS function under resting conditions in subjects with IBS, UMD, and healthy controls. We proposed that (1) IBS patients will show increased sympathetic and decreased parasympathetic activity compared to the healthy controls and (2) ANS measures would be similar in the two patient groups. Results indicated that IBS and UMD patients showed higher levels of sympathetic and lower levels of parasympathetic activity compared to the healthy controls.

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain or discomfort combined with alteration in bowel function. IBS is thought to be a stress-related disorder and affects up to 15% of the US population, mostly females (Drossman et al., 2002). Currently, the exact cause of IBS is unknown. However, altered brain-gut interactions and dysfunction of the autonomic nervous system (Mayer et. al., 2006; Posserud et al., 2006) are hypothesized to contribute to the symptoms seen in IBS. The autonomic nervous system (ANS) regulates the gastrointestinal (GI) system through its three divisions, the sympathetic, parasympathetic and enteric branches. Direct control of the GI system by the enteric system is regulated via communication with the sympathetic and parasympathetic nervous systems.

The autonomic nervous system is a key regulator of stress-response. It is responsible for the body's involuntary and automatic response to stress. Sympathetic activity is activated during times of stress when it helps mediate arousal, activation, and energy mobilization. Parasympathetic activity promotes growth, energy storage and homeostasis, restoring stability to the body's internal environment. The ANS plays a central role in the pathophysiology of stress-related disease. When a body experiences stress, sympathetic nervous system responses are activated and homeostasis is disrupted. Eventually the body will seek to restore homeostasis through allostasis, inducing physiological or behavioral changes via the regulatory mechanisms of the body. Alteration of the hypothalamic-pituitary-adrenal axis hormones and the autonomic nervous system are common in restoring homeostasis. These changes may be adaptive during acute stress, however maintenance of allostatic changes over a long period may result in allostatic load contributing to the exhaustion and the wear and tear of the body.

Thayer et al. (2006) hypothesized that the ANS plays a role in a wide range of somatic and mental diseases. Modulation of the ANS in response to stress includes increased sympathetic nervous system (SNS) activity and decreased parasympathetic nervous system (PNS) activity. Heart rate variability (HRV) and skin conductance (SC) are commonly used to measure ANS function. Skin conductance is a measure of stress response and monitors changes in the electrical properties of the skin associated with sweat gland activity. Heart rate variability is a measure of the beat-to-beat variations in heart rate and an indicator of regulation of autonomic activity. Reduced HRV has been used as a marker of reduced vagal activity. Vagal activity refers to the parasympathetic innervation of the heart mediated by the vagus nerve. Using a model of neurovisceral integration, Thayer (2006) described how the ANS and parasympathetic nervous system may be associated with regulation of allostatic systems. Decreased vagal function is associated with increased allostatic load and poor health, therefore, vagal activity plays an important inhibitory function in the regulation of allostatic systems (Thayer et al., 2006).

Although stress and emotional regulation are hypothesized to be relevant components in IBS there have been a few studies examining the body's physiological response to stress by measuring ANS output. Tillisch et al. (2005) tested the differences in ANS function during rest and during a visceral stressor (rectosigmoid balloon distension) in IBS patients and healthy controls. IBS patients showed a greater SC response to visceral distension than the controls and reduced high frequency HRV across both conditions. High frequency HRV is a measure of the parasympathetic nervous system. Tillisch et al. concluded that IBS patients have altered autonomic responsiveness to a visceral stressor, with increased sympathetic and decreased parasympathetic activity.

Reduced high frequency HRV associated with increased general sympathetic activity has also been described in patients with unipolar major depression (UMD). Davydov et al. (2007) studied differences in baroreflex sensitivity, heart rate, heart rate variability (HRV), and systolic blood pressure under resting conditions in a sample of subjects with UMD who were taking antidepressant medications and who were in partial remission and compared them with a sample of healthy controls. UMD patients showed higher general sympathetic activity (higher systolic blood pressure and more low frequency HRV) and lower parasympathetic-related activity (higher heart rate and reduced high frequency HRV). Conclusions were that ANS mechanisms may be impaired in patients with depression and may contribute to their increased cardiac risk.

One study related depression to IBS by examining the ANS function in IBS patients with comorbid anxiety/depression disorders. Jarrett et al. (2002) compared women with IBS who had a history of anxiety or depressive symptoms to those without either disorder. Indicators of cardiac parasympathetic activity, autonomic nervous system balance, and general autonomic activity were examined. Among women with IBS, those with a positive history of anxiety or depression had lower parasympathetic activity than those

without a previous history of anxiety or depression. Similar differences were seen in the women without IBS. Jarrett et al. concluded that a history of anxiety and depressive disorders is associated with lower parasympathetic activity, both in women with IBS and controls.

In this current study, the primary focus was to look at ANS function in patients with IBS. Our aim was to test differences in ANS function under resting conditions between subjects diagnosed with IBS to subjects diagnosed with UMD and a group of healthy controls. Because of previously noted findings on ANS activity in IBS patients, depressed patients, and IBS patients with comorbid anxiety/depression disorders, we hypothesized that (1) IBS patients will have increased SNS and decreased PNS activity compared to the healthy controls (reduced high frequency HRV and higher systolic blood pressure and heart rate) and (2) ANS measures would be similar in the two patient groups. Based on these hypotheses we examined autonomic function at baseline in female IBS patients and compared them to baseline measures in female depressed patients and healthy controls. Psychological assessments for anxiety were also compared in the three subject groups.

Methods

Participants

There were three subject samples used in this study: 16 subjects diagnosed with irritable bowel syndrome (IBS), 17 with major depressive disorder, and 17 healthy controls, all female. The groups did not differ significantly in age. Data for the IBS subject sample were collected from an ongoing study of irritable bowel syndrome, stress response, and Traditional Chinese Medicine. The subjects were recruited by flyers and newspaper advertisements. Age ranged from 18-59 (mean=35.1) years, with 13 Caucasian, one Pacific Islander, one Hispanic, and one Asian American participants in this study.

Data for the depressed subject sample were collected from a previous study of yoga as a complementary treatment of major depression (Shapiro et al., 2007). The diagnosis of major depressive disorder of mild to moderate severity was confirmed with the Mini-International Neuropsychiatric Interview ("MINI") (Sheehan et al., 1998). All participants were taking antidepressant medication and were in partial remission at the time of the study. Partial remission was defined by scores between 7 and 18 on the 17-item Hamilton Depression Scale (HAM-D17; Hamilton, 1967). Age ranged from 20-50 (mean=33.9) years, with 14 Caucasian and three Asian Americans.

The data for the healthy controls were obtained from a previous study of a sample of 220 healthy subjects (Goldstein et al., 2008). Age ranged from 22-50 (mean=34.2) years, with 14 Caucasian and three Asian participants in this study. Healthy subjects were screened to exclude individuals with any psychiatric disorder. All subjects in the three subject groups were screened to exclude a history of heart disease, diabetes, or drug use likely to affect cardiovascular functions.

Psychological Measures

In all the samples, during the screening visit, information was collected on health history and demographic characteristics, and subjects completed personality scales. The scale common to all subjects was The Spielberger Trait Anxiety Inventory (STAI), measuring the general disposition to experience anxiety frequently (Spielberger, 1979). The STAI scores were compared between the three subject groups. The Hospital Anxiety Depression (HAD), a reliable instrument for detecting states associated with depression and anxiety (Zigmond et al., 1983) was also administered to the IBS subjects. A within-subjects comparison of the HAD scores was conducted in the IBS group.

Physiological Measures

The Biopac System (Goleta, CA) hardware was used to obtain physiological data from subjects under resting conditions in the laboratory. Continuous surface electrocardiography (ECG) was recorded to confirm the sinus origin of the beats. ECG electrodes were placed on the right wrist and two inches above the left ankle of the subject. Beat-to-beat arterial blood pressure (BP) was estimated using the Finapres (Ohmeda, CO, USA). After attachment and calibration of the transducers, the baseline data were recorded for 20 minutes in depressed and healthy subjects and 10 minutes in IBS subjects in a resting condition.

Heart rate variability was measured in three commonly standardized frequency bands: very low-frequency (VLF) power (0.0075-0.075 Hz), low-frequency (LF) power (0.075-0.125 Hz), and high-frequency (HF) power (0.125-0.50 Hz). The fractional very low-, low-, and high-frequency powers were calculated by dividing the individual values by the total power after log transformations. The following HRV ratio calculations were made: the ratio of low-frequency to high-frequency power after log transformation is represented as Low/High ratio. The ratio of low-frequency to total frequency power after log transformation is represented as Low/Total where total frequency is a sum of VLF, LF and HF power. The ratio of high-frequency to total power after log transformation is represented as High/Total where total frequency is a sum of VLF, LF and HF. High-frequency HRV is a measure of respiratory sinus arrhythmia, indicative of parasympathetic control of the heart (vagal tone). Low-frequency HRV is indicative of a mixture of parasympathetic and sympathetic control of the heart (Davydov et al., 2007).

Measures of baroreflex sensitivity (BRS) have also been shown to be useful indicators of vagal function. The baroreflex changes heart rate and total peripheral resistance to help regulate and maintain a stable blood pressure. BRS signifies how the autonomic nervous system adapts to changes in blood pressure (BP) by measuring the slope of the change in the cardiac inter-beat interval. A measure of baroreflex sensitivity (BRS) was obtained by the "sequence" method (Di Rienzo et al, 2001a). Using the Biopac software, RR-interval (time between beats) and systolic pressure time series were scanned to identify sequences during which both RR-interval and associated systolic pressure increased or decreased over a successive sequence of three or more beats. A minimum change of 3ms for the RR-interval and 1 mm Hg for systolic pressure per beat was used to calculate the mean of the least square linear regression slopes (BRS, ms/mm HG) for up (+) or down (-) BP sequences. This value represented by SLOPET signified the total BRS value averaging the slopes in both up and down sequences.

Data analysis

The independent variable in this study was experimental group (3 levels). Age was used as a covariate. The dependent variables in this study were measures of autonomic nervous system (ANS) functions. Mean heart rate (HR) and mean blood pressure (BP) for the baseline period as well as indices of heart rate variability (HRV) and baroreflex sensitivity (BRS) were examined. Systat (v. 10.2) software was used to analyze the data. Analysis of variance (ANOVA) was used to test for differences between the three experimental groups. Follow-up pairwise comparisons of the three groups with the Scheffe correction were also conducted.

Results

A significant main effect of experimental group on heart rate ($p=0.016$) and mean systolic blood pressure ($p=0.043$) was revealed. The IBS subject group exhibited the highest values for heart rate ($M=75.2$, $SE=2.4$) and systolic blood pressure ($M=125.0$, $SE=4.7$) at baseline. Figure 1 shows the means and standard error (SE) of heart rate and systolic blood pressure for the three subject groups.

Table 1: Means and standard error (SE) of dependent physiological measures for the subject groups.

Variables	Healthy	IBS	Depressed	*P*
HR (bpm)	65.3 (2.30) [a]	75.2 (2.38) [b]	71.2 (2.30) [a]	0.016
SBP (mmHg)	108.1 (4.58) [a]	125.0 (4.72) [b]	18.3 (4.58) [a]	0.043
Low/High	0.800 (0.038) [a]	1.106 (0.040) [b]	1.102 (0.038) [b]	<0.001
Low/Total	0.299 (0.008) [a]	0.342 (0.008) [b]	0.339 (0.008) [b]	<0.001
High/Total	0.376 (0.006) [a]	0.314 (0.007) [b]	0.310 (0.006) [b]	<0.001

Note: Groups with different superscripts were significantly different ($p < 0.05$) (Scheffe's test).

HR = heart rate (bpm); SBP = systolic blood pressure (mmHg); Low/High = ratio of low to high frequency HRV; Low/Total = ratio of low frequency to total power HRV; High/Total = ratio of high frequency to total power HRV

Effects of experimental group on the heart rate variability (HRV) measures were also significant ($p<0.001$). At baseline healthy controls exhibited the lowest sympathetic activity while the patient groups exhibited higher levels of sympathetic activity as indicated by their reduced high frequency HRV ratios and elevated low frequency HRV ratios. High/total frequency power ratios were similar between the IBS group (M=0.314, SE=0.007) and depressed group (M=0.310, SE=0.006) and generally reduced in comparison to the controls (M=0.376, SE=0.006). Low/total and low/high frequency power ratios were elevated in the two patients groups compared to the controls indicating higher levels of sympathetic activity. Figure 2 shows the means and standard error (SE) of the HRV measures for the three subject groups.

Overall comparison of the three groups shows that subjects with IBS and with Depression were most similar in their values for the physiological measures of the autonomic nervous system. In comparison to the healthy controls, IBS subjects and depressed subjects yielded higher sympathetic nervous system

Figure 1

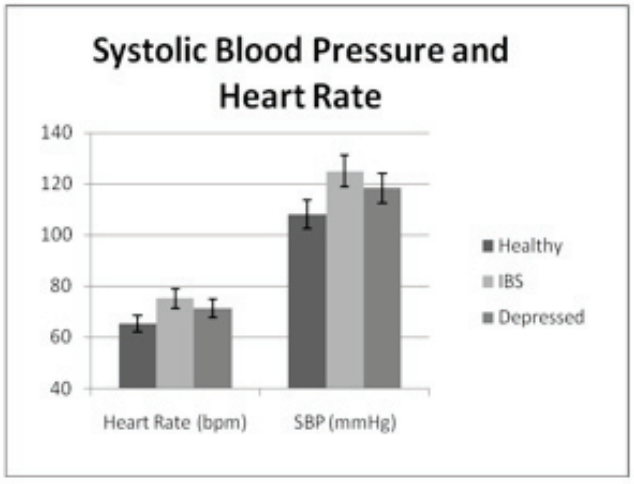

Figure 1. Means and standard error (SE) of heart rate and systolic blood pressure for all the subject groups.

Figure 2

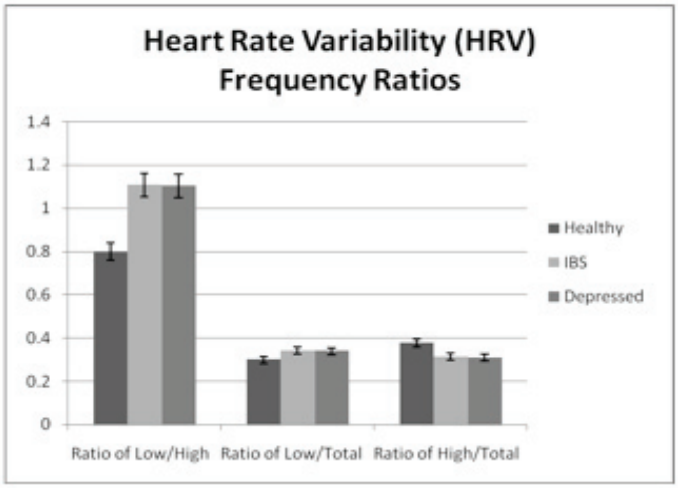

Figure 2. Means and standard error (SE) of heart rate variability measures for all the subject groups.

activity as seen by higher mean systolic blood pressure (SBP) values, higher LF/HF power ratios, higher LF/total power ratios, and lower parasympathetic nervous system activity as seen by the lower HF/total power ratios. Table 1 summarizes the findings for the baseline physiological differences in the three subject groups.

Discussion

This study demonstrated evidence of decreased parasympathetic tone and increased sympathetic activity in a sample of IBS female patients and related results to similar findings in a sample of patients with UMD. Compared to healthy controls, both patient samples exhibited elevated heart rate, systolic blood pressure, and sympathetic nervous system activity (ratio of low to high frequency power HRV) and reduced vagal tone (high frequency HRV). These findings are consistent with the general findings of decreased parasympathetic and increased sympathetic activity among patients with IBS (Tillisch et al., 2005) and decreased parasympathetic activity in patients with UMD (Davydov et al., 2007) and co-morbid anxiety/ depression disorders in controls and IBS patients (Jarrett et al., 2003).

The depressed patients included in this study were taking antidepressant medications and medications taken by the IBS patients were not stringently controlled for although blood pressure and pain medications were screened for and were exclusion criteria for the study. Three of the subjects in the IBS sample had abnormally high blood pressure readings on the day of the lab visit.

Our study expands on previous literature by explicitly examining and measuring autonomic function in both clinical groups of depression and IBS and in healthy controls. In addition to finding reduced parasympathetic activity in the patient groups as indicated by the reduced high frequency HRV ratio, we also found a difference in ANS balance between the patient groups and healthy controls as indicated by the low to high frequency HRV ratio comparisons. Implications of this research suggest that parallels between these two clinical populations exist and that altered emotional states such as depression can affect ANS balance. Baseline measures of autonomic function in the clinical groups could be valuable predictors of stress response. Further research is needed to understand the relationship of ANS imbalance and HRV to specific gastrointestinal symptoms seen in IBS. These further explorations would be useful in understanding the pathophysiology and potential treatments of IBS as well as depression. The parallel findings in the two clinical populations suggest that altered emotional states, such as depression, affects ANS balance and that treatments designed to treat autonomic imbalance be further developed for IBS.

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Determining the Link Between Temperature Perception Mechanisms and Feelings of Social Connection

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Abstract

Phrases such as "warm the heart" and "icy stare" suggest a relationship between temperature perception mechanisms and feelings of social connectedness or rejection. We examined the effect of temperature on the accessibility of, and memory for, inclusion ("love," "hug") and exclusion ("reject," "abandon") words. Sixty-five undergraduates were randomly assigned to wear a warm, cold, or neutral vest and then completed a reaction time task and a surprise memory recognition test. Results revealed a significant temperature by word type interaction on the reaction time task and the memory task. Specifically, participants wearing the cold, compared to the neutral, vest were quicker to detect rejection words, while participants wearing the warm, compared to the neutral, vest showed increased memory for social inclusion words. This implies a difference in the mental processing of 'warm' and 'cold' emotions and a possible coping mechanism against 'cold' memories.

Introduction

People use several temperature-related words and phrases to describe experiences of feeling socially connected or socially disconnected from others. Typical phrases that use temperature-related words to describe emotions include "warm the heart," "in the heat of the moment," and "left out in the cold." Additionally, 'warm' and 'cold' can serve as important descriptors of personality when labeling individuals (Asch, 1946). The question is whether the emotions that are evoked by these and other phrases literally feel warm or cold to people. It is unclear whether these phrases are simply metaphorical or developed because of connections between these social feelings and actual feelings of warmth or cold. Do certain emotions actually produce changes in temperature perception or, equally plausible, do changes in temperature perception induce certain emotions?

Solomon Asch (1946, 1958) originally suggested that abstract mental concepts might be developed from concrete physical examples after studying 'warm' and 'cold' as powerful personality traits involved in forming impressions of individuals. Asch demonstrated that students asked to form impressions about a person described by a short list of adjectives associated personality traits such as 'generous,' 'happy,' 'sociable,' 'humorous,' and 'popular' with individuals who were described as 'warm' rather than 'cold.' The pairing of temperature-related words and more abstract, complex concepts may be explained by a possible inherent predisposition to use bodily experiences to help describe and understand these more complicated notions (Lakoff, 1987). Recent studies have also suggested that the understanding of metaphors is bidirectional rather than unidirectional. For example, Zhong and Liljenquist (2006) found that people not only described criminal activity with adjectives related to cleanliness (e.g., "having a clean record"), but also felt the need to physically clean themselves after recollecting past wrongdoings. If thoughts of cleanliness can prime abstract concepts related to morality and reflections of morality can prime physical feelings of cleanliness, then it is plausible that the same bidirectional relationship would be experienced between temperature and abstract concepts of social interaction.

The development of these mental associations between the physical and psychological manifestations of an abstract concept may begin early in life, such as when an infant experiences the warmth (i.e., physical warmth) and trustworthiness (i.e., abstract concept of trustworthiness) of a parent or caregiver (Williams & Bargh, 2008). Cognitive linguistics theories suggest that internal abstract concepts are mentally visualized by utilizing comparisons to tangible objects, possibly playing a vital role in early language development (Mandler, 1992). Additionally, embodiment theories suggest that tangible objects and intangible events that produce the same or similar emotions are stored comparably in memory (Niedenthal, Halberstadt, & Innes-Ker, 1999). The expectation that a mental connection between both the literal and metaphorical forms of a concept exists is also supported by the neurobiological finding that the insular cortex is involved in the processing of both physiological and psychological 'warmth' (Meyer-Lindenberg, 2008). Eisenberger, Lieberman, and Williams (2003) similarly found that social rejection activates areas in the brain known to be involved in the regulation of physical pain. This longstanding interest in the psychological manifestations of tangible experiences prompted a recent investigation into the specific bidirectional relationship between temperature and social emotions.

Williams and Bargh (2008) tested the hypothesis that tangible experiences of warmth or cold directly influence "interpersonal warmth," or feelings of friendliness and trust that we would typically describe as 'warm' feelings. They conducted two experiments that primed participants with one of two temperature conditions (hot vs. cold) and then tested for psychological effects of the temperature manipulation. In the first experiment, each of the 41 participants was asked by a confederate to hold either a hot cup of coffee or an iced coffee on the elevator ride up to the laboratory. Participants then filled out a personality questionnaire asking them to rate an anonymous person on various personality traits. Williams and Bargh found that participants who had held the hot cup of coffee rated the anonymous person as significantly warmer than the participants who had held the iced coffee. Importantly, this effect did not carry over to ratings of traits that were not associated with the warm-cold dimension, suggesting that the results are not due to an overall improvement in mood among participants holding the hot cup of coffee. In the second experiment, each of the 53 participants was asked to hold a hot or cold therapeutic pad and rate its effectiveness, after which they were given the option of receiving compensation in the form of a gift for themselves or a gift for a friend. Participants who had held the hot pad chose the gift for a friend significantly more often than participants who had held the cold pad.

Zhong and Leonardelli (2008) conducted experiments that investigated the effect of inducing social exclusion has on feelings of coldness among participants (i.e., the direction opposite Williams and

Bargh (2008) if considering the bidirectional metaphorical relationship between temperature and social emotions). In the first experiment, each of the 65 participants was asked to recall a memory of social inclusion or exclusion (defined as feelings of acceptance and rejection, respectively) and then later asked to estimate the temperature of the room. Participants who had recalled an experience of social exclusion gave significantly lower estimates of the room temperature than the participants who had recalled an experience of social inclusion. In the second experiment, an experience of social exclusion was or was not induced by having each of the 52 participants play an online ball-tossing game that they believed was with other participants (but was actually computer-controlled). Participants then rated their preference for particular hot, neutral, or cold foods and drinks. Zhong and Leonardelli found that participants who had been socially excluded during the game expressed a stronger desire for hot foods but an equal desire for all other foods than those who had not been socially excluded.

We wanted to further validate the connection between physical and psychological warmth and cold by inducing a more substantial change in temperature and determining the effect of this temperature change on thoughts of social inclusion and exclusion. Neither Williams and Bargh (2008) nor Zhong and Leonardelli (2008) included a neutral temperature condition in their two experiments and both groups relied on self-reported dependent variables. To correct for these potential problems, we decided to include a neutral temperature condition and add a performance-based dependent variable in our experiment. Williams and Bargh were effective in eliciting positive reactions from participants who touched hot compared to cold objects and Zhong and Leonardelli had success in obtaining lower temperature estimates in those who relived a rejection compared to an inclusion experience. Accordingly, we predicted that participants in our experiment would be quicker to respond to social inclusion words when wearing a heating vest than when wearing a neutral or cooling vest and quicker to respond to social exclusion words when wearing a cooling vest than when wearing a heating or neutral vest. This is due to the fact that participants wearing the heating vest were expected to prime thoughts of social inclusion while participants wearing the cooling vest were expected to prime thoughts of social exclusion. We additionally included a brief memory recognition test to determine whether the warm, neutral, or cold vests had any remnant effects.

Method

Participants

Sixty-five female undergraduates from the University of California, Los Angeles participated in the experiment in exchange for one unit of research participation credit. Because of well-known differences in temperature preferences between males and females, we decided to only examine females in this first study to reduce variability in response to warm or cool temperatures.

Design

The experiment was a two-way, mixed-model design. The between-subjects independent variable was the temperature of the vest and the within-subjects independent variable was the types of words the participants saw on the screen. The dependent variables were the reaction times to particular words on the computer screen and the number of particular words recognized on the surprise memory test. Both of these variables were measured on a ratio scale.

Materials

Heating/cooling vests. A fleece heating vest with an internal heating device and a polyester cooling vest capable of holding four ice-packs were used to manipulate temperature perception. The exact temperature shift induced by each of the two vests was not calculated because merely a general

temperature effect was desired. An ace bandage was used to hold the vest as closely to each participant's body as possible.

MacStim computer program. A Mac computer and keyboard were used for testing via a MacStim program. The MacStim program included two similar tasks in which a new word would appear on the screen every few seconds. The first task was short and designed to be a practice task. The remaining task was approximately three minutes long and designed to gather the relevant reaction times to the various types of words. Logs kept track of reaction times to each word.

The words/non-words lists. The lists of words included non-words, which were originally real words that had either one or two letters changed (e.g., movies became 'mocies'), as well as real words. The real words included social inclusion words (e.g., 'love,' 'hug,'), social exclusion words (e.g., 'reject,' 'abandon,') and neutral words (e.g., 'table,' 'answer;' see Appendix for the full lists of words).

Recognition memory test. A paper recognition memory test that included social inclusion, social exclusion, and neutral words, some that had been present during the computer task and some that had not been, was provided to each participant to complete by hand after computer testing. The ten social inclusion words and ten social exclusion words on the memory test each included five that had been in the script and five that had not.

Procedure

Participants were tested individually between August 2008 and November 2008. Upon entering the lab, each participant was seated in a windowless room facing a Mac computer and told that they would be categorizing a series of words and nonwords while wearing a warm, cold, or neutral vest. Participants were told that we were interested in the effect of temperature perception on the ability to quickly distinguish between words and nonwords. The direction to distinguish between words and nonwords was designed to prevent participants from discovering the actual purpose of the task. While each participant read and filled out the consent form, the experimenter prepared one of the three randomly assigned temperature conditions by turning on the heating vest for the warm condition, placing the four ice-packs inside the cooling vest for the cold condition, or doing nothing to the cooling vest for the neutral condition. Although the experimenter had to be aware of the temperature of the vest (i.e., because the temperature could be felt when placing the vest on the participant), the experimenter read directions to the participants from a pre-written script to avoid changes in instruction between participants as a result of the experimenter's knowledge of the temperature condition. The appropriate vest was put on the participant while she was standing and the ace bandage was wrapped tightly around the body underneath the participant's breasts and secured tightly. After receiving the instructions, each participant completed two computer tasks that required her to distinguish each word in a series as a word or nonword as quickly as possible. The first computer task was a practice task that was completed while the experimenter watched to ensure that the participant understood instructions. Then each participant completed the second, full-length computer task alone. The experimenter reentered the room to save the reaction times after the task was completed. After finishing the computer portion of the experiment, each participant removed her vest and filled out a questionnaire containing a surprise recognition memory test. Each participant was debriefed upon completion of the experiment.

Results

Data from three participants were lost due to technical difficulties. As such, data from the remaining 62 participants were used in the analyses reported below.

Reaction Time

Figure 1 and Table 1 show the reaction times to social inclusion words and social exclusion words in the warm, neutral, and cold temperature conditions. To examine whether reaction times to social inclusion or social exclusion words differed as a function of wearing the warm, neutral, or cold vests, we conducted a repeated measures analysis of variance (ANOVA) with word type (social inclusion, social exclusion) as the within-subjects variable and condition (warm, neutral, cold) as the between-subjects variable. For ease of interpretation, reaction times to the neutral words were subtracted out of the reaction times to the social inclusion and exclusion words.

Overall main analysis. A repeated measures ANOVA on participants' reaction times indicated a significant main effect of word type, $F(1, 59) = 52.05$, $p < .001$. Specifically, participants were significantly quicker to respond to social inclusion words than social exclusion words. However, there was no significant main effect of temperature condition, $F(2, 59) = .29$, $p = .75$. There was also no significant interaction, $F(2, 59) = 1.83$, $p = .17$; however, the results suggest a trend toward an interaction. To explore this trend more closely, post-hoc repeated measures ANOVAs were conducted to determine which of the three temperature conditions differed significantly from one another.

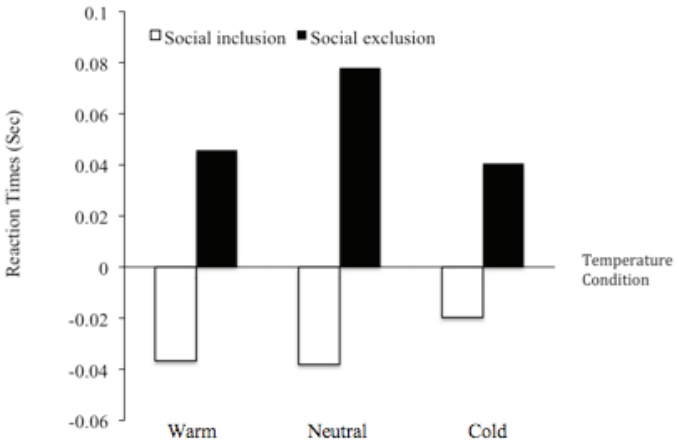

Figure 1. Reaction times to social inclusion words and social exclusion words as a function of temperature condition with the neutral baseline subtracted out.

Cold vs. neutral. A repeated measures ANOVA on participants' reaction times to social inclusion words and social exclusion words in the cold and neutral temperature conditions indicated a significant main effect of word type, $F(1, 39) = 42.07$, $p < .001$, but no significant main effect of temperature condition, $F(1, 39) = .24$, $p = .63$, replicating the findings in the main analyses. This main effect, however, was qualified by a significant interaction, $F(1, 39) = 4.23$, $p < .05$., This pattern suggested that participants were quicker to respond to social inclusion words during the neutral temperature condition compared to the cold temperature condition and were quicker to respond to social exclusion words during the cold compared to the neutral temperature condition; however, neither of these simple effects were significant on their own ($t(39) = .91$, $p = .37$, $t(39) = 1.40$, $p = .17$, respectively).

	Social inclusion		Social exclusion	
Condition	Mean	Standard Deviation	Mean	Standard Deviation
Warm	-0.04	0.08	0.05	0.10
Neutral	-0.04	0.07	0.08	0.09
Cold	-0.02	0.06	0.04	0.08

Table 1. Means and standard deviations for reaction times (sec) to social inclusion and exclusion words with the neutral baseline subtracted out.

Warm vs. neutral. A repeated measures ANOVA on participants' reaction times for social inclusion words and social exclusion words in the warm and neutral temperature conditions indicated a significant main effect of word type, $F(1, 39) = 39.01$, $p < .001$, but no significant main effect of temperature condition, $F(1,39) = .52$, $p = .48$, and no significant interaction, $F(1, 39) = 1.11$, $p < .30$.

Cold vs. warm. A repeated measures ANOVA on participants' reaction times for social inclusion words and social exclusion words in the cold and warm temperature conditions revealed a significant main effect of word type, $F(1,40) = 24.70$, $p < .001$, but no significant main effect of temperature condition, $F(1,40) = .09$, $p = .77$, and no significant interaction, $F(1,40) = .60$, $p = .44$.

Memory

Figure 2 and Table 2 show the percent of social inclusion words and social exclusion words recognized in the warm, neutral, and cold temperature conditions. A repeated measures ANOVA of the surprise recognition memory test results compared memory for social inclusion words and social exclusion words and the neutral baseline was again subtracted out for ease of interpretation. The memory results analysis included words that were not present in the task for two reasons. First, the preliminary repeated measures ANOVA of memory for words present in the computer task revealed no significant main effect of word type, $F(1,62) = .08$, $p = .78$, no significant main effect of temperature condition, $F(2, 62) = 2.10$, $p = .13$, and no significant interaction, $F(2, 62) = .94$, $p = .39$. Second, we were interested in examining whether participants would mistakenly recognize social inclusion or social exclusion words that they had not seen previously, as this would suggest that these types of cognitions had been primed by the temperature condition.

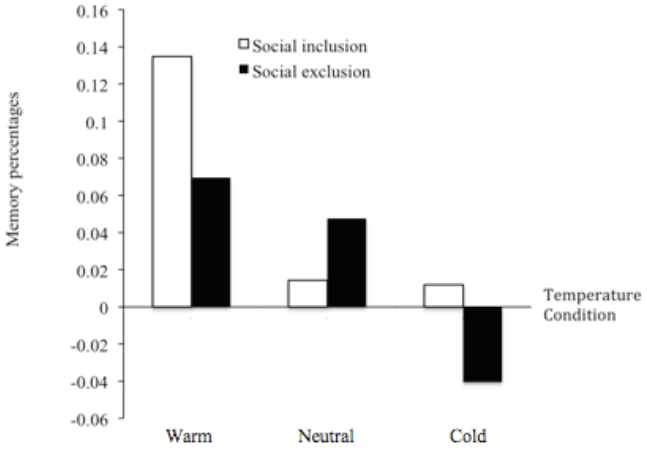

Figure 2.Percent of social inclusion words and social exclusion words recognized as a function of temperature condition with the neutral baseline subtracted out.

	Social inclusion		Social exclusion	
Condition	Mean	Standard Deviation	Mean	Standard Deviation
Warm	0.13	0.22	0.07	0.14
Neutral	0.01	0.17	0.05	0.17
Cold	0.01	0.17	-0.04	0.17

Table 2. Means and standard deviations for memory recognition (%) of social inclusion and exclusion words with neutral baseline subracted out.

Overall main analysis. A repeated measures ANOVA on memory scores indicated no significant main effect of word type, $F(1, 62) = 1.83$, $p = .18$, but a significant main effect of temperature condition, $F(2, 62) = 3.27$, $p < .05$, such that participants in the warm temperature condition remembered significantly more words than participants in the neutral or cold temperature conditions. Additionally, there was a trend toward a significant interaction, $F(2,62) = 2.21$, $p = .12$, prompting post-hoc repeated measures ANOVAs to determine which of the three temperature conditions differed significantly from each other.

Warm vs. neutral. A repeated measures ANOVA compared memory for social inclusion words and social exclusion words in the warm and neutral temperature conditions indicated no significant main effect of word type, $F(1, 42) = .34$, $p = .57$, no significant main effect of temperature condition, $F(1, 42) = 2.42$, $p = .13$, and a marginally significant interaction, $F(1, 42) = 3.20$, $p = .08$. Participants in the warm temperature conditions thought they remembered more social inclusion words than participants in the neutral temperature condition, $t(42) = 2.02$, $p = .05$, but there was no difference in the number of social exclusion words recognized, $t(42) = .48$, $p = .64$.

Cold vs. neutral. A repeated measures ANOVA for memory in the cold and neutral temperature conditions indicated no significant main effect of word type, $F(1, 40) = .14$, $p = .71$, no significant main effect of temperature condition, $F(1, 40) = 1.01$, $p = .32$, and a marginally significant interaction, $F(1, 40) = 2.81$, $p = .10$. Participants in the cold temperature condition thought they remembered fewer social exclusion words than participants in the neutral temperature condition, $t(40) = 1.70$, $p = .10$, but there was no difference in the number of social inclusion words recognized, $t(4) = .05$, $p = .96$.

Warm vs. cold. A repeated measure ANOVA for memory in the cold and warm temperature conditions indicated a significant main effect of temperature condition, $F(1, 42) = 5.83$, $p < .05$, such that participants in the warm temperature condition recognized a greater number of words than participants in the cold temperature condition. There was also a significant main effect of word type, $F(1, 42) = 6.61$, $p < .05$, indicating that social inclusion words were better recognized than social exclusion words. Finally, there was no significant interaction, $F(1, 42) = .08$, $p = .78$.

Discussion

The results for reaction times revealed that participants were quicker at responding to social exclusion words when primed with a cold temperature than when primed with a neutral temperature. Likewise, participants were quicker at responding to social inclusion words when primed with a neutral temperature than when primed with a cold temperature; however, participants were not quicker at responding to social inclusion words when primed with a warm temperature than when primed with a neutral temperature. The results for the surprise memory test reveal that participants in the warm temperature condition thought they saw more social inclusion words than participants in the neutral temperature condition but there was no difference in the number of social exclusion words recognized; however, participants in the cold temperature condition thought they saw fewer social exclusion words than participants in the neutral temperature condition but there was no difference in the number of social inclusion words recognized. These results support our hypothesis in that participants in the cold condition were quicker to respond to social exclusion words and participants in the warm temperature condition better recognized social inclusion words. Even the fact that participants in the warm condition were not any quicker to respond to social inclusion words and participants in the cold temperature condition seemingly forgot social exclusion words support our hypothesis if explained in terms of differences in mental processing between ‘warm’ and ‘cold’ emotions.

Although there were substantial differences between this experiment and the experiments performed by Williams and Bargh (2008) and Zhong and Leonardelli (2008), the results of this experiment actually support the conclusions of others quite well. Interestingly, the warm temperature condition in this experiment was found to have no impact on participants' reaction times to social inclusion words. On the other hand, these same participants remembered more social inclusion words than participants in the neutral and cold temperature conditions. Williams and Bargh achieved increased feelings of trust and friendliness for participants who had held a hot coffee or a hot therapeutic pad minutes before being tested, so they only tested the aftereffect (i.e., being tested after ending contact with a warm object) of a warm temperature condition and not the immediate effect (i.e., being in contact with a warm object and being tested at the same time). The present experiment tested both the immediate effect (i.e., reaction time task) and aftereffect (i.e., the recognition memory test) for the warm temperature condition, but only found a positive effect during the surprise memory test after the heating vest had already been removed as opposed to during the computer task when the heating vest was still being worn. Therefore, the results of this study and the results of Williams and Bargh not only confirm each other (i.e., they both found aftereffects, not immediate effects, of the warm temperature condition) but also suggest that physical and psychological warmth may be processed differently than physical and psychological cold in that warmth may be processed such that it has only a lasting (i.e., after the fact) rather than immediate effect.

The fact that a cold temperature condition decreased reaction times to social exclusion words is consistent with Zhong and Leonardelli's (2008) finding that a memory of social exclusion makes participants feel colder (and later estimate lower room temperatures). In fact, the results produced in the current study and Zhong and Leonardelli study support the hypothesized bidirectional nature of the temperature and social emotions relationship: the present experiment suggests that cold temperature priming can induce thoughts of social exclusion, while the Zhong and Leonardelli study suggests that recollections of social exclusion can induce the sensation of colder temperatures. The failure of participants in the cold temperature condition to recognize social exclusion words on the surprise memory test appears to be inconsistent with Zhong and Leonardelli's finding that a memory of social exclusion or induced social exclusion causes one to feel physically cold. However, it is possible that the reason participants did not recognize as many social exclusion words is because they naturally regulate their memories in order to avoid the negative consequences of social exclusion. In other words, the preference for not remembering social exclusion words could be a general human coping mechanism that allows us to avoid specific, painful memories of social exclusion. Therefore, physical and psychological cold are processed differently than physical and psychological warmth such that cold has both an immediate effect (i.e., faster reaction times) and an aftereffect (i.e., decrease in memory as a possible defense mechanism), whereas warmth only has an aftereffect (i.e., increase in memory). The difference in the aftereffects for warmth and cold (i.e., warmth increased memory and cold decreased memory) also suggests different mental processes for each.

The present experiment not only complemented the studies performed by Williams and Bargh (2008) and Zhong and Leonardelli (2008), but contributed substantially to the recent investigation into the relationship between temperature and social emotions. This was the first study to include a neutral (control) temperature condition when directly testing the relationship between temperature and thoughts of social inclusion and social exclusion. It lends support to the hypothesis that people are inclined to form mental relationships between simple bodily sensations (i.e., temperature) and more complex abstract concepts (i.e., acceptance and rejection). It also supports the hypothesis that the physical and psychological manifestations of both warm and cold temperatures (i.e., literal warm and cold temperatures and abstract concepts of 'warm' and 'cold,' such as a 'warm'-hearted person) are neurobiologically similar, stored comparably in memory as embodiment theories suggest (Niedenthal, Halberstadt, & Innes-Ker, 1999) and/or both processed in the insular cortex or other parts of the brain. Neurobiologically similar physical and

psychological mental representations of the same concept would be expected to affect one another when manifested in actions, thoughts, etc. Assuming that the social inclusion and social exclusion words in this experiment are highly related to psychologically abstract concepts of 'warmth' and 'cold,' respectively, the results of the current study support this relationship between the physical and psychological mental representations of the same concept (i.e., 'warmth' or 'cold'), which suggests that they are neurobiologically similar.

A couple of limitations affect the generalizability of the experiment. Only women participants were used in the experiment because it was judged that women may demonstrate heightened sensitivity to temperature conditions and we only wanted to use one gender in this first study to limit variability in responses. This leaves open the possibility that men would react differently or more strongly. Additionally, the weather in Los Angeles, California during the experiment was far from normal autumn weather. It was not uncommon for daytime temperatures to be remarkably warm (between 85 and 95 degrees), perhaps reversing the comfort levels of participants in the warm, neutral, and cold temperature conditions (i.e., it is assumed that the cold condition is inherently more uncomfortable than the warm condition, but this may not have been true on a 95 degree day). Future studies could include men and/or test participants year-round in order to combat the limitations of this experiment. Alternatively, future research could utilize alternative ways of influencing temperature perception (such as changing room temperature) or testing for thoughts of social inclusion or social exclusion (surveys, interactions with other participants, etc.). Indeed, there still remains numerous ways that the relationship between temperature and social emotions could be tested, perhaps some of which will produce weaker results (or stronger results) for reasons that have yet to be discovered.

This study is the first study to use a neutral (control) temperature condition in order to establish a connection between temperature and thoughts of social inclusion and social exclusion. The results suggest that there is a reason individuals use temperature-related words to describe emotions, people, etc. (i.e., he turned the 'cold' shoulder), as our participants responded to social inclusion and social exclusion words differently depending on the temperature condition. While a great deal of research still needs to be done to determine if temperature can influence affect and/or social interactions, or if positive or negative experiences and/or social interactions can produce changes in temperature perception, three experiments have now suggested a general link between temperature perception mechanisms and social emotions. It still remains an interesting and largely unknown reason why these two important parts of life would be interwoven, but the results imply that temperature can affect one's emotions. This leaves an array of related future research topics to investigate.

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Beyond Machos and Jotos:
Latino Male Perceptions of Masculinity, Contact and Attitudes toward Male Homosexuality

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Abstract

Do heterosexual and gay Latino men differ in their attitudes toward gender roles and homosexuality? A study comparing 6 homosexual and 6 heterosexual Latino men was conducted with a brief survey including measures on their attitudes toward male homosexuality and gender roles as well as open-ended questions about definitions of masculinity. Based on previous research (Herek & Gonzalez-Rivera, 2006; Mirande, 1997), three predictions were made. Compared to heterosexual Latino men, gay Latino men will 1) endorse less traditional gender roles and 2) define "masculinity" in terms of such stereotypically masculine characteristics as size and aggressiveness rather than dominance over women. In addition, 3) both gay and heterosexual Latino men who have more contact with homosexual people will have less negatives attitudes toward homosexuality than other men. The findings supported all three of the predictions. A better understanding of how Latino men define masculinity and feel toward homosexuality will provide insight into the complexity of gay Latino identity.

Introduction

Gay Latino men in the United States are susceptible to discrimination for both their race and their sexual orientation. The dual effect of sexual prejudice and racism on the construction of gay Latino identity has not been examined extensively in research. Previous research has shown that homophobia, racism and financial hardship can each contribute to mental health difficulties among gay Latinos (Diaz, Ayala, Bein, Henne, & Marin, 2001). Research has also found that gender non-conformity among gay Latinos is related to higher levels of mental distress. Specifically, gay Latino men who identify as effeminate report more negative experiences of discrimination than gay Latino men who do not consider themselves to be effeminate (Sandfort, Melendez, & Diaz, 2007). These findings highlight a need for research on the cultural constructs that shape the identity of gay Latino men and the prejudice they face.

Latino Masculinity

What does being a man mean to Latino men? The Latino male way of being has been historically associated with machismo, which English-language dictionaries define as a strong or exaggerated pride in one's masculinity. Anglo scholars have often viewed traditional masculine attributes of assertiveness, competitiveness and individualism in Latino men as hyper-masculine generalizations of aggressiveness,

womanizing, sexism and oppression toward women (Mirandé, 1997). Is machismo the same as Latino masculinity? Torres, Solberg, and Carlstorm (2002) measured the constructs of machismo, masculinity, and gender role identity in a sample of 150 Latino men. They found that Latino men see masculinity and machismo in diverse ways and that it is inaccurate to stereotype Latino men as having a singular view of masculinity. This work contributes to the definition of Latino masculinity as variable and unique to the individual man. An important shortcoming of prior work, however, is that it has not included the experiences of homosexual men. In the work by Torres et al., 91% of the participants were heterosexual. The gay Latino perspective has thus far been ignored in the study of masculinity.

In a study on sex role positions among Latino gay and bisexual men, it was found that sexually versatile men were more likely to take a passive sexual role (i.e. being penetrated in anal sex) when they perceive a sexual partner to be more masculine, more macho, more aggressive, taller, possessing a larger penis, more handsome, or darker skinned than themselves (Carballo-Diéguez, Dolezal, Nieves, Diaz, Decena, & Balan, 2004). Among non-heterosexual Latino men, perceived masculinity was correlated with attributes of aggressiveness, height, penis size, attractiveness and skin color. This study shows that homosexual Latino men may be likely to emphasize physical characteristics that are attached to stereotypes of masculinity.

Latino Attitudes Toward Homosexuality

Latino culture has often been stereotyped as traditional, morally conservative, religious and consequently homophobic (Garcia, 1998). Studies have also suggested that Latinos possess strict gender roles and rigidity (Dube & Savin-Williams, 1999; Lippa & Tan, 2001). In a landmark study of Mexican-American attitudes toward homosexuality, Herek and Gonzalez-Rivera (2006) found that among Mexican Americans, higher scores on a measure of homophobia correlated positively with being male, low formal education, religiosity, being a parent, lack of personal contact with gay people and acculturation. An important finding is that these characteristics were shown to be strong predictors of negative attitudes toward homosexuals only among respondents who identified as Mexican-American (not just Mexican) and/or those who report speaking only English. This pattern suggests that these negative attitudes may be a product of acculturation or an interaction between Latino and American culture. Traditional gender roles were found to be the most significant predictor of negative attitudes toward gay people.

Lippa and Tan (2001) refer to Latino culture as a traditionally "gender-polarized culture" in which people are socialized to act in a way that exhibits large differences in how men and women act. In a cross-cultural study of over 200 men and women who identified as Asian, Latino, or White, they found that Latinos showed larger homosexual-heterosexual differences on gender-related items than did Whites. This finding points to an association between gender roles and sexual orientation, where homosexuality may be attached to a gender-based interpretation, in Latino culture.

Homosexuality in Latin America. This gender-based interpretation of homosexuality in Latino culture has also been discussed by scholars both in the U.S. and in Latin America. Guzman (2006) argued that the nature of homosexuality is much different in Puerto Rico and other Latin American countries than in the United States. It is a difference of doing versus being. Guzman argued that men who engage in sexual relations with other men are not necessarily labeled as "gay." The Latin American system allows men to have sex with other men without carrying the stigma or the burden of a homosexual identification (Guzman, 2006).

Rather, a man's perceived masculinity is what matters. Hyper-masculine men who have sex with men are assumed to be or accepted as heterosexual, and it is perceived that effeminate men who have sex with men desire to be women because of their gender non-conformity. (Garcia, 1998; Guzman, 2006). The

nature of homosexuality in Latin America is thought to have a significant effect on the identity development of gay Latinos in the U.S.

Gay Latino Identity. Research suggests that the identity development process for gay Latinos is more complex and more difficult than for non-Latino gay man because of the cultural importance placed on religion, family and hetero-normative behaviors (Garcia, 1998). Dube and Savin-Williams (1999) found that gay Latino youth reported being aware of same-sex attractions at an earlier age than African-American and White youth. They attribute this to the "intensification of gender roles in Latino culture" (p. 1395), that is gay Latino are aware earlier of their homosexual feelings because they are inconsistent with strict gender roles in their social environments. Dube and Savin-Williams also found a strong negative association between disclosure of sexual orientation and internalized homophobia for Latino youth; those young men who had yet to disclose their sexual orientation to their families displayed more homophobic attitudes. This may be a way of these youth deflecting their homosexuality by projecting attitudes that oppose such behavior. These findings suggest that Latino gay youth may be aware of their sexuality earlier than youth from other ethnic groups because of their internal inconsistency with strict gender roles, but they can not disclose their sexual orientation because of the hetero-normative attachments to gender, that is hegemonic masculinity.

Contact

According contact theory, more contact or interaction with an out-group will increase the understanding, acceptance and/or tolerance of that out-group (Allport, 1954; Pettigrew, 1998). In a large-scale study of over 500 participants, Herek and Capitanio (1996) found that heterosexual men and women reporting more interpersonal contact with gay men had more positive attitudes toward gay men than those without contact. This study shows a correlation between heterosexuals' contact with gay men and positive attitudes toward gay men. This relationship has not been explored among Latino men.

Social Identity

Social identity theory states that the way one categorizes all people, including oneself, into in-groups or out-groups affects one's perceptions, attitudes and behavior toward them (Tajfel & Turner, 1986). Applying the theory to this study, straight Latino men will view gay Latino men, based on their sexual orientation, as part of an out-group because it deviates from what is considered normal in Latino culture. In turn, straight Latino men will perceive gay Latino men as different from themselves and therefore treat them differently. Latino ethnicity will likely be overlooked as in-group quality if traditional gender expectations are high because importance will be placed on the perceived normalcy of heterosexuality.

The Present Study

The present study of gay and heterosexual Latino men was designed to test hypotheses derived from available research and theory. The research examines the association between participants' own sexual orientation and their contact with homosexuals with their perceptions of Latino masculinity, their endorsement of traditionally Latino gender roles, and their attitudes toward male homosexuality.

Hypothesis 1. In defining masculinity, heterosexual Latino men will emphasize dominance over women. Specifically, the heterosexual participants will respond with adjectives that describe the gender norms common to Latinos such as dominance over women. I expect for the heterosexual participants to define their masculinity in relation to their sexual orientation and the subsequent gender norms. In contrast, homosexual Latino men will define masculinity in terms of traditionally masculine personal characteristics such as size and aggressiveness.

Hypothesis 2. Heterosexual Latino men will have more traditionally Latino gender-role beliefs than homosexual Latinos. Specifically, heterosexual Latino men will score higher on the Mirande (1997) Sex Role Inventory (MSRI) than homosexual Latino men. This hypothesis is based on the idea that male homosexuality is often interpreted as indicating femininity, especially among Latino men. If one possesses and expresses an aspect of identity that is likely to be viewed as gender non-conforming, then he is likely not to support beliefs that perpetuate such ideology that oppose this very own identity.

Hypothesis 3. Both heterosexual and homosexual Latino men who have less contact with homosexual people will have more negative attitudes toward male homosexuality than individuals who have more contact with homosexual people. Specifically, high and low contact participants will differ significantly in their scores on the Herek (1994) Attitudes toward Gay Men (ATG) scale. The rationale behind this hypothesis is contact theory, which argues that more contact or interaction with a perceived out-group will increase the understanding, acceptance and/or tolerance of that perceived out-group (Allport, 1954; Pettigrew, 1998).

Method

Participants and Recruitment

Two groups of men, who identify ethnically as Latino but differ in sexual orientation, were recruited to participate. A very low turnout of participants was experienced. It may be attributed to the sensitive subject matter. Also, a minority sample within a minority sample was sought (Gay Latino men), limiting the turnout rate further. Men who identify sexually as homosexual were recruited at the University of California at Los Angeles (UCLA), in a Chicano/a Studies course and a Gay Latino student organization, but only six agreed to participate. Men who identify as heterosexual were recruited at the UCLA campus in a Chicano/a Studies course and a Latino student organization. While nine from this group agreed to participate, only six of the participants' responses were used in order to keep an equal number between the comparison groups. Ages of all participants ranged from 18 to 25 years, with a mean of 21 years (SD = 1.66).

At the time of recruitment, potential participants were informed of the study's eligibility requirements: that they must be an adult male, at least 18 years of age, who identifies as Latino or Chicano. All participants met the criteria. Researchers approached participants by asking if they would be willing to fill out a brief survey on Latino male social attitudes. Upon agreement, participants were given the options of completing the survey on the spot or returning it in a prepaid envelope at a later time.

Procedure

Participants were given an information sheet detailing the purpose of the study as well as their rights as a participant in the study. Upon agreeing to participate in the study, participants were given a survey and a pencil if filling it out on the spot or a pre-addressed stamped envelope if returning it at a later time. The survey included questions assessing participants' general attitudes toward traditional Latino gender roles, their attitudes toward male homosexuality and their perceptions of masculinity. The survey ended with general demographic questions. The entire survey took no more than 20 minutes for each participant to complete. After completion of the survey, participants were debriefed, thanked for their participation, and offered candy as a small reward.

Measures

The survey had four sections concerning, gender-role beliefs, attitudes toward male homosexuality, perceptions of masculinity and demographics.

Measures Related to Gender-Role Attitudes

Mirande (1997) Sex Role Inventory (MSRI). The MSRI is a 50-item scale, which assesses one's agreement with stereotypically traditional Latino gender role beliefs. Its items assess concepts taken from traditional Latino sayings and folk stories that reflect traditional Latino cultural values toward men and women. Illustrative items include "men should never cry or show emotions" and "women should never question men." Participants are asked to indicate the extent to which they agree or disagree with each statement on a 5-point scale ranging from strongly disagree (1) to strongly agree (5). In a study of 108 diverse Latino men across ages, incomes, education levels, and regions, Mirande (1997) reported that a factor analysis of the MSRI identified: Factor 1 – Traditionalism, Factor 2 – Toughness, and Factor 3 – Sensitivity. Factor loadings ranged from .50 to .78, which suggests high internal validity and high reliability for internal consistency.

Measures Related to Homosexuality

Attitudes toward male homosexuality. The ten-item version of Attitudes Toward Gay Men (ATG, Herek, 1994) assesses homophobia or negative attitudes toward male homosexuality. Items on the ATG scale include such statements as "male homosexuality is merely a different kind of lifestyle that should not be condemned" and "the idea of male homosexual marriages seems ridiculous to me." Participants are asked to indicate the extent to which they agree or disagree with each statement on a five-point scale ranging from strongly disagree (1) to strongly agree (5). Higher scores indicate higher levels of sexual prejudice. In a study on people of Mexican descent, Herek and Gonzalez-Rivera found negative attitudes to be consistent with high religiosity and conservative political ideologies, which suggest high construct validity (2006).

Personal contact with homosexual men and women. Participants were asked, "How many gay/lesbian friends or family members do you have?" Five response options ranged from none to 11 or more. Participants were also asked, "How often do you interact with gays or lesbians?" Five response options ranged from never to sometimes to often.

Sexual Orientation. To assess sexual identity, participants were asked to indicate their sexual identification by checking heterosexual, homosexual, bisexual or other. To assess sexual behavior, participants were asked, "In the past year, were your sexual partners: women, men or both?" Response options were only men, mostly men, men & women equally, only women, mostly women and not applicable. Finally, to assess sexual attraction, participants were asked, "In general, are you attracted to women, men or both?" Response options were only men, mostly men, men & women equally, only women, mostly women and not applicable.

Measures Related to Masculinity

Beliefs about Masculinity. To assess perceptions of masculinity, participants were asked two open-ended questions. First, participants were asked think about the concept of a "masculine man" and to list six adjectives that they thought describe a masculine man. Second, participants were asked think about the ideal of a "real man" and to list six adjectives that they thought describe a real man.

Personal Masculinity. Participants were asked, "How you would rate your own masculinity based the description you have gave before?" Five response options ranged from feminine to moderate to masculine.

Demographic Measures

The questionnaire included items to ascertain participant age and highest educational level. Religiosity was also assessed by asking, "How religious are you?" and providing five response options ranging from not at all to extremely. Acculturation was also assessed in three ways. First, participants were asked, "how often do you speak Spanish?" with five response options ranging from never to sometimes to often. Main language spoken has been historically used to assess Latino acculturation. They were also asked "were you born in the United States?" and "were your parents born in the United States?"

Results

The first hypothesis stated heterosexual men will define masculinity in relation to power over women and other traditional gender roles while gay men will use physical characteristics to define masculinity. The data partly supported the first hypothesis. When defining masculinity, the gay men emphasized physical characteristics of the male body. All of the men used either "buff," "muscular," "fit," "well-built" or "athletic" in their description of a masculine man. "Tough" and its synonyms "brave" and "strong," were used by more than half of the men. No other patterns were found in gay men's descriptions of masculinity. The part of the first hypothesis about heterosexual men was not supported by the data. None of the heterosexual men even mentioned dominance over women in their descriptions of masculinity. Four of the six heterosexual men listed "confident" in their description of a masculine man. Other common descriptors included "respected," "hardworking," and "a leader." A qualitative analysis was used to investigate this hypothesis because it was determined the best way to stimulate examination of this explorative yet complex construct. Table 1 lists the adjectives used most by these men to describe what they think it means to be a man.

Table 1

Latino Men's Descriptors of Masculinity

	Gay Men's Most Popular Responses	Straight Men's Most Popular Responses
Descriptors of a "masculine" man	1. Muscular 2. Athletic 3. Fit 4. Tough 5. Strong 6. Brave	1. Respectful 2. Resourceful 3. Hard-working 4. Provider 5. Leader 6. Strong

Note: These are the most used descriptors by each group to answer the same question of masculinity perception.

The second hypothesis stated heterosexual Latino men will have more traditional Latino gender-role beliefs than homosexual Latinos. It was supported by the data. The gender-role beliefs of these men were evaluated by comparing the mean scores of the homosexual men on the MSRI to those of the heterosexual men. The MSRI scores ranged from one to five. Homosexual men ($M = 2.56$, $SD = 0.38$) scored lower than heterosexual men ($M = 3.75$, $SD = 0.43$) on the MSRI. The statistical significance of this comparison was not calculated due to the simplicity of the statistics used to analyze this difference. Table 2 shows that homosexual men support traditional Latino gender roles less than heterosexual men.

Table 2

Latino Men's Gender Role Beliefs as measured by Scores on the Mirande (1997) Sex Role Inventory (MSRI)

	Gay Latino Men	Straight Latino Men
Mean	M = 2.56	M = 3.75
Standard Deviation	SD = 0.38	SD = 0.43

Note: Mean scores on the MSRI range from 1 to 5. Each participant's score on this measure were averaged into the corresponding group.

The third hypothesis stated both heterosexual and homosexual Latino men who have less contact with homosexual people will have more negatives attitudes toward male homosexuality than individuals who have more contact with homosexual people. The data also supported the third hypothesis. To test the relationship between attitudes toward homosexual people and contact with homosexual people, scores on the ATG scale were first recoded (in order for higher scores to represent more favorable attitudes toward gay people) and compared to amount of interaction with gay men and/or lesbians. We found a significant positive correlation: $r(12) = 0.44$; $p < 0.01$.

Discussion

The first hypothesis was partially supported because the gay men did define masculinity in terms of physical characteristics while the straight men did not define masculinity in terms of dominance over women. Nonetheless, the second hypothesis that straight Latino men hold more traditional gender-role beliefs than do gay Latino men was supported. The third hypothesis that contact with gay people correlates with attitudes toward them was supported.

First, it was found that gay Latino men define masculinity in terms of stereotypically hypermasculine physical characteristics like size and strength. This finding coincides with previous research which found that gay Latino men tend to take a passive sexual role (i.e. being penetrated in anal sex) when they perceive a sexual partner to be more masculine, more macho, more aggressive, taller, possessing a larger penis, more handsome, or darker skinned than themselves (Carballo-Diéguez at al., 2004). Were these men adopting a passive role when defining masculinity as being muscular and athletic? It could be that they define masculinity in such stereotypical ways because they, in fact, reject it as just that a stereotype. Were they describing masculinity or the stereotype of masculinity? It is difficult to differentiate the two at this point. It is plausible that they are defining the stereotype of masculinity and therefore, excluding themselves from that interpretation. Future studies that could replicate or challenge this finding are important to this line of study. The second part of this hypothesis was not supported. On this same measure of masculinity perception, it was found that straight Latino men define masculinity in terms of personality characteristics such as confidence and integrity rather than dominance over women. In fact, gender dynamics were not even mentioned in straight Latino men's descriptions. These straight men seem to be defining a respectable person regardless of gender. This finding is in line with previous

research that claims Latino masculinity is fluid and unfairly assumed to be "machismo" (Mirandé, 1997; Torres et al., 2002). It would be interesting to see how they would have answered the question if they were asked, "What makes a real woman?" Future research could look how both Latino men and women of different sexual orientations define masculinity and femininity. It could be that gay Latino men actually posses a more stereotypical perception of masculinity by possibly excluding themselves from that perception due to their internalized association of homosexuality with gender non-conformity or "gender failure." This question could lead to whole new direction in the study of Latino men's perceptions of gender and sexuality.

Second, it was found that straight Latino men support more traditional gender-role beliefs than do gay Latino men. This finding is consistent for gay Latino men because of the relationship between gender and sexual orientation in Latino culture (Guzman, 2006). Homosexuality is often interpreted as gender non-conformity because men are expected to be attracted to women and vice-versa. Therefore, those who possess such identity or practice such behavior will reject other aspects of an ideology that rejects their very own. That is, gay Latino men may support less traditional gender-role beliefs because their own identity rejects such ideology. Therefore, they reject other aspects of this traditional ideology of gender. The first two findings can seem contradictory for the straight Latino men. The straight men did not define masculinity in terms of dominance over women yet they supported more traditional gender roles than do the gay men. It could be the case that these men support a sort of "sexist benevolence" or a modern chivalry that is actually patronizing. The provider role, that some straight men take, actually handicaps women in a way that asserts they must be helped because they are incapable of doing it alone (Mirande, 1997). This interpretation could explain the coexistence of a non-dominating definition of masculinity with traditional gender roles.

Finally, a correlation was found between attitudes toward gay people and contact with gay people. That is, those with more positive attitudes toward gay people also report more contact or interaction with gay people. This correlation was true for both gay and straight participants. It may be the case that these men, especially the straight men, have more positive attitudes toward gay people because they are naturally open-minded and tolerant and therefore have more contact with gay people. It could also be that the contact and interaction they have with gay people has built tolerance toward this group, which has been explored in great amount in previous research (Herek, 1994). It may be a bit of both these speculations but nevertheless, this still points to an association between contact and positive attitudes or tolerance.

There are a few limitations for this study. These men were recruited from a liberal environment: a university campus. This may have lead to a selection bias. A more thorough study would require a much larger sample size and a more diverse selection process. Ideally, research on Latino masculinity should ensure participants from various socioeconomic statuses and levels of education. Also, the low number of participants is another limitation. The lack of participants yields some statistical significance and the potential of being generalized. Nevertheless, the results mark an important contribution to a growing field of study. The circumstances that lead to the limited amount of participants highlight the challenges of conducting research with minorities within minorities.

Garcia (1998) calls for "research that examine[s] gender constancy between gay and heterosexual Latino men [to] tell us more about the complexities of Latino gender roles, expectations, and behavior" (p. 110). Using both qualitative and quantitative methods, this study did just that by approaching Latino gay identity as a complex product of socio-cultural constructs about gender and sexuality. This study pulled together the previous research on masculinity, homosexuality and gay identity among Latinos by demonstrating that the perceptions of masculinity and attitudes toward homosexuality are related.

Understanding the differences in perceptions and attitudes of homosexual and heterosexual men will provide insight into the socialization of gender and its influence on the interpretation of homosexuality in Latino culture. This study fills in the gap of previous research literature where these concepts where never explicitly studied in relation to each other.

A better understanding of how Latino men perceive masculinity along with gender roles and male homosexuality demonstrates a need for intergroup understanding and tolerance necessary for the complexity of gay Latino identity. The purpose of this study is not to stereotype heterosexual Latino men as homophobic or sexist but rather to demonstrate the need for understanding between homosexual and heterosexual Latinos in order to promote tolerance. This growth of tolerance will aid in reducing the amount of prejudice or homophobia experienced and its subsequent effects on gay Latino identity. Prejudice often grows from misunderstanding or lack of any understanding. If heterosexual Latino men tend to harbor negative attitudes toward male homosexuality, it may be because they do not interact positively if at all with homosexual people. Therefore, there is a need from more contact or interaction between Latino men, both gay and straight, to understand each other better. The rationale behind this goal is to reduce prejudice toward gay Latinos from their own ethnic communities. This reduced prejudice can make the identity development of gay Latinos less difficult and complex.

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