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Beliefs Behind the Scars

The Role of Entity Beliefs About Emotion in Non-Suicidal Self-Injury

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ABSTRACT. Non-suicidal self-injury (NSSI; deliberate harm to the body without the intent to die) in college-age individuals is a growing concern. While an increasing body of research aims to describe the functions of NSSI, little is known about why certain individuals turn to such damaging and potentially life-threatening behaviors. This study aimed to understand why some individuals turn to these behaviors. Specifically, it was hypothesized that NSSI may be associated with entity beliefs about emotions (i.e., that one's emotional response is uncontrollable). Participants completed a negative emotion induction task by recalling an argument and subsequently reported their beliefs about these emotions and their urges to engage in NSSI. Results showed a significant correlation between urges to engage in NSSI and entity beliefs. These findings suggest that entity beliefs about emotions are a correlate of—and potentially a risk factor for—NSSI. Understanding the factors that may drive the urge to engage in NSSI may inform new ways of treating these individuals. Improved treatments may decrease the alarmingly high rate of NSSI in college-age individuals.

1. Introduction

Non-suicidal self-injury (NSSI) is defined as causing deliberate harm to the body without the intent to die (Nock, 2009; Barrocas et. al., 2011). This type of harm results in damage of the skin tissue to the point of scarring (Gratz, 2001). Common types of self-injury include cutting or burning the skin, scratching the skin to draw blood, and picking wounds to the degree of interfering with healing (Gratz, 2001). Although other behaviors such as tattooing or piercing the body result in alteration of the skin tissue, NSSI is commonly seen as a non-culturally sanctioned behavior (Barrocas et al., 2011) due to this specific type of skin alteration (American Psychiatric Association, 2013).

NSSI is commonly found in individuals with psychiatric diagnoses, including major depression, anxiety disorders, post-traumatic stress disorder, eating disorders, and many types of personality disorders (Haw, Hawton, Houston, & Townsend, 2001; Klonsky, 2007; Suyemoto, 1998); NSSI is a particularly common symptom of Borderline Personality Disorder (BPD; Klonsky, 2007; Barrocas et al., 2011). Moreover, NSSI rates in samples of those diagnosed with BPD can be as high as 90% (Zanarini et al., 2008; Baroccas et al., 2011). However, NSSI is not a symptom solely confined to specific psychiatric disorders. NSSI rates are as high as 38% in college-age samples as well (Rodham & Howton, 2009), which indicate the need to examine the properties of this unsettling phenomenon in non-clinical samples.

The Role of Emotion Regulation in NSSI

NSSI in young adult populations is a growing concern. Although many laymen believe that the driving force behind NSSI stems from the need to get attention or to experience physical pain (Suyemoto, 1998), the research suggests that NSSI may arise from emotion regulation deficits (Davis et al., in review). Emotion regulation is essential to healthy functioning (Werner & Gross, 2009; De Castella et al., 2013). It is a skill that is acquired early in life (e.g. infants shifting their gaze in an effort to autonomously regulate emotions; Gross & Thompson, 2007) and often operates without having to make a conscious effort to do so (Hopp, Troy & Mauss, 2011; Mauss, Bunge & Gross, 2007). Emotion regulation consists of the ability to use cognitive strategies to change one's response to emotions (Troy, Wilhelm, Shallcross & Mauss,

AUTHOR'S NOTE: *This study would not have come to fruition without the unwavering support of the Emotion and Emotion Regulation Lab. Thank you to Tchiki Davis for the constant push for excellence, to Iris Mauss for continual inspiration and to Maya Kuehn for encouragement to conduct this study.*

2010). Emotion regulation influences which emotions individuals will have and how they will experience these emotions (Gross, 1998).

Many researchers (see De Castella et al., 2013; Troy et al., 2010) agree that a fundamental strategy for successful emotion regulation is the ability to reappraise a situation. Reappraisal is the ability to change how one thinks about a situation, specifically an emotion-eliciting one. Through this cognitive change, one is able to change the emotional meaning and significance of the situation (De Castella et al., 2013). For example, Gross (1998) conducted a laboratory study examining the role reappraisal plays in emotion regulation. Participants who were instructed to use reappraisal when watching an emotional film clip reported less negative emotions subsequent to watching the clip than did those who were told to "just watch" (Troy et al., 2010). Reappraisal as an emotion regulation strategy proves to be effective for clinical populations as well. Reappraisal has been found to be negatively associated with depressive symptoms (Troy et al., 2010; Garnefski & Kraaij, 2006; Garnefski, Kraaij & Spinhoven, 2001). Moreover, in a study conducted over a 10-week treatment period, men who received intervention treatment exhibited increased reappraisal, which in turn was associated with decreased depressive symptoms (Carrico, Antoni, Weaver, Lechner & Schneiderman, 2005). Indeed, these studies support the importance of emotion regulation as a means to healthy functioning.

Conversely, poor emotion regulation refers to the inability to change one's emotional reactions to a situation (Davis et al., in review; Aldao, Hoeksema, & Schweizer, 2010; Gross & Thompson, 2007). Initial evidence suggests that NSSI is often associated with poor emotion regulation abilities (Crowell et al., 2012; Slee et al., 2008). Those with a history of NSSI report engaging in NSSI for various reasons that commonly include: to relieve negative emotions such as depression, anxiety, guilt; to punish self (Lloyd-Richardson, Nicholas, Dierker & Kelley, 2007); to distract from negative thoughts (Nock, 2009); to relieve feelings of numbness (Barrocas et al., 2011). An explanation of this behavior proposes that emotion-eliciting situations and events can produce extreme arousal in individuals who have poor emotion regulation strategies (Davis et al., in review; Chapman, Gratz & Brown, 2006; Linehan et al., 2007; Nock, 2009). NSSI reduces this high arousal (Klonsky, 2007).

Reduction of arousal is thought to come, in part, from the production of endogenous endorphins (producing an opioid-like feeling) that alleviate negative emotional states (Favazza & Conterio, 1988; Richardson & Zaleski, 1986). The pain produced by self-injurious behavior may produce a rush of endorphins, culminating in a pleasant, relaxed state (Favazza & Conterio, 1988; Richardson & Zaleski, 1986). Thus NSSI increases levels of endogenous endorphins. Conversely, conducting research analyzing cerebrospinal fluid from individuals with a history of NSSI, Stanley et al. (2010) found that some individuals who engage in NSSI have lower levels of endorphins. Individuals who engage in NSSI to experience physical pain (i.e., as a way to punish oneself) may be those who have low levels of these opioids and in turn experience more intense pain, rather than a reduction of psychological pain (Stanley et al., 2010). Indeed, both findings support the understanding that endorphins influence the pain threshold, and that NSSI is used as a way to relieve emotional tension.

Individuals may also turn to NSSI as a way to quell ruminative thoughts (Selby & Joiner, 2009). Rumination—continually thinking about the causes and outcomes of negative, emotional events (Nolen-Hoeksema, 1991)—may make it harder to direct attention away from emotion-eliciting situations (Selby & Joiner, 2009). Minor distractions that shift attention in an effort to regulate emotions (i.e., an intense workout or cold shower) often prove to be insufficient in lowering high arousal. An individual may need to engage in something that is more intense or shocking to the system. More specifically, a jolt to the system, such as severe pain to the body, may be the only way to shift focus away from the intense emotions resulting from an external situation (Selby & Joiner, 2009).

Functional Models of NSSI

Prior research has tended to focus mainly on the functions of NSSI, and numerous models of the function of NSSI have been proposed. In a meta-analysis, Klonsky (2007) concludes that NSSI serves seven main functions: to decrease or alleviate negative emotions and/or high arousal (Affect-regulation model); to abate the experience of dissociation (Anti-dissociation model); as a way of fighting urges to attempt suicide (Anti-suicide model); as a cry for help (Interpersonal-influence model); to demonstrate or affirm boundaries between oneself and others (Interpersonal boundaries model); as a way to express anger felt towards the self (Self-punishment model); and as a way of generating exhilaration comparable to extreme sporting (Sensation-seeking model).

Another functional model of NSSI, the Cascade

model (Selby & Joiner, 2009), posits that NSSI functions as short-term emotion regulation, but also explains why it is not effective in long-term emotion regulation. Due to heightened focus on emotional stimuli, individuals may experience greater negative affect which becomes harder and harder to regulate. Jolting the system through NSSI may be the only effective way to short-circuit this emotional cascade. However, this is only a temporary solution, and the shame or embarrassment surrounding the injurious behavior may thereby perpetuate the emotional cascade (Selby & Joiner, 2009).

Nock (2009) offers an additional hypothesis for the function of NSSI and suggests that NSSI that may contribute to a cyclical pattern of self-injury. Nock proposes that the reason why people may engage in NSSI is that it is a fast and often very accessible way of regulating emotions (Pragmatic Hypothesis; Nock 2009). Importantly, self-injurers report feeling little or no pain while they engage in NSSI (Pain Analgesia/Opiate Hypothesis; Nock, 2009), therefore creating a cyclical pattern of using a fast technique to regulate emotions that becomes easily reinforced by pain analgesia.

A New Idea: Beliefs about Emotions

Much of the past research has focused on functional models of NSSI and how poor emotion regulation may be the driving force leading individuals to intentionally harm themselves. However, it's unclear exactly why poor emotion regulation would increase risk for NSSI. Recent research (see Tamir, John, Srivastava & Gross, 2007; De Castella et al., 2013) indicates that people hold different implicit beliefs about emotion: either emotions are an experience that can be regulated or changed (incremental beliefs) or emotions are something that cannot be controlled or changed (entity beliefs; De Castella et al., 2013). Research has shown that people who hold entity beliefs about emotion are less likely to engage in adaptive emotion regulation (i.e., reappraisal; Tamir et al., 2007). Beliefs about controllability, in any domain, impact daily functioning. For individuals holding incremental beliefs, goals appear to be more attainable, and strategies for attaining these goals are implemented (Tamir & Mauss, 2011).

However, a distinction must be made between beliefs about general controllability and beliefs about personal controllability of one's own emotions. Individuals may believe that in general people can control their emotions, while they themselves are powerless to control their own emotions. Subsequently, this belief of lack of personal controllability may contribute to feelings of personal failure (Tamir & Mauss, 2011). Continually experiencing feelings of personal failure may limit the ability to problem solve (Tamir & Mauss, 2011). Therefore, individuals may engage in NSSI to alleviate negative emotional states (through the release of endogenous endorphins). Thus, individuals who believe they are powerless to control their emotions may engage in NSSI for reasons that the functional models propose. In addition, individuals may engage in NSSI as a way to distract themselves from negative thoughts or feelings. However, NSSI is only a temporary solution; engaging in this behavior only provides temporary relief from the current emotions. It does not solve the root cause. In sum, although past research has primarily focused on the functions that NSSI serves, burgeoning research indicates the need to further investigate its relationship with beliefs about emotion.

Limitations of prior research have impacted a wide range of domains including research, theory, and treatment (Suyemoto, 1998). Conducting studies with inpatient populations or adolescents create biased results as NSSI has a higher prevalence in these populations (Suyemoto, 1998). Therefore, it is imperative to expand the scope of research to non-clinical samples due to research findings indicating that NSSI is not solely limited to adolescents or individuals in inpatient settings. Furthermore, prior research has failed to control for pivotal variables such as depression and anxiety. Due to the salience of depression in many psychiatric disorders, it is crucial to control for depression to indicate the rates of NSSI in the general public that may not necessarily been diagnosed with depression. Moreover, standardized measures of self-reported emotions and emotion regulation assess variables in general (e.g. "I feel sad"; Beck, Steer & Brown, 1996). Assessing variables in general may be confounded due to memory bias (Robinson & Clore, 2002). Therefore, it is of utmost importance to assess these variables in real time through lab-based emotion inductions. This greatly reduces memory bias and may provide a more accurate understanding of the relationship between emotion beliefs and NSSI.

Present Study

Given that emotion beliefs and emotion regulation play a role in coping with negative emotions (Tamir et al., 2007), this study will be the first to examine the relationships between individual beliefs about emotions, emotion regulation, and NSSI. This is achieved through assessing

implicit beliefs about emotions during a mood induction, emotion regulation in general, and NSSI before and after the mood induction. Memory bias may confound results insofar as participants may not accurately remember their emotional beliefs at the time they engaged in NSSI behaviors. To avoid this negative emotions are induced through a recall scenario and both emotion beliefs and NSSI urges are assessed. It is hypothesized that individuals who experience greater entity beliefs about emotions will report greater NSSI frequency, and show greater NSSI urges.

2. Method

Participants

Four hundred and thirty-nine participants (72% female) between the ages of 18 years and 51 years (M = 21, SD = 3.4) were recruited through UC Berkeley's online Research Participation Pool (RPP). Students enrolled in specific courses in the Psychology Department are required to complete a certain number of RPP credits (3 credits for each psychology course they are enrolled in) as part of their final grade. Psychology students can choose what studies to participate in, and those who chose this online study received 0.5 credits.

Measures

Demographics. Participants responded to two general demographic questions asking their gender and their age.

History of NSSI. A single question ("I have hurt myself on purpose several times") from the Schedule for Nonadaptive and Adaptive Personality (Simms & Clark, 2006) was used to measure whether subjects had a history of NSSI. "Several times" was removed so as to allow participants to rate NSSI on a Likert scale (1 = never, 4 = frequently).

Implicit emotion beliefs. Two questions from the Implicit Beliefs Scale (Tamir et al., 2007) were used to assess implicit beliefs in response to the emotion induction: "No matter how hard they try, people can't really change the emotions they have" and "The truth is, people have very little control over their emotions."

Verbal intelligence. Verbal intelligence was assessed through the 40-item Shipley Institute of Living Scale (Zachary, Crumpton, & Spiegel, 1985).

Depression symptoms. Depression symptoms were assessed using the 21-item Beck Depression Inventory (BDI; Beck, Steer & Garbin, 1988).

Anxiety symptoms. Anxiety symptoms were assessed using the 21-item Beck Anxiety Inventory (BAI; Beck, 1990).

Emotion regulation and beliefs. Emotion regulation and beliefs were assessed using the Response Styles Questionnaire (RSQ; Nolen-Hoeksema, 1991), the Emotion Regulation Questionnaire (ERQ 21; Gross & John, 2003), the Behavioral Emotion Regulation Inventory and the Implicit Beliefs Scale (Tamir et al., 2007).

Emotions. Emotions were assessed using the Positive and Negative Affect Schedule (PANAS; Watson, Clark & Tellegan, 1988).

Procedure

Participants read a brief description of the study on the RPP website that indicated that the study was assessing emotion beliefs and emotion regulation. After reading the consent form and agreeing to participate, participants were asked two demographic questions ("What is your gender?" and "What is your age?"). Participants then completed questionnaires assessing verbal intelligence (Shipley Institute of Living Scale), depression (Beck Depression Inventory), anxiety (Beck Anxiety Inventory), and emotion regulation and beliefs (Response Style Questionnaire; Emotion Regulation Questionnaire; The Behavioral Emotion Regulation Inventory; Implicit Beliefs Scale), respectively, and single question assessing their history of NSSI ("I have hurt myself on purpose many times.") After completing these assessments, participants watched an instructional video clip on how wooden bowls are made. The purpose of this video clip was to neutralize participants' emotions before completing the rest of the study and to establish a baseline for each participant. Participants were then asked to rate how strongly they were feeling certain emotions, using the PANAS scale, after watching the bowl-making clip. Participants were then asked to recall an argument that they had had with a significant other or a friend. They were given a minimum of one minute to focus on the argument and to reflect on how they felt in the moment of the argument and what their beliefs about their emotions were at that time. After one minute, a "next" button appeared on the screen to allow participant to continue with the study.

Participants were then asked to again rate how strongly they were feeling the emotions that they rated before the clip, again using the PANAS scale, and also how they responded internally to these emotions. To conclude the study, participants watched a positive, mood-altering

film clip to repair any negative emotions or thoughts that may have arisen during their participation. A clip from Planet Earth was chosen, depicting the change of winter to spring on Mt. Denali. In the unlikely event that longer lasting negative effects occurred from participating in the study, each participant was given a list of campus resources, such as numbers for the Counseling and Psychological Services at the Tang Student Health Center, suicide and crisis hotline, and resources on identifying depression and anxiety.

3. Results

Manipulation Check

In order to assure that the mood induction increased negative emotions, paired t-tests were computed to compare the average of negative emotions prior to mood induction to the average of negative emotions post mood induction. Participants experienced significant increase in sadness ($M = 4.22$, $SD = 2.54$) post mood induction compared to the baseline assessment ($M = 2.00$, $SD = 1.72$), $t(424) = -16.90$, $p < 0.01$. Similarly, participants experienced an increase in anxiety ($M = 3.82$, $SD = 2.456$) post mood induction compared to the baseline assessment ($M = 1.60$, $SD = 1.35$), $t(425) = -17.87$, $p < 0.01$. There was no significant increase in anger post mood induction $t(427) = 0.72$, $p = 0.11$.

Primary Analyses

A total of 439 participants completed the study with 19% reporting a history of NSSI. It was predicted that NSSI frequency would be associated with entity beliefs about emotions. An ANOVA revealed that there was no effect of entity beliefs about emotions on NSSI frequency $F(1,432) = 2.02$, $p = 0.16$. However, consistent with our hypothesis that individuals who experience greater entity beliefs about emotion will show greater NSSI urges, a Pearson's correlation confirmed this hypothesis $r(420) = 0.14$, $p < 0.01$.

4. Discussion

Research investigating NSSI has primarily focused on the functions that NSSI serves. However, little research has been conducted to understand why individuals initially turn to NSSI as a form of emotion regulation rather than culturally sanctioned and safer methods. Due to recent research attention on belief systems, specifically in the emotion domain (see Tamir & Mauss, 2011; De Castella et al., 2013), this study explored whether individuals with a history of NSSI may turn to this behavior due to beliefs that they cannot control their emotions.

Main Findings

Contrary to the first hypothesis that individuals who experience greater entity beliefs about emotions will report more frequent NSSI behaviors, results showed that entity beliefs were not associated with frequency of NSSI behaviors. However, consistent with the second hypothesis that individuals who experience greater entity beliefs about emotions will show greater NSSI urges, results showed that there was an association between entity beliefs and NSSI urges.

The finding of an association between entity beliefs about emotions and NSSI urges expands the framework of NSSI beyond the functional models that prior research has investigated. Previous research has primarily focused on why NSSI is such an effective form of emotion regulation (see Stanley et al., 2010; Kemperman et al., 1997) and what functions these behaviors provide. Results from this study suggest that while understanding the functions of NSSI are important, having insight into individuals' beliefs about why they choose such harmful, and potentially life-threatening methods of emotions regulation is paramount in understanding NSSI. In sum, research must expand its inquiries to encompass a broader, yet deeper understanding of the origins of NSSI.

In addition to our hypothesis that individuals who experience greater entity beliefs about emotions will show greater NSSI urges, analyses showed that for some emotions individuals responded differently to subtypes of entity beliefs ("no matter how hard they try, people can't really change their emotions" and "the truth is, people have very little control over their emotions"). While this only held true for anxiety measured post mood induction, this finding adds further evidence for the need to better understand the origins of NSSI as well as the role certain emotions play in emotion regulation.

Theoretical Interpretations of Main Findings

It is logical to assume that NSSI behaviors and NSSI urges would stem from a common underlying cause, i.e. urges to engage in NSSI are an antecedent to the behavior. However, the results of this study did not confirm that NSSI behaviors and NSSI urges both are associated with underlying entity beliefs about emotions. While this may seem contrary to expected results, it is imperative to remember the role that lab-based emotion

inductions play in real-time measurements of emotions, beliefs, and NSSI urges.

While studies often measure urges to engage in NSSI in general (in general, how strong are your urges to hurt yourself?), this was the first study to assess urges to engage in NSSI in response to a negative emotional situation. This was executed through the mood induction where participants were asked to recall a painful argument with a significant other or friend. Due to real-time measurement of urges alongside questions assessing entity beliefs about emotion, this study was able to reduce the effects of memory bias often found when assessing NSSI behaviors, yielding data to better test the hypotheses.

In sum, through inducing negative emotions and measuring urges to engage in NSSI concurrently, data were obtained that were not distorted by memory bias, thus confirming the association between entity beliefs about emotion and urges to engage in NSSI. However, due to the ethical principal of beneficence, a conclusive association between entity beliefs about emotion and NSSI behaviors could not be confirmed.

Limitations and Future Directions

It is inevitable that research studies will have limitations, yet with every limitation comes the promise of greater knowledge for future studies. While this study assessed NSSI, only one single-item measure was used to assess NSSI ("I have hurt myself on purpose"). No criteria were given as to what constituted NSSI. Subjects may have believed that they have not engaged in NSSI since their NSSI behaviors may not be "typical" behaviors. Therefore, we cannot be sure whether in fact more of the participants may have a history of NSSI.

A second limitation to this study was the inability to have participants engage in NSSI behaviors during this study. Since doing so would raise ethical concerns this was not possible. While urges to engage in NSSI were measured in real time, NSSI behaviors were assessed in general. No restrictions were set to include only participants who had engaged in NSSI within a certain time frame, e.g. in the last 6 months. Therefore, when a participant responded to the statement "I have hurt myself on purpose" it could not be determined whether he or she may have engaged in NSSI recently or many years ago. Similarly, although participants were provided with a Likert scale to measure the frequency of their engagement in NSSI (1 = never, 4 = frequently) it could not be determined whether these behaviors occurred occasionally in the past or were happening in the present.

Likewise, this study used correlational tests to assess the data. Correlation does not imply causation and the direction could not be determined from this type of test. Therefore, it is unknown whether individuals experience urges to engage in NSSI because they believe that they were powerless to control their emotions, or individuals who hold entity beliefs about emotions are at a higher risk for using maladaptive coping strategies.

A further limitation was the sample used for this study. UC Berkeley is among a handful of highest ranked universities in the world. Therefore, the pressure to excel in academic achievement may be more extreme at this institution compared to many other colleges and universities, thus leading to higher levels of stress in students. While current research indicates that NSSI rates may be close to 40% in college age individuals (Rodham & Hawton, 2009), further studies with diverse samples and detailed background information are needed to replicate the finding of an association between entity beliefs and urges to engage in NSSI and to confirm generalizability.

Although no study is without limitations, this study's initial findings may help inform how treatment is conducted with individuals who have a history of NSSI. This study supports prior research findings that individuals who engage in NSSI exhibit poor emotion regulation strategies. The findings of increased anxiety and sadness post mood induction further support the belief that individuals who engage in NSSI have poor emotion regulation abilities. Reappraisal—the ability to change how one thinks about a situation—is paramount in successful emotion regulation, and may be an emotion regulation strategy that these individuals lack. Teaching individuals who have a history of NSSI urges or behaviors to learn to view and understand emotion-eliciting situations from different perspectives may decrease not only negative affect but NSSI urges or behaviors as well.

Moreover, the findings of increased anxiety and sadness post mood induction illustrate NSSI's ineffectiveness to change how an individual thinks about a situation or to understand the root cause of the emotional pain. An individual may engage in NSSI believing that the behaviors will permanently alleviate negative affect. Instead, NSSI only provides temporary relief from the negative emotions, leaving the cause of the emotions themselves unresolved. This lack of resolution may lead individuals to believe that ultimately they have no power to change their emotions. This confirms Klonsky's (2007) Affect-Reg-

ulation model in which individuals engage in NSSI to decrease or alleviate high arousal or negative emotions. Through this cycle of emotional turmoil and temporary relief, followed by a surge of negative emotions (resulting from the originally unprocessed emotion), an individual comes to believe that he or she in truth has no control over his or her emotions. Therefore, it is imperative that individuals with poor emotion regulation abilities not only challenge negative emotions but also challenge their belief systems. Furthermore, helping individuals process the situations that cause negative emotions to arise, as well as teaching reappraisal skills and promoting healthy coping strategies, may decrease the high rates of NSSI in the young adult population and in turn decrease misdiagnoses.

5. Conclusion

This study expands upon previous research to investigate the link between emotional beliefs and NSSI. The findings of this study support the hypothesis that individuals who experience urges to engage in NSSI hold beliefs of powerlessness to control emotions. Most importantly, this study validates the power of real-time measures to assess the often secretive behaviors and their emotional correlates; furthermore, it proposes a new way of treating individuals who have a history of NSSI that eliminates misdiagnosis and treats what may be the root of the problem. This study was the first of its kind to assess current NSSI urges through lab-based mood induction. However, because of ethical limitations, an association between NSSI and entity beliefs could not be established. It is hoped that with the increase in new and creative ways to conduct psychological research, future studies will not only continue to work within the ethical guidelines, but also find creative ways to assess hard to measure co-variables such as entity beliefs about emotion and NSSI behaviors.

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Effects of Activated Self-Concepts on Advertisement and Brand Attitudes

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ABSTRACT. Consumers often choose to purchase a brand based on its associations with perceived reference groups. The brand imbued with a personality then helps consumers create or maintain various types of self-concepts. Escalas and Bettman (2003) have found that people who tend to self-enhance prefer brands that are associated with aspirational groups while people who tend to self-verify prefer brands that are associated with membership groups. Extending upon their findings, the present study investigates how priming self-enhancement goals or self-verification goals influences consumers' attitudes toward brands and advertisements that are associated with either membership groups or aspirational groups. The results demonstrate that while priming does not have a direct correlation with ad and brand attitudes, the combination of priming and preexisting self-motivation tendencies significantly influence brand and ad preferences. Participants who agreed with the self-enhancement statement had more positive attitudes for the brand and the advertisement associated with aspirational groups, and participants who agreed with the self-verification statement had more positive attitudes for the brand and the advertisement associated with membership groups. In addition, the degree to which participants identified or wished to identify with the reference groups was positively correlated to their ad and brand attitudes. The findings' marketing and self-concept implications are discussed.

1. Introduction

In recent years, consumer research has paid increasing attention to the psychological benefits that people seek in brands (Bearden & Etzel, 1982; Belk, 1998; Chan, Berger, & Boven, 2012; Englis & Solomon, 1995; Fournier, 1998; Gasana, 2009; Landon, 1974; White & Dahl, 2006). Marketers have realized that tapping into consumers' self-goals or needs is a key instrument in building a connection between brands and consumers and eventually turning it into brand loyalty (Keller, 1993). Not surprisingly, a considerable number of studies have been published on the relationship between the self-concept and brand usage in consumer behavior, illustrating that consumers use brands to construct or maintain their self-concepts (Escalas & Bettman, 2005; Graeff, 1996; Grubb & Grathwohl, 1967; Kleine, Kleine, & Kernan, 1993; Reed, 2002).

One important aspect of this process derives from brands' associations with reference groups (Childers & Rao, 1992; Escalas & Bettman, 2003). Reference groups not only imbue brands with certain types of personality but also influence consumers' preferences and evaluations. Consumers use reference groups to judge whether there is a fit between their self-concepts and the brand's personality and make purchase decisions accordingly. If the self-concept of the consumer and the brand personality are congruent, a self-brand connection is formed and results in a brand preference. Because a self-brand connection is not easily changeable and can turn into brand loyalty, investigating the relationship between the self-concept and brand preferences can have significant marketing implications. This thesis contributes to the literature by examining self-enhancement and self-verification, which are aspects of self-goals, and their effects on consumers' attitudes toward brands that are associated with various reference groups.

The Self-Concept

The self-concept is denoted as "the sum of an individual's thoughts and feelings about herself or himself with respect to others" (Choi & Rifon, 2012, p. 640). There has been much debate in the self-concept literature about whether the self-concept is fixed or flexible, but studies

have suggested that self-concepts are dynamic and malleable (Markus & Wurf, 1987). Those who argue that the self-concept is dynamic state that the self-concept comprises a set of various self-conceptions that becomes salient depending on social and contextual factors (Aaker, 1999; Markus & Kunda, 1986). For example, the self that is salient during a meeting with a professor may be composed, intellectual, and polite while the self that is salient during a road trip with friends may be adventurous, spontaneous, and goofy. Following this idea, the theory of the working self-concept proposes that while people have stable self-concepts that are present throughout, situational factors significantly influence which particular aspect of the self-conceptions becomes accessible (Aaker & Lee, 2001). In other words, the self that an individual carries at any given moment consists of a set of core and tentative self-conceptions that become active in memory based on social circumstances.

The malleability of the self-concept implies that the self that is salient when forming a self-brand connection can be influenced by situational cues. Previous studies have found that it is possible to make an advertisement more effective or a brand more likable by presenting cues that will elicit a certain type of self in consumers (Chang, 2010; Yi, 1990). In this paper, I use this concept to explore how activated self-concepts can affect people's attitudes towards various advertisements and brands.

Various types of the self such as the actual self, the ideal self, and the avoided self affect self-conceptions (Markus & Nurius, 1986). This paper focuses on the ideal self and the actual self. The actual self pertains to how an individual perceives him or herself, and the ideal self pertains to how an individual wishes to perceive him or herself (Sirgy, 1982). People who prioritize their actual selves are more likely to look for self-verifying feedback from others to maintain their current self-concepts whereas people who focus on discrepancies between the actual self and the ideal self are more likely to look for self-enhancing feedback (Higgins, 1987). The ideal self includes a range of positive selves that are not included in the current self-concept (Markus & Nurius, 1986). Ironically, the range of ideal selves is constrained and usually derives from types that are visible in the media and individual's social background and experiences (Markus & Nurius, 1986). This notion has important implications for marketers and suggests that they can utilize appropriate cues to make certain types of possible selves accessible.

Some researchers in the past have attempted to distinguish the relationship between the actual self or the ideal self and brand connections (Landon, 1974; Mehta, 1999). For example, Landon (1974) examined how the actual self and the ideal self affect consumers' purchase intent and tried to determine whether one type of self has a greater influence than the other. He found significant individual differences in the correlations between the type of self-concept and purchase intent and concluded that there is no dominant type of self that affects purchase decisions more than the other. One of the study's limitations is that it used product categories rather than real brands to correlate them to actual or ideal self. It is unclear how strong of a personality a generic product could have had compared to a brand which is commonly imbued with a distinct personality. The present study overcomes this limitation by using a brand as a stimulus rather than focusing on a product category.

Nevertheless, Landon (1974) provided important concepts in terms of possible moderating factors. He conjectured that individuals who follow "the actualization school" would show a higher correlation between the actual self-image and purchase intention because they strive to preserve their actual self-concepts. On the other hand, those who follow "the perfection school" would show a higher correlation between the ideal self-image and purchase intention because they would want to present themselves most positively by purchasing the product. Consumer psychology has developed considerably since then, and his conjecture has been confirmed true (Escalas & Bettman, 2003). Here I interpret "the actualization school" as self-verification and "the perfection school" as self-enhancement.

Individuals rely heavily on social approval in maintaining their self-concepts, indicating that this effort will also transfer to their consumer behavior (Grubb & Grathwohl, 1967; Hogg & Terry, 2000; Reed, 2002; Westen, 1992). Indeed, researchers found that various aspects of self-conceptions including interdependence (Chang, 2010) and public self-consciousness (Miller & Cox, 1982) have significant impacts on consumer behavior.

Self-Enhancement and Self-Verification

Self-enhancement theories state that individuals desire to present themselves in a positive manner and seek out favorable feedback regardless of whether the evaluations are accurate or inaccurate (McCaslin, Petty, & Wegener, 2010). On the other hand, self-verification denotes individuals' tendency to seek out feedback that

confirms their self-views (Swann, 1987) and is rooted in people's need for the world to be predictable and controllable (Swann, 1990).

The two self-goals play important roles in consumer behavior. Significant findings by Escalas and Bettman (2003) showed how these two self-goals influence the types of reference groups that consumers choose when they form their attitudes toward various brands. They found that individuals with high self-enhancement goals are more influenced by aspirational groups while individuals with high self-verification goals are more influenced by membership groups. Their studies are used as a framework to develop the hypotheses of the current study.

Reference Groups in Consumer Behavior

Denoted as "a person or group of people that significantly influences an individual's behavior," a reference group plays an important role in consumer behavior by lending its associations and meanings to brands and products (Bearden & Etzel, 1982, p. 184). Reference groups can be categorized into membership groups, aspirational groups, or dissociative groups, and they influence consumer attitudes in numerous ways (Escalas & Bettman, 2003). This paper focuses on the membership group, a group to which individuals belong, and the aspirational group, a group to which individuals wish to belong. Consumers use brands that are associated with their membership groups to fit in the group and signal their ties (Escalas & Bettman, 2005). For example, a member in a dance company may signal his or her membership group identity by wearing a New Era fitted hat or Vans which are commonly-used brands by dancers.

Unlike membership groups, which require a sense of belonging and interactions with other members, aspirational groups require little or no interaction. Nonetheless, aspirational groups can heavily influence people's brand preferences when they match people's ideal selves (Cocanougher & Bruce, 1971). Celebrities are often used as aspirational group members when endorsing brands. Reference groups can also include, but are not limited to, people who are wealthy, successful, or intelligent. People often use brands that are associated with aspirational groups to forge the ties with the groups and thus project a favorable self-image (Choi & Rifon, 2012). For example, a person who aspires to portray himself as more masculine may choose to drink Jack Daniels instead of other brands because of its association with masculinity. The power of each reference group may depend on moderating factors, such as whether the product is consumed publicly or privately and whether the product is functional or symbolic (Bearden & Etzel, 1982).

The associations between a brand and a reference group can be real or fabricated. Therefore, marketers can forge an association and reinforce it by presenting the brand in ways that are consistent with the reference group. Using the reference group's images to advertise the brand is one of the many ways to create and reinforce the brand identity borrowed from the group. The present study presents print advertisements with reference group images to show how these can create certain brand associations.

Self-Brand Connection

The self-brand congruency theory combines all of the aforementioned elements—the working self-concept, self-goals, and reference groups—to explain how consumers form connections to brands. This widely-accepted theory states that consumers are more likely to prefer products that match their self-concepts, whether the self-concepts in question are actual or ideal (Chang, 2002; Hong & Zinkhan, 1995; Kleine, Kleine, & Kernan, 1993). Since brands borrow their identities from reference groups, the psychological connection between individuals and their membership group or aspirational group plays a key role in this process (White & Dahl, 2006). The desire to identify with the membership or aspirational group is heavily reflected on people's consumption behavior. Previous studies have found that individuals create stronger self-brand connections to brands that are consistent with their reference group image and even consider the act of consuming symbols an essential part of membership group bonding (Escalas & Bettman, 2005). To fulfill the ideal self by associating themselves with aspirational groups, individuals also seek values such as prestige or exclusivity in brands and transfer their associations to their self-concepts (Solomon, 1983).

Priming Effects

Because the self-concept is malleable, situational cues can elicit certain aspects of the self and influence individuals' purchase behaviors (Chang, 2010). Various factors such as context, group salience (Lessig & Park, 1978), self-goals (Escalas & Bettman, 2003), degree of independence (Chang, 2010), and public self-consciousness (Miller & Cox, 1982) have been found to affect the

self-brand congruency. Some researchers have applied this concept to consumer research and studied how activating an aspect of the self influences consumer behavior (Aaker & Lee, 2001; Chang, 2010; Forehand & Deshpandé, 2001). For example, Chang (2010) primed either independent or interdependent self-concept using magazine articles that came before the print advertisement and found that people primed with independent self-concept preferred a larger product assortment range. Other studies have found that advertising can prime certain product attributions to influence consumers' judgments of the products (Yi, 1990).

However, while much is currently known about the self-brand congruency, there has been little empirical research into what would happen if a certain kind of self-goal were purposely activated by priming cues. The present study attempts to fill this gap by investigating how activation of self-goals will impact consumers' attitudes toward brands and advertisements that are associated with reference groups.

Hypotheses

Based on the literature review and theoretical development, the following hypotheses were formulated.

Hypothesis 1: People whose self-enhancement goals are activated will have more positive attitudes toward a brand/advertisement associated with an aspirational group while people whose self-verification goals are activated will have more positive attitudes toward a brand/advertisement associated with a membership group.

Hypothesis 2: The extent to which individuals feel like they belong or wish to belong to a group will influence their attitudes toward the brand/advertisement associated with the reference group.

The present study is not only based on the premise that the study will extend Escalas and Bettman's (2003) previous findings, but also on the premise that understanding whether self-goals can be primed to influence consumers' attitudes will help us learn about the extent to which the self-concept is malleable and contribute to the consumer psychology literature.

2. Method

Participants

The study was conducted via online survey using Qualtrics software sponsored by Princeton University's Survey Research Center. A random sample of 848 undergraduate students from Princeton University was invited to participate via email. They could access the survey through a unique link included, and each link created an ID and ensured that no participants took the survey more than one time. Of the 383 responses received, 213 responses were fully completed and used for analysis. Participation was voluntary, and there was no compensation.

The participant sample included 86 Caucasians (40.4%), 11 Hispanics (5.2%), 38 African Americans (17.8%), 66 Asians (31.0%), and 12 others (5.6%). They comprised 80 male participants (37.6%) and 133 female participants (62.4%). In terms of class year, participants included 58 freshmen (27.2%), 61 sophomores (28.6%), 36 juniors (16.9%), and 58 seniors (27.2%).

A generic tablet was chosen as the stimulus product after careful consideration. As a commonly used product among the participant population, a tablet can be used privately or publicly and have both symbolic and functional values. Therefore, it was assumed that using a tablet for the study could minimize potential confounding variables. The tablet image used in the advertisements was not of an easily recognizable brand. Additionally, Chang's studies (2010) which also included priming aspects of self-concepts used printers, a private good, in the first study and shoes, a public good, in the second study and concluded that the findings were not affected by product types.

I chose Princeton students to represent the membership group and rich or successful people to represent the aspirational group. Each reference group had two different images to increase external validity.

For the membership group, the generalizability and degree of membership were important factors to consider. Because Princeton University students generally tend to have a strong school pride, it was predicted that most students would feel a strong sense of belonging to the Princeton community. One of the pictures, which will be referred to as the "study image," featured two Princeton students lying down on the grass on campus studying (Figure 1). The other picture, which will be referred to as the "sports image," included a group of Princeton students displaying school spirit at a football game (Figure 2). To enhance the advertisement's associations with the membership groups, the tagline, "Princeton students' new go-to tablet. Join the community," was included.

For the aspirational group, the generalizability and degree of aspiration to belong to the group were im-

portant factors to consider. Previous studies have shown that most college undergraduates have affluent and successful images among their ideal selves (Markus & Nurius, 1986). It could be inferred that Princeton students who tend to be ambitious and success-driven also fit the category. Therefore, using wealthy and successful people to represent the aspirational group without indicating any specific profession seemed appropriate. Specifically, one picture, which will be referred to as the "rich image," included a group of well-dressed, attractive people at a luxurious beach house (Figure 3). The other picture, which will be referred to as the "success image," consisted of a group of businesspeople in suits at a firm (Figure 4). To enhance the advertisement's associations with the aspirational groups, the tagline, "Strive for extraordinary," was included. For the control group, a print advertisement featured the brand without a reference group (Figure 5).

All advertisements included a general product description, but it was intentionally presented in a small size font for membership group and aspirational group advertisements to make participants focus on the reference groups and the taglines. Only the control group used the product description as the focus component of the advertisement.

Procedure

Participants first filled out a consent form in which they were informed that the study was "regarding self-goals and consumer behavior." The purpose of the first task was to prime participants with either self-enhancement or self-verification goals. Participants were randomly assigned to either self-enhancement or self-verification prime condition and were instructed to read a short paragraph about a fictitious Princeton student named Robert. In the self-enhancement prime condition, Robert was described as a typical self-enhancer in domains that students can relate to such as academics, extracurricular activities, and close relationships. For example, the paragraph stated that he "preferred professors who gave him positive feedback even when he knew his work could be improved," "avoided situations in which people could criticize his skills as a dancer," and "loved spending time with his girlfriend who always gave him a lot of compliments." The full prime story can be viewed in Appendix A.

In the self-verification prime condition, Robert was described as a self-verifier in the same domains. For example, he "preferred professors who gave him constructive criticism than those who only said positive things," "liked it better when people confirmed his view about his skills as a dancer," and "loved spending time with his girlfriend who knew him well intuitively" (Appendix A). The stories were carefully constructed in order to ascertain that neither of the conditions made Robert seem unlikeable or incompetent. The domains and the name of the character were kept constant for both stories to avoid confounding variables.

After reading the story, participants were given the following instruction: "Reflect on your life and write about moments when you thought or behaved like Robert did." For each condition, a short description of self-enhancement goals ("trying to present yourself in the most positive light, seeking positive evaluations, etc.") or self-verification goals ("preferring others to see you as you see yourself, seeking to confirm your self-view, etc.") was included to lead participants to respond in accordance with the definition of each self-goal (Appendix A). This step was intended to make participants empathize with Robert by making similar experiences in their lives salient and thus enhance the priming effect.

Participants in the control group (n = 56) skipped the prime task. The control group was included in the study in order to set a reference point to which the results from the membership group advertisement condition and the aspirational group advertisement condition results could be compared.

In the next task, participants were told that they will see a print advertisement of a new tablet and were instructed to examine the advertisement and the brand carefully (Appendix A). Regardless of which self-goal prime condition, participants were randomly assigned to either membership group advertisement or aspirational group advertisement featuring the new tablet. After the exposure to the brand advertised in one of the conditions, participants were asked to complete a questionnaire. The questionnaire asked participants to indicate their attitudes toward the brand on the dimensions of "good," "likable," "pleasant," "positive," and "high quality," adopted from Holbrook and Batra (1987). Their attitudes toward the advertisement were measured on the dimensions of "interesting," "good," "likable," "favorable," and "pleasant," adopted from MacKenzie, Lutz, and Belch (1986). The ratings were on Likert scales of 1 (strongly disagree) to 7 (strongly agree). The brand and ad attitudes scale items can be viewed in Appendix B.

In addition to their attitudes about the brand and

the advertisement, participants also rated the relevance of the reference groups in the advertisement to themselves. Participants who saw one of the membership group advertisements were presented with the statements that read, "I associate with people in the ad," and "I enjoy being a Princeton student" (Appendix C). Participants who saw one of the aspirational group advertisements were presented with the statements that read, "I aspire to be like people in the ad," and "People in the ad look like successful people." Again, participants rated how much they agreed with the statements on Likert scales of 1 (strongly disagree) to 7 (strongly agree). These questions were designed to account for variability in participants' ratings of the brand and the advertisement. If the hypothesis by Escalas and Bettman (2003) holds true, then it is reasonable to assume that responses can vary depending on how much participants actually viewed Princeton students as their membership group and rich or successful people as their aspirational group. Therefore, these measures provided an additional way to analyze the validity of the results.

The questionnaire then asked participants about their purchase intent for the brand. Given that participants were unfamiliar with the fictitious brand and were exposed to the advertisement only once for a brief moment, it was decided that the purchase intent question should be more conditional. Therefore, the purchase intent question for the three dimensions asked, "What are your chances of buying the product the next time you need to purchase a tablet?" They then rated their purchase intent on Likert scales of 1 to 7 with three dimensions, which were "likely" to "unlikely," "possible" to "impossible," and "probable" to "improbable." The modified purchase intent question and the measures were adapted from the work by Yi (2010). The purchase intent question is included in Appendix D.

After answering the questions about the brand and the advertisements, participants were asked to read two statements about self-goals and indicate where they stand. This measure aimed to examine whether there was a correlation between the prime paragraph participants read and self-goals they reported and if not, to understand participants' predominant self-motivations. On one end of the seven-point Likert scale, the statement read, "It is important that people see me in the best possible light" to represent self-enhancement goals. The other end read, "It is important for me to have accurate information about my strengths and weaknesses" to represent self-verification goals. The two scale items were borrowed from Escalas and Bettman's (2003) experiment, but instead of having two separate scales, it was presented as a continuum in order to make people choose one or the other. The self-goals scale is presented in Appendix E.

Participants in the control group, who were not exposed to a prime condition, saw the version of the print advertisement that purposely did not include any reference group but featured the product by itself with the same product description. After viewing the advertisement, participants in the group rated their attitudes toward the brand, advertisement, and purchase intent in the same way as in the other conditions. They also indicated their predominant self-goals in the same way.

Lastly, all participants answered a few demographics questions about gender, race, and class year and were fully debriefed. Participants were asked whether they formulated any hypothesis while taking the survey. Of the few who attempted to guess the hypothesis, none of the participants guessed it correctly.

3. Results

Manipulation Checks

When participants were exposed to either the membership group advertisement or the aspirational group advertisement, the advertisement they saw included one of the two images chosen for the reference group. The images were varied for each reference group in order to increase external validity, and thus two aspects of the reference group advertisements needed to be checked: 1) Did participants identify with people in the two images of each reference group to a similar degree? 2) Did participants perceive the people in the advertisements as representative of their membership group and aspirational group? A one-way test ANOVA was used to check the two variables.

Membership Group Ads. For participants exposed to the membership group advertisement, either the study image or the sports image was included in the advertisement. Between the two images, there was a significant preference for the study image over the sports image regardless of the prime condition. Regarding ad attitudes, the results indicate that the advertisement using the study image was perceived as more pleasant, $F(2, 130) = 4.36$, $p < .05$. Brand attitudes toward the study image were rated more positively than the sports image for items which indicated

that the tablet in the study image was perceived as marginally more likable, $F(2, 130) = 2.62$, $p < .1$, pleasant, $F(2, 130) = 5.18$, $p < .01$, and high quality, $F(2, 130) = 7.95$, $p = .001$. Other items on brand and ad attitudes were not significantly different, but as shown in Table 1, the study image generally had higher scores than the sports image. Although the preference toward the study image was surprising at first, it may be the case that the sports image did not depict a context in which people will use a tablet. Although the degree of membership participants felt for each group was not significantly different, it may also be the case that the sports image represented a more specific type of students who whom not all participants identify with. On the other hand, people in the study image could have appealed to more participants because people in the image were in a situation where using a tablet could be appropriate and were more general and relatable to all Princeton students.

For the membership group advertisement, the measures used in the study to check the degree were the two statements on seven-point Likert scales: "I associate with the people in the ad" and "I enjoy being a Princeton student." The mean score was 3.72 (SD = 1.54) for the first statement and 5.75 (SD = 1.19) for the second statement, indicating that participants generally enjoyed being Princeton students. As shown in Table 2, no significant difference was found between the two images in terms of how much participants associated with the people in the group or enjoyed being Princeton students. The mean for the first statement could be due to several possible reasons. Because the images used for the membership group advertisement could not represent the entire student body at Princeton, other salient factors such as race or student types could have affected the degree of membership. One participant commented that she did not associate with the group because most of the people in the group were Caucasian while she was African American.

Aspirational Group Ads. For participants exposed to the aspirational group advertisement, either the rich image or the success image was included in the advertisement. Between the two images, there was a significant preference for the success image over the rich image. Participants rated the brand in the success image as more likable, $F(2, 130) = 2.776$, $p < .1$, positive, $F(2, 130) = 3.412$, $p < .05$, and high quality, $F(2, 130) = 8.662$, $p < .001$. They also indicated that it was more likely, $F(2, 130) = 6.682$, $p < .005$, and probable $F(2, 130) = 4.295$, $p < .05$, that they will purchase the tablet in the success image than rich image (Table 1). Participants reported that they perceived the rich image advertisement as more interesting, $F(2, 130) = 3.355$, $p < .05$, showing that interesting advertisements do not necessarily lead to more positive evaluations.

The measures used to check the degree of aspiration were the following statements on seven-point Likert scales: "I aspire to be like people in the ad" and "People in the ad look like successful people." The mean score for the first statement was 3.65 (SD = 1.45) and 5.21 for the second statement (SD = 1.24). Without doubt, people in the success image (M = 5.55, SD = .942) were perceived as more successful, $F(1, 64) = 9.17$, $p < .005$, than people in the rich image (M = 4.65, SD = 1.47). There was no significant difference in how much participants wished to belong to the group in each image.

Table 1. Comparison of images within ads using membership groups (MG) and aspirational groups (AG)

		Study MG	Sports MG	Sig	Rich AG	Success AG	Sig
Brand Attitude	Good	3.97 (1.09)	3.85 (1.31)	.599	3.69 (1.29)	4.21 (1.14)	.017
	Likable	4.56 (.960)	4.15 (1.52)	.077	3.69 (1.44)	4.17 (1.03)	.066
	Pleasant	4.76 (.987)	3.94 (1.21)	.007	3.96 (1.17)	4.12 (1.29)	.590
	Positive	4.65 (1.01)	4.44 (1.38)	.251	3.85 (1.29)	4.45 (1.21)	.036
	High Quality	3.56 (1.11)	3.03 (1.40)	.001	3.77 (.276)	4.45 (.215)	.000
Ad Attitude	Interesting	3.29 (1.34)	3.15 (1.46)	.861	3.77 (1.56)	2.83 (1.40)	.038
	Good	3.53 (1.24)	3.15 (1.33)	.424	3.12 (1.18)	3.62 (1.41)	.279
	Likable	4.09 (1.16)	3.88 (1.57)	.439	3.50 (1.61)	3.88 (1.27)	.293
	Favorable	3.79 (1.18)	3.56 (1.54)	.709	3.35 (1.36)	3.93 (1.37)	.208
	Pleasant	4.47 (.222)	3.62 (.222)	.015	3.73 (1.45)	4.10 (1.30)	.450
Purchase Intent	Likely	2.35 (1.43)	2.32 (1.61)	.742	1.75 (1.06)	2.98 (1.35)	.002
	Possible	3.94 (1.58)	3.59 (1.83)	.583	3.46 (1.56)	4.17 (1.59)	.182
	Probable	2.65 (1.48)	2.53 (1.84)	.901	2.04 (1.11)	3.02 (1.24)	.016

Note. Standard deviations appear in parentheses.

Table 2. Attitudes towards membership group ads

Attitudes toward Membership Group Ads	Study	Sports	Sig	Total
Degree of Membership	3.79 (1.51)	3.65 (1.59)	.751	3.72 (1.54)
Degree of Membership Enjoyment	5.82 (1.14)	5.68 (1.25)	.660	5.75 (1.19)

Note. Standard deviations are in parentheses.

Table 3. Attitudes towards aspirational group ads.

	Rich	Success	Sig	Total
Degree of Aspiration	3.46 (1.56)	3.76 (1.39)	.298	3.65 (1.45)
Degree of Success	4.65 (1.47)	5.55 (.942)	.004	5.21 (1.24)

Note. Standard deviations are in parentheses.

Effects of Priming on Brand and Ad Attitudes

Participants' attitudes toward the brand and the advertisements as a function of the self-enhancement and self-verification primes are presented in Table 4. I predicted that primed self-enhancement goals would make individuals prefer the aspirational group advertisement while primed self-verification goals would make individuals prefer the membership group advertisement. The data that are presented in Table 4 show only limited support for my hypothesis. Participants in the self-verification prime condition rated the membership group advertisement marginally more positive than they rated aspirational group advertisement, $F(1, 132) = 3.108$, $p < .1$. There were no significant differences on any of the other dependent measures comparing self-enhancement and self-verification groups.

Table 4. Brand and Ad Attitudes as a Function of Primes

		Self-Enhancement Prime			Self-Verification Prime			
		Aspirational	Membership	Sig.	Aspirational	Membership	Sig.	Total Sig.
Brand Attitude	Good	3.82 (1.29)	3.81 (1.17)	.950	4.21 (1.12)	4.03 (1.23)	.357	.705
	Likable	4.00 (1.30)	4.25 (1.25)	.406	3.97 (1.14)	4.47 (1.32)	.109	.565
	Pleasant	4.12 (1.41)	4.39 (1.05)	.352	4.00 (1.07)	4.313 (1.31)	.298	.921
	Positive	4.32 (1.45)	4.44 (1.16)	.694	4.12 (1.07)	4.66 (1.26)	.080	.328
	High-Quality	3.97 (1.73)	3.306 (1.17)	.051	4.412 (1.28)	3.281 (1.42)	.001	.339
Ad Attitude	Interesting	3.26 (1.60)	3.06 (1.35)	.550	3.12 (1.45)	3.41 (1.47)	.424	.323
	Good	3.35 (1.35)	3.25 (1.25)	.745	3.5 (1.39)	3.44 (1.56)	.848	.929
	Likable	3.82 (1.59)	4.00 (1.35)	.600	3.65 (1.23)	3.97 (1.45)	.353	.763
	Favorable	3.85 (1.46)	3.61 (1.27)	.465	3.56 (1.31)	3.75 (1.48)	.575	.362
	Pleasant	4.12 (1.55)	3.86 (1.15)	.419	3.79 (1.12)	4.25 (1.44)	.164	.119
Purchase Intent	Likely	2.56 (1.42)	2.39 (1.63)	.626	2.44 (1.39)	2.28 (1.40)	.656	.984
	Possible	4.18 (1.82)	3.67 (1.79)	.199	3.62 (1.33)	3.88 (1.62)	.528	.379
	Probable	2.59 (1.37)	2.67 (1.59)	.812	2.71 (1.19)	2.53 (1.30)	.544	.548

Note. Standard deviations appear in parentheses.

Effects of Self-Goals on Brand and Ad Attitudes

In the absence of statistically significant differences on product preferences as a function of my priming manipulation, I reanalyzed the data as a function of people's expressed degree of self-verification and self-enhancement. In the study, participants had been asked to indicate where they stand regarding self-goals. The seven-point scale had the self-enhancement statement on one end and the self-verification statement on the other end (Appendix E). It should be noted that the indication for self-goals occurred after participants had been exposed to one of the two primes and advertisements, and therefore the reported self-goals could have been affected by the prime conditions. For the purpose of this analysis, only participants whose score reflected a strong tendency for self-enhancement goals (1 and 2) or self-verification goals (6 and 7) were included. Participants were dichotomized as self-enhancers or self-verifiers accordingly.

The results of participants' preferences for the advertisements grouped by their standing on the self-enhancement/self-verification individual difference measure are shown in Table 5. In several dimensions, self-enhancers rated the aspirational group advertisement higher than self-verifiers did, and self-verifiers rated the membership group advertisement higher than self-enhancers did. As expected, self-enhancers found the brand in the aspirational group advertisement better, $F(1, 70) = 3.96$, $p = .05$, more likable, $F(1, 70) = 6.63$, $p < .05$, and higher quality, $F(1, 70) = 4.06$, $p < .05$. They also found the aspirational group advertisement as marginally more interesting, $F(1, 70) = 3.00$, $p < .1$, and reported that it was more likely, $F(1, 70) = 4.65$, $p < .05$, and possible, $F(1, 70) = 5.823$, $p < .05$, for them to purchase the brand in the aspirational group advertisement.

Similarly, self-verifiers perceived the brand in the membership group advertisement as marginally more likable, $F(1, 70) = 3.825$, $p < .1$, and the membership group advertisement as marginally more favorable, $F(1, 70) = 3.789$, $p < .1$. It was also more possible for self-verifiers to purchase the brand in the membership group advertisement than self-verifiers, $F(1, 70) = 4.90$, $p < .05$. The results from two scale items, "likable" and "possible," are shown in Figure 6 to represent the general findings. The results from this analysis partially support the original hypothesis because the self-goals reported by participants are a combination of preexisting self-motivations and priming.

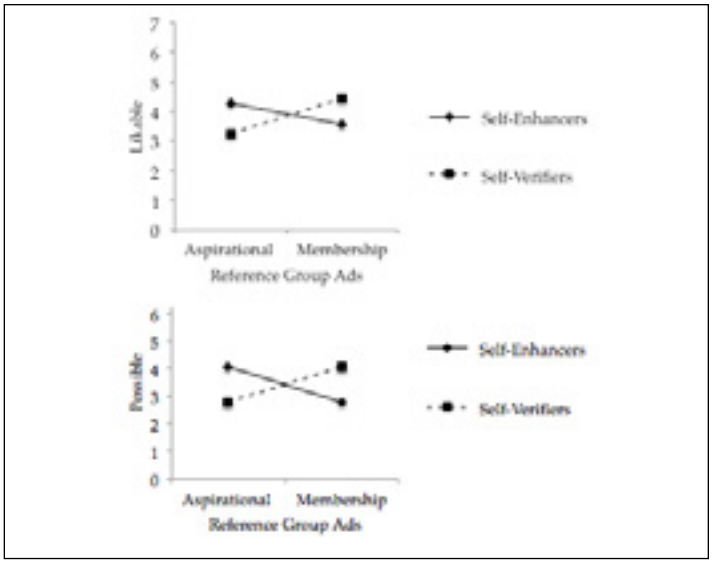
Degree of Aspiration and Membership Association

Adopting Escalas and Bettman's hypothesis (2003), I expected that the degree to which participants saw themselves belonging to the membership group or the degree to which they aspired to belong to the aspirational group would affect their preferences for the advertisement and the brand associated with the reference group. I dichotomized participants who rated the degree of aspiration 5, 6, or 7 as high aspiring and those who rated the degree of aspiration 1, 2, or 3 as low aspiring. For the aspirational group advertisement, ten out of thirteen scale items showed a significant difference and confirmed the hypothesis; high aspiring participants evaluated the brand and the advertisement more positively than low as-

piring participants did (Table 6.). In addition, high aspiring participants perceived the people in the advertisement as more successful, $F(1, 51) = 27.32$, $p < .001$. The degree of aspiration was also positively correlated to the degree of self-enhancement goals $F(1, 24) = 8.754$, $p < .05$.

For the membership group advertisement, I operationalized participants who rated the degree of membership 5, 6, or 7 as high membership and those who rated the degree of membership 1, 2, or 3 as low membership. The results are shown in Table 6. Every measure showed a significant difference and confirmed the hypothesis, indicating that the stronger the sense of belonging participants felt to the group, the more positive they evaluated the brand and the advertisement. Furthermore, participants rated that it was more likely, $F(1, 50) = 8.632$, $p < .005$, possible, $F(1, 50) = 2.945$, $p < .05$, and probable $F(1, 50) = 10.69$, $p < .005$, that they will purchase the product when they felt a greater sense of belonging to the group. The relationship between the sense of belonging participants felt and how much they enjoyed being Princeton students was also positively correlated, $F(1, 50) = 6.017$, $p < .05$.

Figure 6. Brand attitude (likable) and purchase intent (possible) as a function of self-goal reports.



4. Discussion

Summary of Results

The initial analysis found that the first hypothesis was partially supported since participants who were self-verification primed rated the membership group advertisement as more positive. However, participants who were self-enhancement primed did not show a significant priming effect. In addition, a strong relationship was observed between participants' reported self-goals and brand/ad attitudes, revealing that participants who reported to self-enhance preferred the aspirational group advertisement while participants who reported to self-verify preferred the membership group advertisement. Overall, in addition to confirming previous findings that showed that individuals' self-motivations affect their product preferences (Escalas & Bettman, 2003), the results in the present study extend upon them by suggesting the possibility of priming self-motivations.

The study also replicated another finding from previous research and proved that the extent to which participants felt like they belonged or wished to belong to a group affected their brand/ad attitudes (Escalas & Bettman, 2003). In another words, the more participants wished to belong to the aspirational group in the advertisement, the more they preferred the brand and the advertisement. Similarly, the more participants felt they belonged to the membership group in the advertisement, the more they preferred the brand and the advertisement. The findings support the argument that individuals use brands associated with their positive reference groups to form their self-concepts related to the actual self or the ideal self (Reed, 2004; Wright & Sirgy, 1992).

Table 5. Brand and Ad Attitudes as a Function of Self-Goal Reports.

		Aspirational Group Ad			Membership Group Ad			
		Self-Enhancers	Self-Verifiers	Sig	Self-Enhancers	Self-Verifiers	Sig	Total Sig
Brand Attitude	Good	4.20 (.696)	3.50 (1.24)	.050	3.64 (1.39)	3.80 (1.11)	.686	.106
	Likable	4.30 (1.58)	3.25 (1.16)	.012	3.57 (1.45)	4.43 (1.19)	.054	.002
	Pleasant	4.60 (1.72)	3.70 (.979)	.474	3.93 (1.33)	4.35 (1.14)	.362	.249
	Positive	4.20 (1.51)	3.60 (1.05)	.332	4.57 (1.34)	4.40 (1.05)	.694	.466
	High-Quality	4.40 (1.50)	3.55 (1.43)	.048	2.79 (1.19)	3.15 (1.14)	.436	.057
Ad Attitude	Interesting	3.63 (1.64)	2.80 (1.36)	.098	2.57 (1.40)	2.90 (1.41)	.521	.105
	Good	3.40 (1.23)	2.80 (1.06)	.123	2.86 (1.46)	3.10 (1.17)	.568	.145
	Likable	3.79 (1.30)	3.00 (1.30)	.110	3.43 (1.51)	3.80 (1.40)	.476	.100
	Favorable	3.85 (1.27)	3.30 (1.17)	.177	2.79 (1.21)	3.15 (1.21)	.056	.021
	Pleasant	3.95 (1.43)	3.45 (1.28)	.243	3.43 (1.56)	3.95 (1.15)	.269	.111
Purchase Intent	Likely	2.75 (1.52)	1.80 (1.01)	.035	2.36 (1.67)	2.25 (1.21)	.826	.203
	Possible	4.10 (1.68)	2.80 (1.51)	.018	2.79 (2.12)	4.10 (1.59)	.030	.002
	Probable	3.40 (1.39)	2.20 (.834)	.639	2.36 (1.67)	2.60 (1.27)	.605	.495

Note. Standard deviations appear in parentheses.

Implications

The results from the present study have numerous implications for both marketers and researchers. For marketers, the extended knowledge about the relationship between self-goals and reference groups as the means to form self-brand connections provides useful insight in designing effective marketing programs. Depending on whether a brand is associated with a membership group

or an aspirational group, marketers can strategically position the brand in situations where either self-enhancement goals or self-verification goals are salient. For example, affinity marketing, which incentivizes organizations to endorse a brand or a product to their members, can be effective for brands that are associated with membership groups (Fock, Chan, & Yan, 2011). In a similar manner, a brand associated with aspirational groups can benefit from engaging in product placement at events such as museum galas where people are likely to focus on self-enhancing.

Although more research is necessary to confirm the strength of the priming, advertisers may also benefit from eliciting self-enhancement goals or self-verification goals within the advertisement. As previous research shows, numerous advertising components such as the tagline and content surrounding the advertisement in a magazine can affect consumers' attitudes toward an advertisement or a brand (Snyder & DeBono, 1985). Therefore, using a tagline that is conducive to self-enhancement or publishing a self-improvement content page before presenting the advertisement for a luxury brand may help shape consumers' attitudes toward the brand and the advertisement. It should be noted that a single advertisement will not help change the predominant brand identity that already exists, but by consistently associating a brand with a reference group and the appropriate self-goal, the brand can increase its brand equity.

Table 6. Brand and Ad Attirudes as a Function of Degree Aspiration and Membership

		Low Aspiring	High Aspiring	Sig	Low Membership	High Membership	Sig
Brand Attitude	Good	3.62 (1.10)	4.50 (.762)	.001	3.70 (1.15)	4.48 (1.08)	.001
	Likable	3.40 (1.15)	4.73 (.919)	.000	3.83 (1.36)	4.74 (1.10)	.010
	Pleasant	3.55 (.876)	4.69 (1.26)	.000	3.92 (1.25)	5.00 (1.00)	.001
	Positive	3.65 (.975)	4.92 (1.08)	.000	4.06 (1.17)	5.22 (1.17)	.000
	High Quality	3.97 (1.51)	4.50 (1.45)	.166	2.67 (1.12)	4.13 (1.14)	.000
Ad Attitude	Interesting	2.68 (1.27)	3.50 (1.45)	.017	2.53 (1.28)	4.00 (1.09)	.000
	Good	2.95 (1.18)	3.96 (1.28)	.002	2.77 (1.19)	4.17 (1.03)	.000
	Likable	3.10 (1.30)	4.35 (1.50)	.001	3.26 (1.31)	4.74 (1.29)	.000
	Favorable	3.25 (1.32)	4.27 (1.22)	.002	2.89 (1.12)	4.57 (1.16)	.000
	Pleasant	3.35 (1.25)	4.35 (1.47)	.004	3.47 (1.34)	4.83 (1.30)	.000
Purchase Intent	Likely	2.15 (1.33)	2.62 (1.50)	.191	1.89 (1.21)	3.13 (1.84)	.003
	Possible	3.35 (1.67)	4.38 (1.44)	.012	3.31 (1.86)	4.26 (1.63)	.049
	Probable	2.38 (1.21)	2.69 (1.23)	.305	2.17 (1.21)	3.39 (1.75)	.002

Note. Standard deviations appear in parentheses.

These findings also contribute to the self-concept literature. Many researchers have debated whether people have a rigid preference for self-enhancement or self-verification and whether the two self-goals contradict or can compromise each other. This thesis provides further support for the argument that the working self-concept arises from a set of self-conceptions and can change depending on the context and situational cues individuals face. However, as the results showed, it is the combination of both predominant self-concept and environmental factors that come into play to shape one's working self-concept. The strength of priming may influence how malleable a self-concept can be, and this topic still needs more investigation.

Limitations and Future Research

This research should be interpreted with a several limitations in mind. First, since Princeton University is an elite Ivy League school, it could be argued that the membership group already fulfills the role of aspirational group. While it is true that many Princeton students may pride themselves on attending one of the top universities, I argue that it no longer acts as an aspirational group once students are admitted. In addition, because they have already acquired membership in Princeton, future career aspirations become more apparent and important to students. Nonetheless, there was a significant difference in participants' preferences between images used within the same reference group (study image vs. sports image; rich image vs. successful image), and future studies could benefit from conducting a pretest to find images that represent participants' membership group and aspirational group more accurately.

Second, it is possible that participants' general attitudes toward the brand were low due to lack of exposure to the brand in the past. A few participants commented it was unlikely that they would purchase the new brand of tablet because they only buy tablets from well-established brands such as Samsung or Apple. Even though a fabricated brand was used to facilitate manipulations, brand image or brand loyalty for existing brands could have been too strong for the brief exposure of a new brand to persuade them to switch. Future studies could enhance the validity of the findings by choosing another product to replicate the study.

Third, granted that the indication of self-goals in the study partially confirmed the hypothesis, it is unclear to what extent priming affected participants' report because the question about self-goals was asked after they had been exposed to the prime and the advertisement. As of now, it can only be assumed that the indication resulted from a combination of priming and preexisting self-goals, but it is possible that stronger cues could

prime people's salient self-goals and directly influence their brand / ad attitudes. Future research should examine whether primes can only enhance individuals' preexisting self-goals or can make them focus on one self-goal over another regardless of their predisposed tendencies. Studying this topic would also contribute to the literature of self-concept by providing evidence for great extent of malleability.

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Investigating the Cognitive Enhancing Effects of Modafinil in Rats using a novel Self-Ordered Sequencing Touchscreen Task

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ABSTRACT. Reports of the cognitive enhancing effects of modafinil have been inconsistent across human and rodent literatures. Cognitive enhancements are typically observed on specific tasks above a certain difficulty threshold, being more pronounced in subjects with impaired baseline performance. The current study employs a novel touchscreen task in rodents analogous to Cambridge Neuropsychological Test Automated Battery (CANTAB)'s self ordered spatial working memory task (SWM) in humans, where pro-cognitive effects of modafinil were observed (Müller et al. 2013). Consistent with previous literature in humans, results showed modafinil selectively enhanced performance on the more difficult trial types. This improvement was unaccompanied by changes in correct or error latency measures. The current touchscreen paradigm proves to be more sensitive to modafinil's enhancing effects on spatial working memory than other current existing pre-clinical tests in rodents. This task can be combined with lesion and neurochemical assays in the future to narrow down the neural basis and molecular pathways underpinning modafinil's cognitive enhancing effects. Its close resemblance to CANTAB SWM touchscreen task used to test human patients makes it a useful preclinical tool to measure self-ordered spatial working memory.

1. Introduction

Modafinil is a wake-promoting agent licensed to treat narcolepsy, with putative cognitive enhancing effects. In humans, modafinil has yielded enhancements on tasks of attention, working memory and executive control in non-sleep-deprived healthy adults, and in neuropsychiatric populations such as ADHD and schizophrenia patients (Müller et al., 2013; Turner et al., 2003; Turner et al., 2004). However, others have failed to either replicate attentional enhancements in healthy adults, or have yielded heterogeneous results (Randall, Fleck, Shneerson, & File, 2004; Randall, Shneerson, Plaha, & File, 2003; Müller, Steffenhagen, Regenthal, & Bublak, 2004; Randall et al., 2005). The rodent literature is similarly inconsistent, with some positive findings on cognitive enhancement of abstract rule learning (Béracochéa et al., 2001; Béracochéa, Celerier, Peres, & Pierard, 2003; Béracochéa, 2002; Ward, Harsh, York, Stewart, & McCoy, 2004), sustained attention, executive control (Chudasama & Robbins, 2004; Morgan, Crowley, Smith, LaRoche, & Dopheide, 2007), and object recognition (Redrobe, Bull, & Plath, 2010), while others showed increased impulsivity and worsening of performance by reducing sustained attention (Liu, Tung, Lin, & Chuang, 2011; Waters, Burnham, O'Connor, Dawson, & Dias, 2005), or differential findings depending on baseline performance level of subjects (Eagle, Tufft, Goodchild, & Robbins, 2007).

Three main factors may be contributing toward this mixed profile of modafinil's cognitive enhancing effects. First, enhancement may be subject dependent. Greater cognitive-enhancement is typically observed in poor-baseline-performers, in both healthy young human adults (Esposito et al., 2013), and ADHD patients (Turner, Clark, Dowson, et al., 2004). Eagle et al. (2007) mirrored this finding in rats using a stop-signal-reaction-task (SSRT), and found modafinil at 10 and 30mg/kg improved performance of rats with slow baseline SSRT, and not those with fast baseline SSRT. This suggests baseline performance may determine modafinil's potential cognitive enhancing effects.

Second, enhancement may be task dependent, with key factors being task specificity and task difficulty. Randall et al. (2005) assessed whether previous discrep-

ancies between their negative findings and Turner et al. (2003, 2004)'s positive findings was partly due to inconsistent use of selection of Cambridge Neuropsychological Test Automated Battery (CANTAB) tests. They replicated Turner et al.'s positive results only by using the same tasks (Digit-Span, Pattern Recognition Memory) at 100mg and 200mg doses of modafinil, suggesting only certain tasks in non-sleep-deprived young healthy adults are sensitive to effects of modafinil. Moreover, Müller et al. (2013) found that modafinil enhanced performance of healthy volunteers on CANTAB self ordered spatial working memory task (SWM) by reducing error-rates only at their newly implemented, more difficult, 10-box and 12-box levels. This result suggests that Turner et al. (2003)'s failure to detect modafinil's enhancement on SWM may have been a ceiling effect without using these more difficult levels. Similarly, Morgan et al. (2007) found a dose dependent improvement in response accuracy and impulsivity control in rats on three choice visual discrimination task only when sustained attention load was increased by making the task more difficult with unpredictable stimulus onset and duration. However, modafinil induced enhancements related to task difficulty may interact with task specificity as pro-cognitive effects have not been observed in versions of the 5 choice serial reaction time task for rats, irrespective of task difficulty.

Third, the relationship between modafinil's cognitive enhancing effects and impact on impulsivity remains unclear. Turner et al. (2003) found increased correct response and reduced error rates were accompanied by increased response latency, suggesting modafinil affected speed accuracy trade off. Slowed response latency was also observed in ADHD (Turner, Clark, Dowson, et al., 2004) and schizophrenia patients (Turner, Clark, Pomarol-Clotet, et al., 2004), implicating modafinil reduces response impulsivity. In contrast, Müller et al. (2013) used a novel 6 move solutions version of Stockings of Cambridge-task and found modafinil's improvement on accuracy was unaccompanied by any effect on latency, suggesting speed error trade off is not sufficient to explain modafinil's cognitive enhancing effect. In addition, some rodent literature have shown that modafinil actually increased impulsivity, whether it improved response accuracy (Chudasama & Robbins, 2004) or not (Waters et al., 2005). Therefore further investigation on modafinil's effect on impulsivity and how this affects response accuracy and error rate is needed.

The current study employs a novel self ordered sequencing touchscreen task to investigate modafinil's cognitive enhancing effects in young healthy non-sleep-deprived rats. It aims to pharmacologically validate this novel preclinical test by attempting to emulate and extend the cognitive enhancing effects of modafinil observed in humans (Muller et al. 2013). Whereas previous rodent research mostly used various maze (Béracochéa et al., 2001; Béracochéa et al., 2003; Béracochéa, 2002; Piérard et al., 2006) or odours discrimination tasks (Goetghebeur & Dias, 2009), a touchscreen task bears closer resemblance to the human CANTAB tasks, facilitating translation to clinical studies (Bussey et al., 2012). A validated preclinical test of cognition is important as it can be used in conjunction with lesion (not possible in humans) and biochemical assays, to gain further insight into the mechanistic workings of modafinil. The use of touchscreen ensures close proximity between the animal and the visual stimulus on screen, and maximizes performance despite the rat's poor visual acuity (1.0 cycle/degree) (Bussey et al., 2008). It is more ethical, using appetitive not negative (Ward et al., 2004) reinforcement, and minimizes handling stress during testing which may add variability to results (Piérard et al., 2006). It is ecologically valid as it exploits rats' natural tendency to explore novelty (Horner et al., 2013). Computer automation increases range and precision of parameters measured (Bussey et al., 2012).

It also attempts to address the three previously stated sources of inconsistencies underlying modafinil's cognitive enhancing effects. First, this task was developed because it is analogous to the SWM CANTAB task used by Müller et al. (2013), where positive findings were obtained. Whereas Müller et al. (2013) manipulated task difficulty by introducing new 10- and 12-box levels, the current study divides difficulty into four levels by using 2- or 3-stimulus trials, and each subdivided into delay of stimulus onset and no-delay trials. The hypothesis based on previous literature is modafinil should selectively enhance performance on more difficult trials only (3-stimulus, delay). Second, to assess whether observable differences in modafinil effects is affected by pre-treatment baseline performance, rats were grouped based on training performance into good and poor performers. The hypothesis is modafinil should have a greater cognitive-enhancement-effect in poor performers. Third, latencies are analysed to assess Turner et al (2003)'s hypothesis of modafinil's cognitive-enhancing-effect being mediated by reducing speed of responding. This predicts any performance enhancement should be accompanied

by increased latencies.

2. Method

Subjects

20 male Lister Hooded rats (Charles River, UK) were kept on a reverse day/night cycle (lights on 7pm-7am) and housed four per cage, in a room maintained at constant temperature (20-24°C) and humidity (55±10%). Rats were fed on a restricted diet (standard rat chow, Special-Diets-Services) to maintain 85% of their free-feeding weight, with water provided ad libitum. Prior to training, handling and habituation to the facility took place. Testing was conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986.

Apparatus

Each animal was tested in an automated touchscreen operant chamber (Campden Instruments, Med Associates) . The touchscreen (15-inch), located at the front of the chamber, used optical IT sensors to register responses. The magazine was at the rear of chamber. Illumination of the magazine light paired with a click produced by the tone generator signaled the delivery of a 45mg Noyes pellet (Sandown Scientific). An IR camera above the chamber looked through the transparent lid, enabling monitoring of animal behaviour on computers outside the testing room to minimize interference during testing. The touchscreens were controlled and data collected by Whisker control system (Version 3.6.1, Cardinal and Aitken 2001), using the OWM program written by A. Mar.

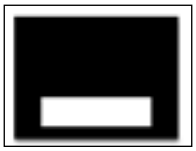
Shaping

Animals learned the basic operation of touchscreen chamber through a series of training stages. Figure 1 shows stimulus and training procedure during shaping trials.

Self-ordered sequencing task

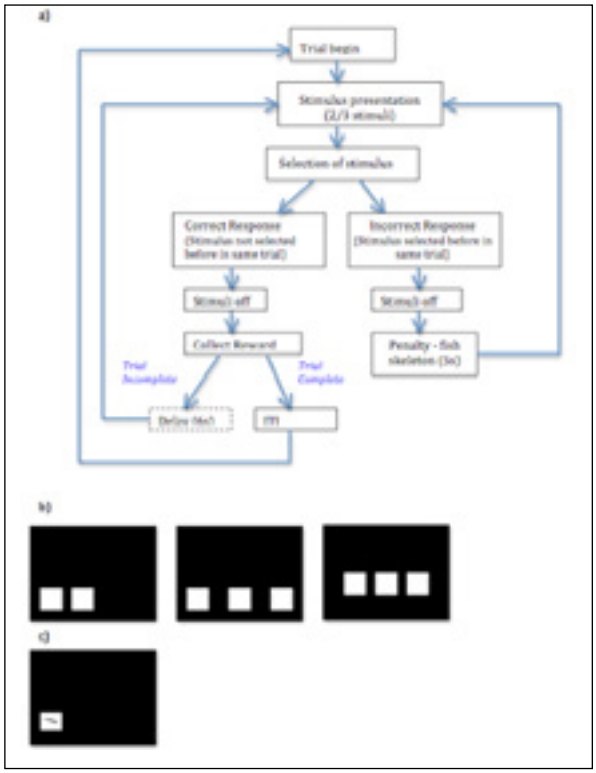
Figure 2a outlines the proceedings of each trial. Each session began with a free pellet; signaled by a click and illumination of magazine. Trial one was self-initiated upon collection of this free pellet, resulting in stimuli presented on screen (Figure 2b). A correct attempt required the animal to select a stimulus it had not selected before within the same trial. It resulted in removal of all stimuli from the screen followed by reward delivery. Re-presentation of the stimuli immediately followed reward collection from magazine on no-delay trials, or after 6-seconds on delay trials. An incorrect attempt occurred when the animal revisited a previously selected stimulus in the same trial (Figure 2c), resulting in a 5-second penalty before re-presentation of stimuli and no rewards. The trial was completed when all stimuli were selected, and a perfect trial if each stimulus was selected once only. An inter-trial-interval (ITI) (25, 30, 35, 40, 45s) was randomly

Figure 1. Stimulus and training procedure during shaping trials.



Note. For shaping sessions, a solid white rectangular stimulus (10 cm-long x 2.5 cm wide) was presented on the screen for each trial. Touching the stimulus resulted in a click and illumination of magazine, followed by reward delivery. Light was switched off upon collection of reward from magazine, and next trial initiated. Each session lasted 100 trials or 60 minutes, whichever elapsed first. Criterion of completing all 100 trials within 60 minutes was reached in 3-4 sessions.

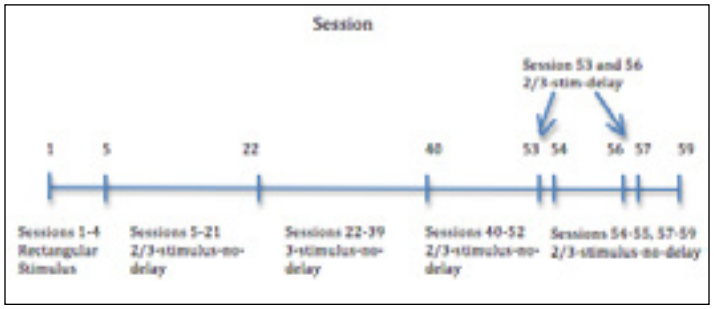
Figure 2. Trial proceedings, stimuli, and response penalty



Notes. (a) Flowchart overview of the self-ordered-sequencing procedure. Dotted line for delay indicates 6-second delay is only present on delay trials, and ITI length is variable. (b) stimuli presentation on touchscreen. Depending on trial type, either 2 (left) or 3 stimulus (middle/right) would be simultaneously presented on the screen. On 3-stimulus trials, stimuli were presented either close together - ≤8cm apart (middle)/far apart - ≥8cm apart (right) distribution of stimuli on screen. Each stimulus was a white square (3cm×3cm) (c) Incorrect response penalty results in fish skeleton presented at the wrong position touched on that attempt.

selected and initiated upon collection of last reward from magazine on any given trial.

Figure 3. Timeline of training schedule prior to modafinil administration



Note. All training sessions consisted of 80 trials or 60 minutes, whichever elapsed first.

Training Schedule

Sessions 1-4 = shaping sessions, training rats to learn the basic operation of a touchscreen chamber
Sessions 5-21 = training using 2/3 stimulus-no-delay sessions. After eighteen sessions, below chance performance (22.2%) on 3-stimulus trials across cohort observed. In order to ensure criterion level performance on 3-stimuus trials could be reached under time constraints before modafinil administration, a 3-stimulus-no-delay trial only stage was added to facilitate learning of 3-stimulus task.
Sessions 22-39 = training using 3-stimulus-no-delay sessions. Eighteen sessions were completed with the last three consecutive sessions showing stabilized above criterion-level performance.
Sessions 40-52 = training using 2/3 stimulus-no-delay sessions. Thirteen sessions were completed, which led to half the cohort reaching stabilised above-criterion level performance for both 2-stimulus (50% perfect) and 3-stimulus trials (22.2% perfect).
Session 53 = probe session 1 using 2/3-stimulus-delay (6-seconds)
Session 54-55 = 2 sessions using 2/3-stimulus-no-delay trials to re-baseline performance before probe session 2
Session 56 = probe session 2 using 2/3-stimulus-delay (6-seconds)
Session 57-58 = 2 sessions using 2/3-stimulus-no-delay trials to re-baseline performance before modafinil study

Drug administration and testing schedule

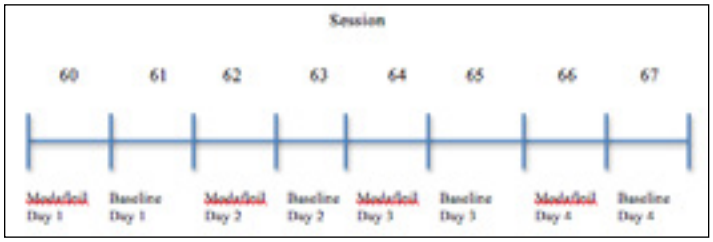
Modafinil was administered using a Latin-Square design (TABLE 1). Modafinil (Eli Lilly – milled) was prepared on each drug day in 10% sucrose suspension and administered orally at doses of 8mg/kg, 16mg/kg, 32mg/kg, or vehicle (10% sucrose suspension). Vigorous shaking of the modafinil suspension prior to dosing was performed, maximising even distribution of drug in solution. Each animal was weighed prior to drug administration, to calculate volume (1ml/kg) needed to reach the specified dose. FIGURE 4 shows modafinil dosing schedule. Latin-Square was completed using once/daily sessions, over eight days.

Table 1. Latin Square (4 x 4) used for modafinil administration.

Group	Day 1 Dose (mg/kg)	Day 2 Dose (mg/kg)	Day 3 Dose (mg/kg)	Day 4 Dose (mg/kg)
1	Vehicle	8	16	32
2	8	32	Vehicle	16
3	16	Vehicle	32	8
4	32	16	8	Vehicle

Note. Rates were divided into four groups, matched for performance based on training. All animals received all doses across four alternating days in a randomized manner.

Figure 4. Timeline of training schedule during modafinil study (post-rtraining).



Note. Modafinil day testing began thirty minutes after modafinil administration, and used 2/3 stimulus-delay sessions with 80 trials or 75 minutes, whichever elapsed first. On drug dosing sessions, session length was increased from 60 to 75 minutes to maximize number of trials completed per session. A drug free 2/3 stimulus-no-delay session (80 trials, 60 minutes) baseline day followed each drug day. This allowed recovery of performance and reduced any potential cumulative drug effects that may confound data interpretation. In total, modafinil study lasted for 8 days, with 4 drug days and 4 baseline days to complete the Latin Square.

Statistical analysis

Performance in the task was calculated using the following measures:

Percentage of perfect trials: number of perfect trials divided by total trials completed for each trial type.
Errors: First-mistake on 3-stimulus trials was broken down by attempt (expressed as a percentage of first mistakes made on attempt two or three); and by where on the screen the mistake was made (left, middle, right). A perseverative error is any mistake made after the first mistake on any attempt. Error rate was number of perseverative-errors expressed as a percentage of the total number of completed imperfect trials of that type.
Latencies: Correct response latency was the time taken for the first correct response on all except first attempts. Error latency was the time taken for a mistake to be made on any attempt. Reward collection latency was the time taken to enter the magazine for reward collection.
Splitting subjects into groups: Based on the hypothesis that

modafinil's cognitive enhancing effects are greater in subjects with more impaired baseline performance, group-split was based on stable performance across training sessions 40-52, prior to drug testing (See Table 2).

Table 2. Group average performance across training sessions 40-52.

Group	% Perfect - 2 stimulus trials	% Perfect - 3 stimulus trials
1 (n=10, Good)	57.93±3.48	28.5±2.04
2 (n=8, Poor)	52.10±2.67	13.5±0.71

Note. Data are shown as group mean ± SEM (n=10 in good group, 8 in poor group). Efforts were made to maximize difference in performance on both 2-stimulus and 3-stimulus trials between the groups, using median of percentage of perfect trials across sessions 40-52 as a variable for splitting. The uneven group size was because groups were determined pre-Latin-Square, based on total of 19 rats with 9 rats in poor group. One rat from poor group failed to complete Latin-Square, thus reducing group size to 8.

For all analyses, trial types was broken down by stimulus-number (2/3) and delay (0/6sec), each analysed at two levels, and group was used as a between-subject variable. Two rats were excluded. One did not complete training due to illness. The other failed to complete the Latin-Square due to insufficient drug to make up the correct dose on the last day of testing. Data of the remaining 18 subjects were analysed.

Statistical analysis was carried out using Windows versions of SPSS (Version 15, SPSS, Chicago), using repeated-measures factorial Analysis of Variance (ANOVA). Skewness and range of values for each variable were checked using SPSS descriptive statistics, and given n is equal across all drug groups, homogeneity of variance was not violated. Normality was checked using Whisker-box-plots for any extreme outliers. Sphericity was checked using Mauchly's test. When violated ($p<0.05$), if Greenhouse-Geisser estimate of sphericity (Epsilon factor) is <0.75, Greenhouse-Geisser correction was used; if Epsilon factor is >0.75, Huynh-Feldt correction was used. No serious violations of any assumptions were found. $p\leq0.05$ was considered to be statistically significant. However, $0.05\leq p\leq0.1$ were also discussed if it helped to interpret any drug effects observed. Dunnett's test was used as a post-hoc analyses for any significant effects of ANOVA of dose, comparing each dose against vehicle. Tukey's HSD was used as a post-hoc analyses investigating between-dose or non-dose related significant outputs of ANOVA.

3. Results

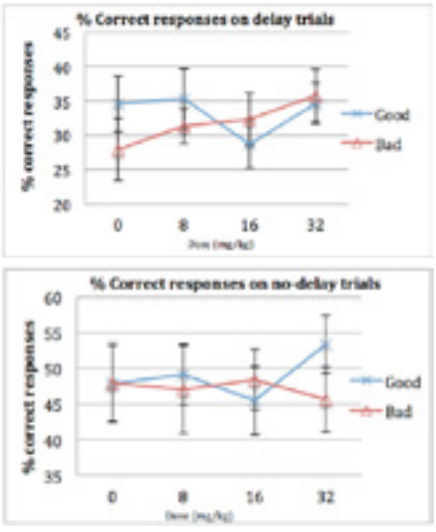
Percentage perfect trials

Across the entire cohort, ANOVA revealed main effects of stimulus-number ($F(1,17)=379.717$; $p<0.001$) and delay ($F(1,17)=38.21$; $p<0.001$), with lower percentage-perfect on delay and 3-stimulus trials relative to no-delay and 2-stimulus trials. Dunnett's confirmed delay introduced a significant reduction in percentage-perfect ($p<0.05$). This suggests both delay and stimulus-number variations successfully manipulated task difficulty, as expected.

ANOVA revealed a stimulus-number × delay interaction ($F(1,17)=17.769$; $p<0.001$), Dunnett's post hoc tests showed delay had a greater impact on performance of 3-stimulus than on 2-stimulus trials ($p<0.01$), in line with the prediction that delay would increase working memory load to a greater extent for 3-stimulus trials compared to 2-stimulus trials.

One comparison was to see if modafinil selectively improved performance of the poor-baseline-performers more than the good-baseline-performers. Using group as a between-subject variable, ANOVA revealed a non-significant trend of dose×delay×group interaction ($F(3,51)=2.596$; $p=0.063$) (FIGURE 5). Linear contrast comparison revealed no significant linear trends ($p>0.05$).

Figure 5. Effect of modafinil on % of correct responses on groups divided by baseline performance.



Note. Data are shown as group mean ± SEM (within-subject experimental design). Factorial ANOVA did not reveal any significant interaction when group was used as a between-subject factor, although there is a near-significant trend ($p=0.063$) of modafinil's differential effect on % of correct responses between the two groups. The trend is interesting on harder delay trials, where modafinil had little enhancement on good group, though improved poor group's performance dose-dependently. This is consistent with the hypothesis that modafinil has greater cognitive-enhancing-effect on poor-baseline-performers on more difficult trial types.

Error Analyses

First-mistakes. For analysis of which attempt in time in a 3-stimulus trial the first-mistakes were made, there were main effects of delay ($F(1,17)=12.437$; $p=0.003$), attempt ($F(1,17)=56.196$; $p<0.01$) and a delay×attempt interaction ($F(1,17)=13.699$; $p=0.002$). All were confirmed by linear contrast comparison indicating significant linear relationships ($p<0.05$). Tukey's post-hoc tests revealed that both delay and attempt had a significant effect on number of first mistakes made, and the interaction was because on delay trials more first-mistakes were made on attempt two, but on no-delay trials, more were made on attempt three.

Factorial ANOVA of which position on the screen first-mistakes in a 3-stimulus trial revealed a delay×position effect ($F(2,34)=7.001$; $p=0.003$). Using group as a between-subject variable, there was a delay×position×group effect ($F(2,34)=3.481$; $p=0.043$). Tukey's post-hoc tests revealed on delay trials, both groups made more first-mistakes on the middle stimulus. In contrast, Tukey's tests found on no-delay trials, whereas the good group made more first-mistakes on the right and fewest on the left, the poor group made more on the left and fewest in the middle. There was no significant difference between sequencing for both groups between baseline and drug days ($p>0.05$). Sequencing data on drug days (TABLE 3) revealed that the poor group, on delay trials, preferred to start in the middle (39.91%-MRL+MLR), but on no-delay trials, preferred to start on the left (48.01% - LMR+LRM). Given first-mistake is the first time a rat revisits a previously visited location, the starting position is more likely to be revisited on following attempts. This shift in starting position preference from middle (delay) to left (no-delay) may account for the difference in number of first mistakes made at each of these locations on different trial types.

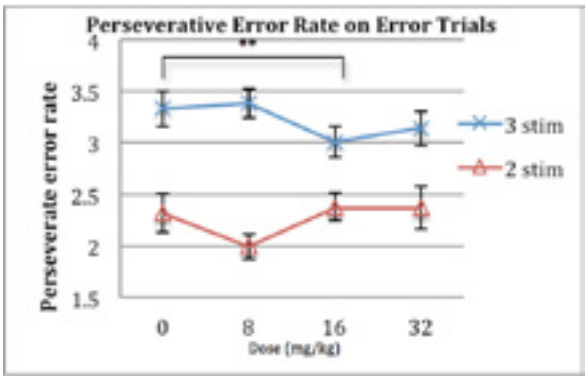
Table 3. Sequencing analysis.

Trial Type	Group	L-M-R (%)	L-R-M (%)	M-R-L (%)	M-L-R (%)	R-L-M (%)	R-M-L (%)
Delay	1 (Good)	15.74 (4.11)	9.96 (2.81)	16.15 (2.54)	17.97 (2.53)	17.16 (3.96)	23.02 (3.82)
	2 (Poor)	24.43 (5.69)	8.76 (1.70)	25.24 (2.74)	14.67 (2.23)	13.42 (2.55)	13.49 (3.14)
No Delay	1 (Good)	10.75 (2.29)	16.46 (3.67)	19.04 (5.15)	24.71 (2.9)	11.38 (2.43)	17.67 (2.59)
	2 (Poor)	21.45 (2.48)	26.56 (3.67)	7.39 (2.02)	21.27 (4.63)	11.48 (3.89)	11.84 (4.76)

Notes. L=left, M=middle, R=right position on screen, in order of tapping of touchscreen. Data are shown as group mean with SEM in brackets.

Preservative mistakes error rate. This was calculated by dividing number of mistakes (excluding first mistakes) by number of imperfect trials. ANOVA showed significant main effect of delay ($F(1,17)=5.298$; $p=0.034$). Linear contrast comparison found a significant linear trend of delay ($p=0.034$), and post-hoc Dunnett's tests revealed a significant effect of delay increasing perseverative-mistake-error-rate ($p=0.034$). ANOVA also found a dose×stimulus-number interaction ($F(3,51)=2.96$; $p=0.041$), Dunnett's test revealed a significant ($p=0.007$) difference between 16mg/kg and vehicle (FIGURE 6). Using group as a between-subject variable, there was no significant interaction of dose×stimulus-number ($F(3,51)=2.009$; $p=0.176$).

Figure 6. Effect of modafinil on perseverative error rate.

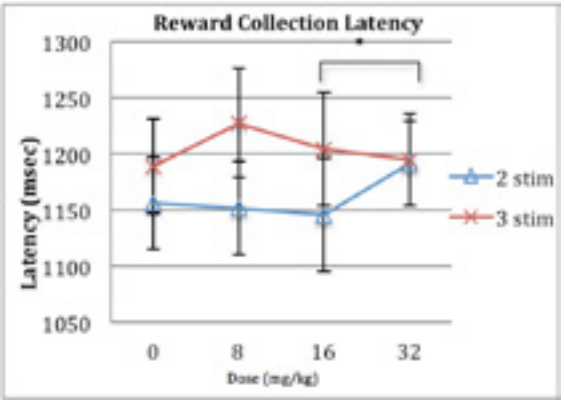


Note. Data are shown as group mean ± SEM (n=18) (within-subject experimental design). Factorial repeated measures ANOVA found a significant dose×stimulus-number interaction ($F(3,51)=2.96$; $p=0.041$), Dunnett's post-hoc found a significant difference at dose 16mg/kg compared to vehicle ($p=0.007$), though not at any other doses ($p>0.05$). ** denotes the significant difference ($p<0.01$). The graph reveals a differential effect of modafinil across 2 and 3-stimulus. On 3-stimulus trials, 16mg/kg significantly reduced perseverative error rate (lowest at 16mg/kg). No significant effects were found for 2-stimulus trials.

Latency Analyses

There were no significant main effects of drug or drug×stimulus-number for either correct-response-latency or error-latency ($p>0.05$). ANOVA revealed a significant stimulus-number×drug interaction for reward-collection-latency ($F(3,51)=2.784$; $p=0.005$) (FIGURE 7). Dunnett's post-hoc did not reveal any significant difference between any doses and vehicle ($p>0.05$). Tukey's revealed a significant difference between 16mg/kg and 32mg/kg ($p=0.019$). ANOVA found no significant main effect of drug when group was used as a between-subject variable ($p=0.07$).

Figure 7. Effect of modafinil on reward collection latency.



Notes. Data are shown as group mean ± SEM (n=18) (within-subject experimental design). Dunnett's post-hoc tests revealed reward collection latency at none of the doses for either stimulus number were significantly different from vehicle ($p>0.05$). Tukey's post-hoc test revealed there was a significant difference between 16mg/kg and 32mg/kg ($p=0.019$), denoted by *. The graph shows whereas 32mg/kg decreased reward collection latency on 3-stimulus trials, it increased reward collection latency on 2-stimulus trials. For 2-stimulus trials, a relatively stable latency was observed across low doses, with a sudden increase at 32mg/kg. In contrast, 3-stimulus trials showed greatest latency at 8mg/kg, whereas at higher doses the latency was comparable to vehicle.

4. Discussion

The main finding of this study was that modafinil significantly reduced error-rate selectively on the more difficult 3-stimulus trials, mirroring the effect found in humans by Müller et al (2013). There was also a near significant increase in percentage-perfect, only on the more difficult delay trials in poor baseline group. Neither was accompanied by any changes in correct/error latencies. There was a significant effect of modafinil on reward collection latency, which may reflect changes in motivation. The effects of modafinil in this study are discussed from both a psychological perspective by assessing its implications for spatial working memory, as well as from a neurobiological perspective by hypothesizing the neural substrates underlying this cognitive enhancing effect.

The finding of reduced error rate only on the harder 3-stimulus trials is consistent with Müller et al (2013)'s finding in humans, where a reduced error-rate was only found on the more difficult 10- and 12-box levels of CANTAB SWM task. From a psychological perspective, this suggests modafinil's pro-cognitive effects may only be observed when cognitive load on spatial working memory is above a critical threshold. This has been found in animal literature using T-maze (Beracochea et al., 2001, 2002, 2003) and Morris Water Maze (Shuman, Wood, & Anagnostaras, 2009). However, these previous studies only found enhanced performance at doses of modafinil (64mg/kg or 75mg/kg) higher than the present study, suggesting the current task may be more sensitive for detecting modafinil's effect on spatial working memory. The significant reduction in error rate found by Müller et al (2013) was by using a clinically relevant plasma concentration of modafinil in humans (200mg), which translates to around 10mg/kg in rodents (Yu et al. 2000), close to the significant effect observed at 16mg/kg in the current study. This suggests the current novel touchscreen study is a successful translation of the CANTAB SWM task in humans for use in preclinical animal studies, and its improved sensitivity to modafinil's cognitive-enhancing-effect makes it superior to existing paradigms assessing spatial working memory in rodents.

Two interesting findings also emerged when comparing modafinil's effect on error-rate and percentage perfect measures. First, the cognitive enhancing effect for each measure was dependent on a different type of difficulty manipulation. Error rate on stimulus-number, percentage perfect on delay. Second, subject's baseline performance only seemed to predict modafinil's effect on percentage-perfect, reflected by a near significant interaction between dose, delay and group, where modafinil improved poor group's performance on harder delay trials more than good performers. Baseline performance did not affect modafinil's effect on error rate. Orthogonality between these two measures suggests they may reflect two different components of spatial-working-memory. Spatial working memory is a complex cognitive process involving many components, such as planning and selection of most efficient strategy mediated by frontal lobe, and the self ordered sequencing nature of the task would have also required efficient mnemonic processing and accurate sequencing encoding mediated by subcortical areas such as hippocampus. Therefore, the two performance measures may reflect different aspects of spatial working memory, with percentage perfect reflecting efficiency of executive planning and error rate reflecting quality of mnemonic encoding. This dissociation suggests each measure may be assessing the efficiency of a different node in neural network underlying spatial working memory. The fact that modafinil improved performance assessed by both measures may imply that it improved overall efficiency of neural network, rather than a single node.

Functional imaging in rats supports modafinil's enhancement of this neural network. Gozzi et al. (2012) used pharmacological magnetic resonance imaging to map circuitry activated by modafinil at 10mg/kg admin-

istered intravenously in rats. They found stimulation of fronto-cortical areas involved in higher cognitive function, and activation of other subcortical areas (e.g. thalamus and hippocampus), suggesting modafinil does act on the neural substrates associated with spatial working memory. Note the dosage chosen is clinically relevant and can be easily translated to human studies.

Neurobiological effects of modafinil on spatial working memory has also been assessed in human patients with frontal lobe lesions, temporal lobe lesions and amygdalo-hippocampectomy, using SWM task with 4/6/8 boxes (Owen, Sahakian, Semple, Polkey, & Robbins, 1995). Whereas frontal lobe patients displayed impairment across all difficulty levels due to failure to adopt the most efficient sequencing strategy, temporal and amygdalo-hippocampectomy groups only showed impairment at the most challenging level, and was unrelated to strategy selection but rather reflect a more fundamental disruption of mnemonic processing. This study also suggested a spatial working memory network consisting of cortical and subcortical nodes. Top-down control exerted by prefrontal cortex is responsible for selecting the most efficient strategy to solve a problem, and the efficiency of each strategy depends on bottom-up feedback of mnemonic processing by subcortical regions. Therefore, selective enhancement observed on harder 3-stimulus trials in the current study suggests that modafinil perhaps enhanced the efficiency of subcortical nodes to improve mnemonic processing, and improved coding accuracy of more complex sequences on harder trials, improving quality of bottom-up feedback.

Owen, Downes, Sahakian, Polkey, & Robbins (1990), using an automated Tower of London task, found patients with frontal lobe lesion could solve the most difficult versions but required more moves per problem than matched controls, suggesting inefficient planning and selection of sequencing strategy. Therefore modafinil could have also improved frontal lobe's top-down control to plan and select the most efficient sequencing strategy to minimize load on spatial working memory, and reduced error rate on harder 3-stimulus trials. Owen et al. (1990) also found that frontal lobe patients did not show an increase in latency before first move on any trial, but there was an increase in latency accompanying correct responses on subsequent moves. The authors interpreted this as increased impulsivity to respond before forming an efficient sequencing plan, therefore, to ensure subsequent responses are correct, more time is spent thinking per move to compensate for lack of pre-planning.

Perhaps the difference in baseline performance between the groups reflected a difference in their frontal lobe executive planning efficiency. Poor baseline group may require more thinking time before beginning of next attempt to ensure a correct response. If this increased planning of the next move can take place during delay and complete before the re-presentation of stimuli for the next attempt in any trial, it could account for why near significant effect of increased percentage perfect is observed only across delay trials, and why this was unmatched by any changes in correct response latency because thinking was completed during the delay. However, this account predicts that on no delay trials, this increased thinking should result in an increase in correct response latency, but this was not observed. This suggests modafinil's cognitive enhancing effect is more complex than simply increasing frontal lobe's planning efficiency, and given that baseline performance did not affect modafinil's effect on error rate, it implies modafinil did not act solely on frontal lobe, but also on subcortical areas in the network underlying spatial working memory.

Finally, enhancement in performance was not accompanied by any changes in correct/error latency measures, inconsistent with Turner et al. (2003)'s proposal of modafinil shifting speed accuracy trade off. However, perhaps speed accuracy trade-off was not as important a factor in optimizing performance for the current non-speeded spatial working memory task, compared to reaction tasks (e.g. Stop-Signal) used by Turner et al. (2003) where subjects were under greater time pressure. Modafinil did affect reward collection latency. This was not a change in general motor readiness or feeding, which should have affected all latencies across all trial types consistently, but was not observed in the present study. In addition, the suggested anorexigenic effect of modafinil (Nicolaidis & De Saint Hilaire, 1993) lacks replication (Morgan, Crowley, Smith, LaRoche, & Dopheide, 2007), making it an unlikely explanation. Perhaps higher doses made the harder 3-stimulus trials more enjoyable and increased motivation. Müller et al (2013) found participants rated the task as more enjoyable after taking modafinil, although subjective ratings are not easy to assess in rats. One potential measure for general motivation is the number of trials completed within each session, although this analysis was not possible as nearly all subjects completed all trials within each session.

The current study did not include any biochemi-

cal assays to investigate modafinil induced neurochemical changes, but some potential underlying neurotransmitter pathways are discussed. Catecholamines are involved in regulation of arousal, and stimulants (e.g. amphetamine) often work by increasing presynaptic release of dopamine and noradrenaline. In contrast, modafinil has been proposed to work post-synaptically as a putative noradrenergic receptor agonist, binding to and enhancing activity of alpha-1 receptors (Akaoka, Roussel, Lin, Chouvet, & Jouvet, 1991) and beta receptors (Lin et al., 1992). Modafinil's effect on dopaminergic system is more controversial. Some animal studies suggested it had little impingement on dopaminergic activity (Redrobe, Bull, & Plath, 2010; Waters, Burnham, O'Connor, Dawson, & Dias, 2005), has low abuse potential and less effect on extrapyramidal motor activities compared to amphetamine, making it safer to use. However, modafinil may modulate dopaminergic activity indirectly, such as by influencing enzymatic activity involved in formation of dopamine from Levo-Dopa (Murillo-Rodríguez, Haro, Palomero-Rivero, Millán-Aldaco, & Drucker-Colín, 2007). Studies using dopamine receptor antagonists such as SCH23390 (D1-receptor-antagonist) and raclopride (D2-receptor-antagonist) have generated mixed findings. Some, using knockout mice models, argued modafinil enhanced dopamine activity by directly binding to D1 and D2 receptors (Young, Kooistra, & Geyer, 2011; Young, 2009). Others suggested indirect activity by inhibiting dopamine transporters (DAT), mimicking the effect of GBR12909 (selective DAT inhibitor) (Young & Geyer, 2010). The latter has been replicated in humans using clinically relevant doses of modafinil (Volkow et al., 2009). Dopamine influences motivation, Young & Geyer (2010) found an increased motivation using modafinil at 16, 32, and 64mg/kg, similar to doses used in the current study.

If faster reward collection latency indicated higher motivation, then the present study only partially supports modafinil's role in enhancing motivation since latency decreased only for 3-stimulus trials, and increased for 2-stimulus trials. Perhaps increased dopamine transmission led to aberrant salience attribution to irrelevant cues in the environment (Kapur, 2003), which distracts focused attention. This may be more problematic on easier 2-stimulus than harder 3-stmulus trials, as the former has lower cognitive load so the subject may be more easily distracted. An alternative hypothesis is rats may have experienced increased anxiety, distracting from limited executive resources for controlling sustained attention (Eysenck, 1979), and human subjects high in trait anxiety were prone to greater distraction by task-irrelevant stimuli (Deffenbacher & Hazaleus, 1985; Forster, Elizalde, Castle, & Bishop, 2013). This seems less plausible than the aberrant salience account, since modafinil has not been shown in animal studies to have anxiogenic effects (Simon, Panissaud, & Costentin, 1994), and it is unlikely that modafinil would selectively increase anxiety on 2-stimulus trials but not on 3-stimulus trials within the same session. However, a biochemical assay assessing modafinil's effect on dopamine transmission is needed to test these speculations.

Limitations

Within-subject design minimised limitations of small sample size on statistical power. However, when group was used as a between-subject variable, sample size was halved which reduced statistical power, so larger sample size would have been favourable. To use difference in number of trials completed as a measure of motivation, either session length should be decreased or number of trials increased to prevent completion of all trials within each session. A time-out should be added so a response made outside a certain time frame is counted as incorrect. This requires subjects to respond quickly to maximize performance, analogous to human reaction-time tasks (e.g. Stop-Signal) used by Turner et al. (2003), allowing better assessment of modafinil's effect on speed-accuracy-trade-off.

Future Experiments

Two further experiments are proposed based on current data interpretation. To assess the contribution of cortical and subcortical structures to performance and whether modafinil affects each region differently, a lesion study can be used, with frontal lobe lesion and hippocampus lesion compared against sham lesioned animals. Receptor antagonists to noradrenergic and dopaminergic receptors can also be used in combination to central microinfusions of modafinil into the prefrontal cortex to investigate the neurobiological specificity underlying the mechanisms of modafinil.

A second experiment modifies the touchscreen task to assesses whether any differences observed in performance on 2 and 3-stimlus trials is attributable to a difference in working memory load imposed by trial type, or executive function governing flexible encoding of positions poked to always minimize working memory load.

To succeed in the current self-ordered-sequencing-task, subject can either choose to encode the position poked or unpoked within a given trial. For 2-stimulus trials, both strategies require memory of one location with no difference on memory load. However, for 3-stmulus trials, different strategies generate different working memory load. A correct attempt two can be achieved by either remembering one previously poked location, or two unpoked locations, the former minimises working memory load. The reverse is true for attempt three. Therefore, subjects who flexibly change their strategy to always minimize their working memory load across attempts within the same trial may perform better on harder 3-stimlus trials, and across delays. The difference between good and poor performers may not be one of working memory but of executive control, suggesting modafinil may enhance executive control by increase flexibility in coding strategy in poor baseline performers.

To assess if this flexible encoding affect performance, a 4-stimulus trial type can be introduced. Attempt three in the 4-stimulus trial always requires encoding of two locations no matter what coding strategy used, which serves as a baseline for comparison since attempts two and four require encoding of either one (easier than attempt three) or three locations (harder than attempt three). None of the attempts within a 3-stimulus trial can be used as a baseline, and therefore it is necessary to use 4-stimulus trials. If animals can flexibly change their coding strategy to always minimize working memory load, then error-rate on attempts two and four should be less than for attempt three (always encoding one rather than two locations), suggesting the task assesses executive function. If animals do not change coding strategy and the task is purely assessing working memory, then depending on whether the animals chooses to encode places poked or unpoked, more errors should be made on attempts four and two, relative to attempt three, respectively. This predicted difference in pattern of error rate enables further specification of the cognitive processes underlying this task.

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How Does NREM Sleep Affect the Consolidation of Declarative Memories?

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ABSTRACT. Non-Rapid eye movement [NREM] sleep has been shown to be an influential process in the consolidation of declarative memories. This hypothesis is derived from a variety of experiments that involve manipulation of sleep cycles around word-pair learning tasks. There are two popular theories that attempt to explain the underlying mechanisms: the synaptic homeostasis hypothesis suggests that slow oscillations seen in sleep EEG indicate the global downscaling of synaptic strengths, leading to an improved signal-to-noise ratio for relevant synaptic connections; the active system consolidation hypothesis suggests that memory traces are actively replayed in order to be reorganised and consolidated, involving a continual interchange of information between the hippocampus and the cortex. There is a considerable amount of data from different study paradigms to support both theories. However, they are not mutually exclusive and attempts have been made to combine the theoretical frameworks together to form an overall model. I also consider the overall modulation of memory consolidation during sleep by the activity acetylcholine, which in turn may be controlled by cortisol at an endocrine level. I give an evaluation of various experimental techniques that are shared across many studies in this subject. I highlight a number of issues with the current methodology, including the implications of correlational data provided by EEG activity and the effect of semantic representations in the brain. An overall analysis of the data suggests that theories in this subject are mostly congruent with each other. There are also implications for other areas: REM sleep may also be involved in declarative memory consolidation, contrary to the classical dual-process model; reconsolidation for declarative memories may turn out to be the same process as consolidation. I give suggestions for further experiments that could be conducted to address some of the current uncertainties.

1. Introduction

Studies have shown throughout history that sleep can affect the process of memory formation. Indeed, many theories postulate that one of the functions of sleep is to facilitate or improve memory formation. Recent experiments have shown that non-REM [NREM] sleep in particular can enhance declarative memory formation. It is suggested that specific neural activity present during the various stages of NREM sleep may contribute to the consolidation of declarative memories (Rasch & Born, 2013).

Memory Consolidation

A classical model of memory involves two different types of memories – short-term and long-term. Short-term memory is very dynamic, but is also very labile and susceptible to degradation either over time (tens of seconds) or though interference. Long-term memory, on the other hand, is difficult to establish, often requires a long learning process, but is stable over months or years and is not usually affected by interference (Atkinson and Shiffrin, 1968). Consolidation is often defined as the process through which short-term memory is stabilised into long-term memory after its initial acquisition from sensory inputs (McGaugh, 2000).

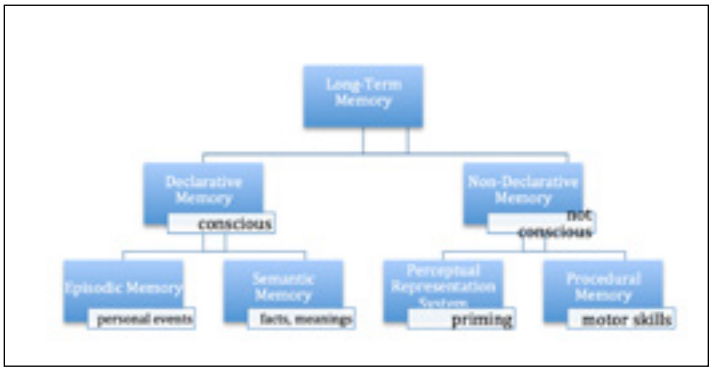
Figure 1. Classical model of the stages of memory formation

Declarative and Non-Declarative Memory

Within the domain of long-term memory, there

are subdivisions containing different types of memory. This is relevant because these have been shown to involve different neurological structures. The most important division is between declarative and non-declarative memory. Declarative memories are memories that a subject can explicitly describe, such as a past event, or the meaning of a word or concept; non-declarative memories are memories that cannot be expressed, and includes things like motor skills, primed responses, habituation etc. Declarative memories have been shown involve the cortex and hippocampus, whereas non-declarative memories involve the cerebellum and amygdala (Squire & Zola-Morgan, 1991).

Figure 2. Divisions of long-term memory, based on (Squire & Zola-Morgan, 1991)



Most of the experiments cited in this paper use methods which test semantic memory as a representation of declarative memory in general. This is also due to the fact that learning tasks such as word-pair association are much easier to be implemented in a controlled fashion compared to episodic events (which would be personal). These experiments usually involve a word-pair learning task followed by a recall test. Non-declarative memory, on the other hand, is usually represented by procedural memory tasks such as a mirror-tracing or finger-sequence tapping. This is sometimes used as a control to highlight the effects of declarative memory alone.

Sleep

Sleep is a process that is characterised by specific patterns of neural activity, usually identified with EEG. There are several stages of sleep, but these can generally be divided into REM sleep – characterised by rapid eye movements – and non-REM [NREM] sleep. NREM sleep has four separate stages, each of which have their own characteristic activity as measured on an EEG. Stages 3 and 4 are classed together as slow-wave sleep.

2. Sleep and Learning Task Performance

Dual-Process Theory. The dual-process theory is the generalisation that declarative memory is enhanced by NREM sleep while non-declarative memory is enhanced by REM sleep (Plihal & Born, 1997). Several different study paradigms have been used to study the differential effects of NREM and REM sleep. The focus here will be on NREM sleep and declarative memory.

Night-half paradigm. This is a classical experimental design that involves splitting the night into two halves. Word-pair learning tasks and procedural mirror-tracing tasks (for declarative memory and non-declarative memory respectively) are introduced before either early sleep or late sleep. The protocols for word-pair association tasks, which are used to analyse declarative memory performance, are usually very similar. For example, this might involve 48 word pairs presented to the subject for learning, with eight pairs discarded (four at beginning, four at end) to account for primacy/recency effects. The subjects are then allowed to rest for a period of time, which may include sleep. Finally, there is recall task where the first (cue) word is presented to test for the associated pair. Mirror-tracing tasks, used to analyse non-declarative memory, may involve the subject being asked to trace around a shape using a pen while only using the reflection of their hands as a guide. This causes a reversal in visual feedback that would improve over time and practice (Gais & Born, 2004a).

Since REM sleep occurs more in the latter stages of the night, and vice versa for NREM sleep, it is hypothesised that a difference in performance in the recall would be due to the effects of the two types of sleep. In one study, a double dissociation was shown: NREM sleep in early sleep improved declarative memory performance, while REM sleep in late sleep improved non-declarative memory performance (Plihal & Born, 1997).

Day-time nap. More recent methods showed that a one-hour daytime nap consisting of only NREM sleep improved subjects' performance in declarative memory tasks but not procedural (non-declarative) memory tasks (Tucker et al., 2006). The control group here simply stayed awake for an hour. This result is important as it shows a causative relationship; it heavily suggests that the underlying mechanisms of NREM might be involved in the consolidation of declarative memories.

Sleep deprivation. Another way to study NREM sleep is through sleep deprivation, though this can be difficult to achieve methodologically. In one study, subjects

were kept awake for 24 hours before a learning task. The result was both reductions in recall in the short term and long term (Gais et al., 2007).

The problem with these results is that they do not tell us anything about the underlying mechanisms NREM sleep on declarative memory. As such, further studies have been conducted to explore this using different experimental techniques.

Theories. Various experiment have given rise to two popular theories that attempt to give an overall model of the mechanisms behind the effects of sleep on declarative memory consolidation: the synaptic homeostasis hypothesis and the active system consolidation hypothesis. Both theories incorporate a wide range of data form different studies. In summary, the synaptic homeostasis hypothesis suggests that the positive effect on consolidation is due to a global downscaling of synaptic strengths (Tononi & Cirelli, 2006), whilst the active system consolidation hypothesis suggests that memory reactivations between the hippocampus and the cortex are actively induced to promote consolidation (Born & Wilhelm, 2012).

3. Synaptic Homeostasis Hypothesis

The synaptic homeostasis hypothesis [SHY] suggests that SWS downscales synaptic strength globally (Tononi & Cirelli, 2006). This indirectly promotes consolidation by depressing weak synaptic connections and thus improving the signal-to-noise ratio of stronger connections that were encoded in wakefulness. The main claims are that: (1) Synapses are globally potentiated during waking, (2) Synapses are globally depressed during SWS, and (3) Synaptic potentiation influences the homeostatic control of SWS.

Downscaling may be necessary because the brain cannot be in a constant state of acquiring new memories and laying down new connections. Since it takes energy to establish a synaptic connection as well as to maintain it, the brain will need a way to prune old or unnecessary connections. The SHY identifies slow oscillations as the key indication of this global downscaling.

Slow Oscillations

Slow oscillations are a characteristic EEG pattern that is seen during SWS (stages 3 and 4 of NREM sleep). They indicate global synchronous transitions between periods of sustained firing and periods of diminished activity, at around 1-4Hz. These patterns are generated in the cortex (Rasch & Born, 2013).

One study showed that the negative phase of slow wave oscillations, as recorded on an EEG, correlate with reduced activity in individual neurones (Vyazovskiy et al., 2009). Another computational study showed that a decrease in the strength of excitatory cortico-cortical connections could account for the decrease global activity seen in SWS, including various aspects of the waveform of slow oscillations (amplitude, slop, incidence of multiple-wave peaks etc.) (Esser et al., 2007). These studies support the SHY as they suggest that the low frequency oscillations are a manifestation of reduced global synaptic activity.

Further experiments highlight the link to memory consolidation. Anodal transcranial direct current stimulation [tDCS] has been used as a non-invasive technique to depolarise neurones in human brains. This was carried out in one study in SWS as a way of inducing slow oscillations in subjects during the retention interval (Gais & Born, 2004a). The result was that retention was increased compared to placebo group. It is interesting that this effect was only seen when tDCS was applied during SWS, and not in waking, nor for a procedural task (non-declarative memory).

In another study, a slow oscillatory potential was applied transcranially at 0.75 Hz during SWS, imitating the normal slow wave activity. The result was that learning performance was significantly improved (Marshall et al., 2006). The opposite effect was seen for tDCS applying theta frequency oscillations at 5Hz, which suppresses normal slow wave activity (Marshall et al., 2011).

LTP

Long-term potentiation [LTP] is presented as a synaptic-level mechanism for the changes in synaptic potential (even though SHY is more vague on the precise mechanism, referring to it as 'LTP-like') (Tononi & Cirelli, 2012). LTP has been widely shown to be a reliable mechanism that explains permanent changes in the strength of synapses. It is essentially a process through which synaptic connections become stronger through repeated and synchronous activation. It occurs when a presynaptic neurone is activated weakly at the same time as a strong postsynaptic depolarization, leading to the strengthening of this synapse and a lowered threshold for activation in future. LTP can be non-associative (for a single input) or associative between multiple inputs, where it is also co-operative and input-specific. The timescale of

LTP is also appropriate as synaptic consolidation occurs over hours.

4. Active System Consolidation

The active consolidation hypothesis is another theory that attempts to explain memory consolidation. This is a more integrative theory that is focussed on the idea of systems level consolidation (Born & Wilhelm, 2012).

Essentially, it involves a bidirectional transfer of information between the cortex and hippocampus over time, which provides the basis for memory consolidation at a systems level. Neural activity, including hippocampal ripples and thalamo-cortical spindles – as measured by EEG – underlie this process. Since there is a finite amount of space in the cortex, old memories are reorganised so that they can be re-integrated with new memories, thus improving efficiency and preventing them from being overwritten. Similar to the SHY, this theory identifies LTP as a potential candidate for changes in synaptic activity at a local scale (Born & Wilhelm, 2012).

Two-Stage Model

The active consolidation hypothesis is based on the two-stage model of memory consolidation (Diekelmann & Born, 2010). This model hypothesises that the hippocampus and the cortex serve distinct roles in memory consolidation. The hippocampus is presented as a short-term store, which is capable of learning very quickly and is highly plastic and volatile. The cortex is presented as a long-term store, which has high stability but is slow to learn. A period of time (days) is needed for the short-term store to teach newly acquired information traces to the long-term store (Buzsaki, 1989).

This theory can also account for how the brain might acquire new memory traces without disrupting existing traces; this is called the stability-plasticity dilemma. The two-stage model offers a solution as it hypothesises that old information is brought out of the long-term store (cortex) into the short-term store (hippocampus), where it is reorganised and integrated with new information. This is then put back into the long-term store (Diekelmann & Born, 2010).

Reactivation of Memories

Reactivation of memories is central to the active system consolidation hypothesis, which suggests that it is the active replay of newly acquired traces during sleep that drives memory consolidation.

Experiments in rats have shown that spatial memory is replayed during SWS (Ji & Wilson, 2007). In this study, rats were exposed to a spatial learning task; neuronal spikes were recorded in SWS following the exposure using a tetrode implanted in the rats' skulls.

The raw recordings from cells show a clear correlation between start time and end time of frames, which are patterns of firing across several neurones in the same recording area. Meanwhile, the distribution of the time differences, with the median to the right of zero, indicates that the cortex fires immediately before the hippocampus.

This could imply that during sleep, the cortex initiates a transfer of information to the hippocampus. The purpose of this could be that select 'packets' of information are extracted from the cortex in order to be reorganised within the hippocampus. This means that when the information is transferred back to the cortex, it is in a much more compact and efficient form, both in terms of storage and later retrieval.

Firing of individual cells was also recorded. The same patterns of firing were seen in both waking and sleeping across multiple cells, again suggesting that there is a replay in the memory that had been formed; the temporal order in particular was preserved. This was seen in both the cortex and the hippocampus.

Studies in humans have also supported the idea of memory reactivations. PET imaging showed that the activity was increased in the right hippocampal and parahippocampal areas in SWS following a daytime navigation task (Peigneux et al., 2004). There was also a positive correlation between cerebral blood flow to these areas and overnight improvement in performance in the spatial navigation.

Memories can also be activated through cueing during sleep (Rasch et al. 2007). In this study odours were presented to waking subjects in conjunction with a visuospatial object-location learning task. The same odour was subsequently reintroduced during SWS, REM sleep or waking. Results showed that there was a significant improvement in recall for the SWS group alone, even though they were not even consciously aware that they had been presented with a cue. Also, fMRI data indicate that there was activation of the left anterior and left posterior hippocampus during odour presentation.

The extraction of old memories from the cortex could manifest as the reactivation of memories seen in some studies. This would explain why cueing reactivations e.g. by odours might improve memory consolidation – as it enhances this interplay between the hippocampus and the cortex.

Long-Term Activity of Cortex and Hippocampus

In a sleep deprivation study, subjects were put into two groups following a learning task; one group was allowed to have a full night's sleep, while the other group was sleep-deprived for 24 hours. The subjects were then imaged using fMRI 48 hours later whilst recalling word-pairs (Gais et al., 2007).

In the sleep group, there was functionally related activity between hippocampal activity and frontal activity, which included the precuneus and the medial prefrontal cortex (mPFC). But in the sleep-deprived group there was no related activity. There was also a significant effect, over a time frame of months, in the overall activation of the hippocampus and the mPFC.

Results indicate that in the sleep group, the hippocampus is more active immediately following learning, but the mPFC is more active over the long term. However, this is opposite in the sleep-deprived group.

A possible explanation here is that the hippocampus could be acting as a teacher for the cortex, which accounts for the higher activity in the normal group. In the sleep-deprived group, on the other hand, the hippocampus is not given an opportunity to carry out this teaching, which accounts for the lower level of activation. Eventually this information is degraded over a few days. Six months later, however, when the normal group is asked to recall word-pairs, they can rely on a well-constructed array of information that is stored in the cortex. But in the sleep-deprived group, since this information was never processed efficiently, the hippocampus is recruited at this stage either as a buffer in the retrieval of old information, or to recommence the proper consolidation of the old memory.

Neural Basis of Reactivations – Ripples and Spindles

EEG recordings have shown that changes in certain patterns (hippocampal sharp wave-ripples and spindles) correlate with the performance in learning tasks. This has lead to the idea that these patterns indicate neural activity that is involved in memory consolidation, or indeed the reactivation of memory traces. Studies have shown phase synchronisation between slow oscillations and the activity of ripples and spindles, which could indicate that the up-phases in slow oscillations initiate information transfer between the hippocampus and the cortex (Contreras & Steriade, 1995).

Hippocampal Sharp Wave-Ripples

Hippocampal sharp wave-ripples [ripples] are high frequency oscillations seen in EEG recordings during sleep. Specifically, they appear to be originating from the hippocampus (Diekelmann & Born, 2010).

One study that investigated ripple activity constructed a spatial discrimination task in which rats were provided reward at the end of a correct selection of route (Ramadan et al., 2009) .The rats were split into three groups: control, trained and pseudo-trained (where they were given a reward regardless of whether they made the right choice). The amount of ripple activity was recorded after each training session, which mostly involved SWS.

The results show a correlation between ripple activity and the training process, which would have instigated memory consolidation. The behavioural aspect was also investigated by comparing ripple activity in day 6 to mean errors in day 7. The rationale was that if ripple activity was correlated with memory consolidation then the peak performance would be the day following the night with the highest mean ripple activity (day 6). The result show a negative relationship, indicating that ripple activity corresponds with better learning task performance.

Spindles

Spindles are found mostly in stage 2 NREM sleep, but can also be present in SWS. These are patterns of oscillatory activity at 10-15Hz that appear for periods up to 3 seconds. Sleep spindles are thought to be generated in the GABAergic neurones of the nucleus reticularis and propagated globally via cortico-thalamic circuits (Rasch & Born, 2013).

Increases in spindle activity have been associated with declarative memory learning (Schabus et al., 2004). This is another observational study involving word-pair learning tasks. The results show that the subjects who had a positive change in the spindle activity between the experimental and control nights achieved better results in the test.

Another study looked at the association of spindle activity with the integration of new memories with old memories. The experiment involved introducing novel words in a learning task that are similar to existing words in the subject's vocabulary (e.g. cathedruke-cathedral) (Tamminen et al. 2010). The sub-experiment of interest was an auditory lexical decision task, where the subject had to decide whether a presented spoken word was a real word or not; real-control words were mixed with real-cue words (cathedral) and nonsense-associated words (cathedruke). The results show a clear positive correlation in the spindle activity in the night's sleep that immediately followed. This supports the hypothesis that memories are indeed reorganised after their initial acquisition, as this task specifically tests the ability of the subject to combine new information with old information (marked by increased lexical competition).

5. Combining the Synaptic Homeostasis Hypothesis and the Active System Consolidation Hypothesis

Though they contend to be independent theories in themselves, the synaptic homeostasis hypothesis and the active system consolidation hypothesis are not mutually exclusive (Diekelmann & Born, 2010).

Genzel et al. attempt to bring the two theories together by focusing on the exact sleep stages at which these various EEG recordings are observed (Genzel et al., 2014). They suggest that the whilst reactivations of memories can occur in all stages of NREM sleep, exchange of information at a global level is likely to be more significant in light sleep (stages 1 and 2 of NREM sleep). Synaptic downscaling, on the other hand, would occur more during the deeper stages of NREM sleep (SWS).

Genzel's theory suggests that synaptic downscaling itself is insufficient as a way of consolidating memories, as it does nothing to integrate or reorganise new and old memories at a systems level. This is especially considering that the SHY is not specific about which neurological structures drive learning. Instead, it focuses on the specific neural environment that sleep produces and its relevance to memory consolidation, at a much more synaptic level.

6. Homeostatic Modulation

Another factor that should be considered is how the brain controls the overall process of consolidation. Sleep itself is initiated by structures in the thalamus, and propagates globally via different neurotransmitter systems. This includes cholinergic, noradrenergic and serotonergic neurones, whose activity in general is decreased during sleep. Acetylcholine in particular has been studied in relation to memory consolidation.

Ach

The activity of acetylcholine [Ach] systems on memory consolidation has been studied using pharmacological methods to modulate its tonal activity (Gais & Born, 2004b). At sleep onset, subjects were given an infusion of physostigmine, a cholinesterase inhibitor that indirectly increases the activity of pathways involving Ach as a neurotransmitter. The result showed a decrease in memory task performance that was specific to declarative memory.

Activation of Ach has been shown to also increase synaptic LTP in in vitro preparations of hippocampal structures (Hasselmo & McGaughy, 2004). This does seem to support the synaptic homeostasis hypothesis as well, as it is possible that the differential activation of Ach between and waking and sleep could account for changes neural activity in general, and hence in synaptic potentiation.

Equally, this can support the active consolidation hypothesis – cholinergic activity could be directing the brain between a state of encoding new memories and a state of consolidation. Specifically, the pathways inhibited by cholinergic activity are feedback circuits in the CA3 as well as projections to CA1 and the cortex; these are circuits that are involved in active consolidation – where information is transferred between the hippocampus to the cortex.

Endocrine Control

The endocrine system has also been implicated in memory consolidation. Cortisol is a glucocorticoid that has a wide range of effects throughout the body. Its level during sleep is usually low, and when this is raised pharmacologically then the enhancing effect of SWS sleep on declarative memory consolidation is reduced (Plihal & Born, 1999). Pre-encoding levels of cortisol also seem to have a positive correlation with memory recall performance (Bennion et al., 2013).

This is reflective of the waking state as highlighted by high Ach levels. Cortisol could act on the brain to increase alertness (by up regulating Ach). From an evolu-

tionary point of view it is beneficial to maximise the capacity of the brain to acquire new information in a stressful situation (as survival may depend on remembering an escape route, for example). Equally, cortisol levels would decrease at night to allow for the switch into a 'consolidation state' to enable these new memory traces to be retrieved more efficiently at a later time.

7. Evaluation of Experimental Technique

Semantic Representations in the Brain

As with any experiment, the methods used to obtain the data must be carefully scrutinised. As has been mentioned earlier most studies used word-pair association tasks as the parameter for learning, with an associated cued-recall task.

A question should be raised as to whether the semantic meaning of words can affect how they are stored in a memory system. There are two popular models of how semantic information is represented in the brain. The network model involves individual concepts that can be connected in any way, such that 'red' might be linked to either 'fire truck' or 'apples' (Collins & Loftus, 1975). The strength of these connections will depend on the relevance between the two concepts. For example, 'eagle' will be better associated with 'hummingbird' than 'squirrel', because they are both birds and can fly etc., but 'eagle' might be better connected to 'squirrel' than 'table' because they are both animals. The feature model, on the other hand, is based on the idea that any object can be broken down into its constituent features, such as a 'squirrel' into 'tail' and 'head' (Tyler & Moss, 2001). Each object is then identified through a statistical analysis of the most relevant features. 'Head', for example, might be attributed to 'squirrel' or 'eagle', but 'wings' will be specific to 'eagle'.

This is relevant because experiments involving learning tasks have used different designs in the word pairing. In Tucker's experiments (2006), these words were semantically related (e.g. clock-hands). However, other experiments involved word pairs of undetermined semantic relationship e.g. two German nouns (Plihal & Born, 1999) or even words that are specifically not semantically related (Schabus et al., 2004).

The dependent variable in most of these experiments is the error rate in recall, and difference of this before and after sleep, to highlight its effects. However, the way that two semantically related words are encoded and stored in the brain is likely to be different than for two words which are not semantically related. Related word-pairs might undergo much further integration over time, in either the network or the feature model. This means that sleep might have a great effect in enhancing the association between these words, compared to non-related words where further processing would not be useful.

Most experiments currently do use a protocol that utilise word pairs that are semantically related words, but even then there is an issue of variability between pairs depending on their semantic meaning. According to the feature model, remembering a word pair that consists of an object ('dog') and a feature of that object ('tail'), for example, would involve simple and direct link between object and component. On the other hand, remembering a word pair that consists of two objects that share similar features ('dog') and ('cat') would involve retrieving two sets of different features, which may involve many different links (tail+nose+etc.). The latter would be a much more complicated process in terms of retrieval, and this might have an influence on performance in recall.

Implications of Correlational Data

Generally, the first step towards uncovering the underlying mechanism behind a particular physiological process is to find a correlation between two elements within that process. However, a very basic but important point is that correlation does not imply causation. A correlation between A and B, for example, could mean that A causes B, B causes A, a third element C causing both A and B, or something much more complex.

This is important because many studies involving EEG activity show correlations, but there is seldom much indication of a causative relationship. The reason behind this is mostly to do this limits on experimental technique – sleep is a very complicated process whose mechanisms we do not understand fully, so it is difficult to a) identify a single mechanistic process and b) manipulate this in human subjects. Though slow oscillation, spindle and ripple activity have all been implicated in memory consolidation, only manipulations of slow oscillations have demonstrated a causative effect (Marshall et al., 2006; Marshall et al., 2011).

Limits of EEG Studies

An EEG study involves recording changes in potential across electrodes that are placed around the skull.

It is a good way of detecting global changes in activity. However, this is still examining neural activity at a very basic level. There could be many interactions happening between brain structures during sleep that do not produce any EEG activity, or more than one process which contribute to the overall EEG trace (such as slow oscillation or spindles).

Factors Affected in Sleep Deprivation

Most sleep-deprivation studies simply involve not allowing the subject to sleep at all following a learning task (Gais et al., 2007). This could be a problem as other stages of sleep could also be involved in physiological processes which we are not yet aware of, but have an effect on cognitive and memory functions indirectly.

One study had attempted to specifically deprive SWS to test for effects on the following nights of sleep. They did this by using an auditory stimulus to disturb the subject enough to prevent them from entering SWS, but without waking them completely; this was guided by a polysomnograph (Ferrara et al., 1999). The subjects received two nights of SWS-deprived sleep followed by a normal recovery night. The result was that though the overall length of sleep did not increase following deprivation, the percentage of Stage-4 NREM sleep and also SWS in general was increased. The results show that there is a need for SWS and that its deprivation will incur a 'debt' that must be paid later on, as evidenced by the increase in the recovery night. The same technique might be applied to a declarative memory task, with an experiment set up to test the effects of SWS deprivation on learning.

8. Overall Analysis

Ultimately, these studies aim to provide a full model of the functions of sleep and how it relates to all types of memories, though this is yet to be achieved. Of the two main theories discussed, the active system consolidation hypothesis is probably more complete as a model, as it brings together much more data involving the interactions of the hippocampus with the cortex. It is worth noting that the results from the vast of majority of studies would either concur with this theory, or provide extensions to its parameters.

Its suggestion of the roles that the hippocampus plays in declarative memory consolidation also fits well with neuropsychological evidence that lesions to this structure lead to amnesia. In patient R.B., for example, localised lesions to CA1 of the hippocampus was accompanied by severe anterograde amnesia but limited retrograde amnesia (Zola-Morgan et al., 1986). Data from other patients show that more extensive damage to the hippocampus and its surrounding structures will lead to more severe retrograde amnesia (Rempel-Clower et al., 1996). The reason could be that CA1 is primarily responsible for teaching of new information to the cortex, and lesions to this area will impair only the encoding of new information. Lesions extending to further areas including CA3 and the dentate gyrus [DG], which are involved in the extraction of memory traces from the cortex, will hence impair the retrieval of old information as well, leading to more severe retrograde amnesia.

Claims made by the SHY regarding the need for synaptic downscaling are probably valid. However, the data is less congruent. One study, for example, suggests that SWS in fact promotes the opposite process of synaptic upscaling (Chauvette et al., 2012). Another study recommends REM sleep instead of SWS stage where synaptic downscaling occurs (Grosmark et al. 2012). Further experiments will be needed to clarify this apparent disparity.

Although these experiments have shown that SWS sleep is primarily involved in declarative memory consolidation, it is likely that other stages, including REM sleep will have an effect either directly or indirectly. A potential role of REM sleep is in synaptic consolidation, which could occur separately from SWS-mediated systems consolidation. A reduced interaction between the cortex and hippocampus (systems-level consolidation) could enable consolidation to occur at a synaptic level, with LTP being enhanced. This is the basis of the sequential hypothesis, which suggests that memory consolidation requires a cyclical alternation between both NREM and REM sleep.

Another concern is that it may be too simplistic to separate information into distinct and independent memory systems. Declarative memories (episodic memory in particular, but perhaps also semantic memory) could be associated with emotional responses, which under the dual system hypothesis are based on completely different neural structures. This raises the question of whether a single memory trace can be processed in two different memory subsystems.

Implications for Reconsolidation

The active system consolidation hypothesis also

has an implication for memory reconsolidation, which is a more controversial topic. Memory reconsolidation is the process through which a stable long-term memory that has become labile is re-stabilised. This has been studied mostly for fear conditioning, a type of non-declarative memory that involves the amygdala and the associated limbic system. However, reconsolidation has also been identified for declarative memory (Forcato et al., 2010).

The puzzling feature for reconsolidation is why established memory traces would be made labile again. The active system consolidation hypothesis would explain this as a part of the normal consolidation process between the hippocampus and the cortex. When a memory is retrieved the information it contains is extracted and transferred to areas where it is needed elsewhere in the brain (such as motor cortex). It is possible that this information is also transferred to the hippocampus to enable this to be updated. The hippocampus will always attempt keep memories updated. If there were new information that is relevant to existing memories, then this would be integrated together and transferred back into the cortex. This means that the old memory would be deleted and replaced with an updated 'version'. This has been demonstrated in evidence which suggests that NREM sleep affects the integration of new memories into old memories (Tamminen et al., 2010). Therefore, reconsolidation could be not a separate process, but a manifestation of the long-term continuation of consolidation.

9. Further Experiments and Conclusions

There are still many questions that are yet to be answered; further experiments could be carried out to test some of these considerations:

Experimental designs should reflect on that the nature of semantic associations could have an effect on the way memories are encoded. Control protocols should be introduced to take into account the semantic meaning of word pairs.

Different experiments techniques need to be developed in order to manipulate activities such as spindle and ripple activity, to demonstrate that these processes have a causative effect on consolidation. As an example, Marshall's experiments (2006, 2011) involved modifying slow oscillation activity using tDCS; this technique could perhaps be adapted to give stimulations that replicate spindles or ripples.

Other neuromodulators, such as noradrenaline and serotonin, may also be relevant in controlling global neural activity. Again, manipulative techniques could be used to investigate their importance in the top-down control of memory consolidation.

The role of REM sleep on declarative memory is unclear. Studies should expand their premises to other stages of sleep, as sleep is likely to be a very integrated process.

Possible interactions with emotional memory as well as other types of non-declarative memory should also be explored. A single memory trace could be represented in more than one memory system; alternatively, two memory systems could interact to encode the same memory process.

On the whole, the studies I have discussed collate well to give a general picture of the mechanisms behind how NREM sleep affects the consolidation of declarative memories. However, more experiments are needed to clarify some aspects of an overall theory, as well as to investigate further the mechanistic details.

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The Effects of Internal and External Expressions of Felt Luck On Performance

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ABSTRACT. Superstitious actions and belief in luck should, hypothetically, have no causal relationship to the actual outcomes of events. However, many individuals still develop and observe superstitions to potentially increase the likelihood that events turn out in their favor (Abbott & Sherratt, 2011). Prior research has shown that individuals with superstitious tendencies tend to have better performance results (Damisch, Stoberock, & Mussweiler, 2010). The present study examines the differences between sources of felt luck, which are broken down into two categories: internal and external. Internal sources of luck are beliefs or actions that exist within the individual; for example, an individual's belief that wearing a shirt would bring them luck. External sources of felt luck are activated from an outside source; for example, someone wishing an individual "good luck." In this study, 60 participants were randomly assigned to a luck group (internal, external, or control). Participants completed the Belief in Good Luck Scale which measures an individual's belief in luck, combined with a personality test, and played a game of miniature golf, during which luck was manipulated based on the group participants were in. The control group made an average of M =1.8 putts, the internal group made an average of M =3.4 putts, and the external group made an average of M =3.3 putts. This study established that participants in luck groups performed significantly better than the control group, but one particular source of felt luck is not superior to another.

1. Introduction

Serena Williams, the world renowned tennis phenomenon, ties her shoelaces in an extremely particular way before every match to bring her luck for the game. Williams has even blamed major career losses on not properly executing her superstitious rituals before matches (Murphy, 2010). While her actions may seem nonsensical; the belief in superstitions has been shown to lead to enhanced performance on cognitive, motor skill, and physical tasks (Damisch, Stoberock, & Mussweiler, 2010).

Superstitions are commonly defined as "actions or inactions that are given in order to affect the probability that a beneficial outcome occurs when, in fact, there is no causal relationship between the action and the outcome" (Abbott & Sherratt, 2011). The drawback of this commonly used definition is that it does not incorporate the notion that both objects and actions can have luck benefits or neither does it imply that the belief in luck is largely irrational. Lindeman and Svedholm's (2012) paper "What's in a Term?" responded to the absence of an effective definition of luck in research. In their paper, the authors recognized that domain-general definitions that apply to a wide variety of contexts don't generally work well in research because they are "too broad in scope to be useful" (p. 248). As a result, Lindeman and Svedholm created a new, narrower definition for luck and superstitious behaviors, which is "an irrational belief that an object, action, or circumstance that is not logically related to a course of events influences its outcome" (p. 250). This study will incorporate luck as defined by Lindeman and Svedholm's, because it offers a focused and narrow conception of luck while encompassing the idea that beliefs and actions can be irrational thoughts.

Sixty-two percent of individuals have been found to observe and develop superstitions (Vyse, 1997, p.18). The most common forms of superstition are carrying a lucky charm, knocking on wood, or crossing fingers for good luck (Rudski & Edwards, 2007).

Evidence indicates that a small percentage, around

7% of American people, unquestionably deny any belief in superstition or luck, (Vyse, 1997, pg.17), but they still make decisions based on underlying beliefs in luck, superstition, and magic (Henslin, 1967). It is possible that these luck decisions operate at an unconscious level of thinking. A study done by Block and Kramer (2009) found that consumers are more likely to make an unsound economic decision when purchasing an item that has a positive superstitious or luck component associated with it. For example; in China, the color red is associated with good fortune. During the Chinese New Year and special family events, the Chinese are willing to spend more money on objects that contain the red because it symbolizes luck and good fortune. Even the participants who denied believing in luck were still more likely to purchase a more expensive object if it had a lucky component associated with it. This study provides evidence that superstition and luck beliefs may operate on an unconscious level.

Superstitions have been typically found to develop when the cost of having a superstition is low compared to its relative benefits (Abbot & Sherratt, 2011). For example, knocking on wood or avoiding the number 13 in order to avoid tempting fate are often not that difficult or time-sensitive to complete. The perceived benefits of engaging in those superstitions are likely to outweigh the low cost of the action. When the belief or action concerning a superstition has a low cost, and the advantages associated with the superstition are high, there are few reasons to not develop superstitious tendencies or believe in luck.

Skinner (1948) and Vyse (1997) found that people and some type of animals engage in superstitions. In 1948, Skinner observed superstitious actions in both pigeons and humans by manipulating their food schedule. First, Skinner activated a behavior in the animals and humans, and then reinforced this action with food. An example of an activated behavior was a tossing motion of the head or a turning of the body in a clock-wise circle. Later, when there was no reinforcement of the behavior, the birds and humans still associated a causal relationship between their actions and the outcomes. As a result of food manipulation birds and humans began to engage in superstitious behaviors. Skinner helped establish that both humans and animals have the potential to engage in superstitious actions. Vyse (1997) conducted a poll on collegiate athletes, from a range of abilities, and found that 84% engage in a luck belief or action before or during a performance. The majority of athletes who engaged in luck beliefs had a lucky object or ritual they completed before the start of a competition.

Superstitious beliefs and behaviors are prevalent in a majority of individuals (Gilovich, 1999). A Gallup Poll conducted in 1990 found that 62% of Americans believe in superstitions (Vyse, 1997 p.18). Why do so many people develop and maintain superstitious or luck beliefs? One explanation is that there are positive performance benefits associated with luck and superstitious beliefs. Previous studies have shown that the belief in superstition can aid an individual in performing better on a task when the action or confidence of luck is manipulated (Damisch, Stoberock, & Mussweiler, 2010). Additionally, some individuals tend to hold a strong belief that they can control a particular outcome even if it is clearly a matter of chance (Langer & Roth, 1975). A study conducted by Langer and Roth demonstrated that an individual who held a higher expectancy of personal success than was warranted for a skill situation carried that confidence into chance situations. Individuals were asked to predict the outcome of 30 coin tosses. The feedback was manipulated, so each individual was right exactly half the time, but the two groups differed by when the feedback of correct coin tosses was distributed. The group who had successful guesses early on in the coin toss overestimated their success compared to the group whose feedback was distributed evenly. Through the use of a coin, the study found that indicators of confidence supported the prediction that individuals believe they can control a particular outcome, even when that outcome is the result of chance (Langer & Roth, 1975). Another Langer's experiment involved a lottery ticket situation. Participants were either given lottery tickets at random or allowed to choose their own ticket. Subjects then had the chance to trade their tickets with other tickets, with a higher chance of paying out. Participants who had chosen their own ticket rather than being given a ticket at random were more reluctant to part with it. Although these lotteries were random, subjects behaved as though their choice of ticket affected the outcome (Langer, 1975). Individuals can unconsciously believe that some external force has allowed them to perform better, and this may be linked to the placebo effect, which functions in a similar way (Rajogapal, 2006).

Patients who are given a placebo treatment, which is used in medical research as a control condition, will sometimes experience a perceived or actual improvement in their medical condition. The placebo effect can foster

actual medical improvement via an individual's perception of improvement. An individual's perception of improvement can be similar to superstition and luck in the sense that when individuals feel like they have a heightened chance of success they achieve higher success even when the action or belief is irrational. The phenomenon of subjective perception of improvement is seen in superstitions in addition to the placebo effect. Different hypotheses have tried to make sense of the underlying psychological mechanisms that create this increased performance outcome.

One hypothesis is that engaging in superstitions "regulates psychological tension and creates a feeling of control and a sense of predictability in otherwise chaotic environments" (Damisch, Stoberock, & Mussweiler, 2010, p. 1014). The feeling of control in an uncertain environment allows people an advantageous sense of ease and reduced anxiety, so those who do not believe in superstitions can be at a disadvantage in an unfamiliar environment as they might be more likely to perform poorly on a task (Keinan, 2002; Schippers & Van Lange, 2006).

Another hypothesis for why individuals engage in luck beliefs is that they fall into the gambler's fallacy mindset. The gambler's fallacy is the mistaken belief that in a series of chance events the probability of one event occurring increases with the number of times another event has occurred in succession (dictionary.com). If individuals have had negative outcomes happen more frequently than normal in the past, they believe future events are most likely to be positive results, so that a balance of luck occurs. For example, when individuals who have had an unlucky streak at a casino assume their luck will turn, they are engaging in the gambler's fallacy. Because there is clearly no relationship between the past and future events this is a mistaken idea. People frequently make unconscious decisions based on these illogical beliefs (Gentsch & Bosbach, 2011). Some individuals fall into this mindset because they are more likely to remember an unlucky sequence of events over the successful succession of events (Darke & Freedman, 1997).

Another reason people engage in superstitions is that some individuals, in general, think they are consistently luckier than others (Vyse, 1997). These internal feelings of personal luck can contribute to an increased illusion of control, an expectancy of personal success probability inappropriately higher than the objective probability would warrant, which can be advantageous in chaotic or unfamiliar situations where a number of variables contribute to success and failure. These feelings of control can lead to heightened performance benefits, because it leads to sense of predictability and perceived self-efficacy (Damisch, Stoberock, & Mussweiler, 2010, p. 1014).

Luck expressions can be broken down into two major categories: external, which are activated from an outside source and internal, which is a belief or an action that exists within the individual. External expressions of luck are explicit statements intended to wish luck on the receiver, such as, "good luck" or "I'm crossing my fingers for you" before he or she participates in a task. These statements become external superstitions if the receiver actually believes they will bring them luck in their endeavors. These expressions can be reflections of superstitious behavior. External expressions of luck are thought to create situations where the participant's motivation to perform well is heightened. One interpretation is that the psychological mechanism underlying external expressions of luck is motivation, which subsequently leads to higher performance on the task because there is more at stake (Damisch, Stoberock, & Mussweiler, 2010). A study showed participants who were exposed to an external expression of luck before a motor-dexterity experiment solved the task significantly faster than the control group (Damisch, Stoberock, and, Mussweiler, 2010). Participants in the superstition activated group solved the task at an average speed of ($M = 191.5$ s, $SD = 117.1$ s), compared to the time it took the control group to solve the same problem ($M = 319.7$ s, $SD = 223.6$ s), $t(48) = 2.0$, $p < .05$, Cohen's $d = 0.72$. The perceived causal mechanism that allowed the superstition activated group to perform the task faster, was an increased level of motivation in the participants.

An internal expression of luck is a belief or an action that exists within the individual, such as a personal pre-exam ritual, or rubbing a rabbit's foot. It has been found that students who engage in an internal expression of luck ritual are generally more relaxed and feel better prepared, resulting in an increase in performance level due to illusion of control (Rudski & Edwards, 2007). Langer defines illusion of control as "an expectancy of personal success probability inappropriately higher than the objective probability would warrant" (1975, p.313). Illusion of control is generally accepted as the central psychological mechanism underlying internal expressions of luck (Drake & Freedman, 1997), as it has been observed that there is a high correlation between internal

expressions of luck and feelings of control (Damisch, Stoberock, & Mussweiler, 2010).

Internal and external superstition performance benefits have been separately studied, but never pitted against each other in the same task. Directly comparing the effects of internal and external expressions of felt luck on performance can help elucidate the underlying mechanisms at work regarding how superstition affects performance. This study directly compares the effects of internal and external expressions of felt luck on performance to investigate the mental processes underlying luck. To examine the mental process underlying luck, this study will divide participants into three groups: external, internal, and control. Depending on the group individuals are placed in, they will have different starting instructions given to them before playing miniature golf. The altered starting instructions will activate different luck components, and then participants will be measured by how many putts out of ten they make.

It was hypothesized that the internal superstition group would perform significantly better than the external group and control group on miniature golf, because the psychological mechanisms of illusion of control and motivation will influence the internal group's performance the most. It is widely accepted that internal superstitions activate illusion of control, but motivation can also potentially be activated with internal superstitions. Motivation can also be activated because individuals who choose to engage in an internal superstition need to believe the action or object is powerful, so they attempt to convince themselves that their object or action is effective in its purpose. Individuals have the motivation to prove to themselves that their internal superstition is lucky, because if the superstition does not bring about the benefits it was intended to, then the superstition is rendered ineffective and useless. For example, an individual might carry a rabbit's foot to a test, anticipating that the object will bring them luck, but if they never perform well on the test, the belief in the object will diminish. This idea suggests that individuals with an internal superstition have a secondary incentive to improve their performance. Because internal superstitions could potentially activate both increased levels of motivation and an illusion of control influence, whereas external superstition may only activate increased motivation. It's likely that internal superstitions would be more successful in yielding a greater improvement in performance than external superstition. Additionally, the external group should perform significantly better than the control group, because the external group is activating motivation, whereas the control group is not purposefully activating any enhancing psychological properties.

Additionally, because I have data on personality from an initial questionnaire, I will examine the correlation between personality and belief in luck. The personality test evaluates five traits: extraversion, conscientiousness, neuroticism, openness, and agreeableness. I hypothesize that there will be a negative correlation between conscientiousness and belief in the luck. Conscientious people are typically diligent, display planned behavior, and think deliberately before making a decision (Costa & McCrae, 1992), and these characteristics do not tend to align with a high belief in luck. Typically, individuals who have a high belief in luck are more laid back, leave things to chance, and believe that there are some factors out of their control. Strong characteristics of a conscientious personality seem to be in direct contrast to characteristics for a high belief in luck.

2. Methods

Participants

Sixty undergraduate students between the ages of 18 and 23 years old from Carleton College, a liberal arts institution, participated in the study. Participants were recruited from introductory level classes, flyers, and word-of-mouth references. 30 males and 30 females were selected, and the average age for all 60 participants was 20.2 years old. The 60 students were randomly assigned to one of three groups: internal superstition, external superstition, and control group, and 20 participants were assigned to each of the three conditions. Participants were given candy and refreshments for participating in the study, and their names were entered in a raffle to win one of five $10 cash prizes.

Materials

All 60 participants were given the Belief in Luck Scale and The Big Five personality questionnaire, which was compiled into one computerized test. The Belief in Luck Scale (Darke & Freedman, 1997) consists of 12 questions measuring an individual's belief in luck. The 12 Belief in Good Luck questions were answered by participants using a 6-point Likert scale. An example of a question on the Belief in Luck Scale is "I consider myself to be a lucky person." The Belief in Luck Scale has

proven to be reliable (alpha = .85) (Darke & Freedman, 1997) and valid, which means that the test measures what it was intended to measure (see Maltby, Day, Gill, Colley, & Wood, 2008). The Belief in Luck Scale questions were interspersed among a battery of other questions, so the participants were unaware of the purpose of the questionnaire. The other 60 questions in the test were personality questions measuring the "Big Five" personality traits. The Big Five personality traits are openness, conscientiousness, extraversion, agreeableness, and neuroticism (Costa & McCrae, 1992). The Big Five personality traits represent broad categories, but are normally defined as: openness reflects the degree of intellectual curiosity, conscientiousness is the tendency to be organized and dependable, extraversion is the feeling of being outgoing and energetic, agreeableness is the predisposition to be compassionate and cooperative, and neuroticism is the tendency to experience unpleasant emotions easily. The Big Five Personality Test has been proven to be reliable (alpha = .87 to .92) and valid (Costa & McCrae, 1992). The Big Five personality test asks questions such as, "I often get angry at the way people treat me." The 60 questions regarding personality were also answered on a 6 point Likert scale with the response ranging from 1 (strongly disagree) to 6 (strongly agree).

The task that all participants completed was miniature golfing on an indoor putting green. Participants stood four feet away from the hole, which was raised an inch and a half off the ground. Each participant placed the ball on the designated line and then they got 10 putts to see how many they could make. Miniature golfing on an indoor green has been used in prior luck research and has been successful at obtaining luck differences (Damisch, Stoberock, & Mussweiler, 2010). All participants used the same golf club, golf ball, and putting-green when completing the miniature golfing task. Each golf shot was taken at a distance of four feet (122cm) on a standardized green.

After the game of miniature putt was completed, all participants filled out an additional four-question survey, which served as a manipulation check. Three of the four questions were answered by the participants using 5-point Likert scale. These questions measured participants' confidence level, luckiness level, and amount of previous experience with miniature golf. The fourth question was a fill-in-the-blank answer that asked participants to write what they thought the purpose of the study was.

Procedure

Before participating in any questionnaires or tasks, all participants were required to read and sign a consent form stating that they agreed to participate. Once the consent form was signed, participants were informed of the three segments of the experiment. Participants were told that the three segments began with an online questionnaire, followed by 10 rounds of miniature golf, and ended with a four-question survey. All subjects were encouraged to ask any questions that arose before or during the task. After all questions were answered, participants began the experiment.

Prior to arriving at the experiment, participants were randomly assigned to a group: internal, external, or control. If participants were randomly assigned to the internal group, they received an email prior to the experiment asking them to bring an item that is personally lucky for them. They were also sent an additional reminder via text or email two days prior to the experimental session. The internal group was asked before the experiment began if they had remembered their lucky object and none of the internal group reported having forgotten a lucky item. Students placed in the external and control group were not required to bring anything to the study, and were not informed that the internal group was required to bring an object.

After arriving, participants were led to a computer behind a screen for privacy purposes. They completed a survey that consisted of 72 questions; all answered on a 6-point Likert-type scale (1 –strongly disagree, 2- disagree, 3- slightly disagree, 4- slightly agree, 5- agree, 6 – strongly agree). Once the online survey was completed, the participants notified the researcher. Participants were then led across the room by the researcher to a putting green. Each participant was handed the same putter and golf ball and was given instructions for the miniature golf portion of the experiment. Participants stood on a standardized green four feet (122cm) away from the hole. The hole was raised one and half inches above the ground, which created a slight incline to the hole. Participants were informed that they had 10 hits from the starting line, marked on the green, to attempt to get the ball in the hole while the researcher kept track of makes and misses. A make constituted as a participant standing in the correct spot and on one swing successfully landing and keeping the golf ball in the hole. A miss was any swing where the golf ball didn't land and stay in the hole. All participants

were given the same instructions, but the starting cue given to them was condition dependent. The control group was told, "I hope you do well, (participant name)! You can start whenever you are ready." The internal group was told, "I hope you do well, (participant name)! Good luck on putting, I hope your lucky object (object name) helps. You can start whenever you are ready." The external group was told, "I hope you do well, (participant name)! Good luck on putting, I have my fingers crossed for you. You can start whenever you are ready."

Once the actual task of miniature putt was completed, the individuals were given the manipulation check survey.

Once the participants finished the entirety of the experiment, they were thanked and given candy and refreshments as compensation. The whole process, on average, took less than twenty minutes per participant. No one misunderstood the task and everyone completed the task in its entirety, so all data was used in the results. After completion participants were also given the option to put their name into a raffle. The five winners of the raffle were given $10 cash prize. All participants finished the experiment in its entirety.

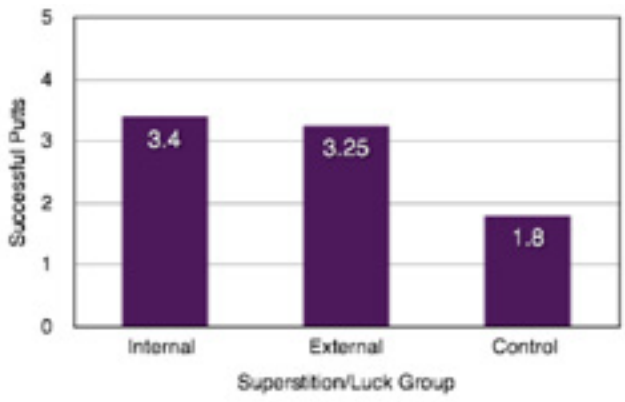
3. Results

The first manipulation check found that 14 out of 60 (or 23%) participants guessed or understood the purpose of the study. Four people in each the control group and external group understood the task was measuring luck in some capacity, and six people in the internal group recognized the purpose of the task. There were no statistical differences between groups regarding participants who guessed the underlying purpose of the study, so the participants were not excluded from the data set.

The second manipulation check measured participants' experience levels in miniature golf, and it was found that the experience level of participants did not vary significantly between any of the three groups. The control group had an experience level of (M =2.25, SD =1.16), the internal group had experience level of (M =2.45, SD =1.28), and the external group had an experience level of (M =2.75, SD =1.62).

The third manipulation check measured participants' confidence levels in regards to miniature golf capabilities. An independent t-test was used to compare the means to see if there was a significant difference in the population's confidence levels. The average confidence level of the control group was significantly lower

Figure 1. Successful Puts Based on Superstition/Luck Group



(M =2.55, SD =1.05) when compared to the average confidence level of the external group (M =3.65, SD =1.24), t(38) =3.03, p <.05. Additionally, the internal group had a high confidence level of (M =3.26, SD =1.28) and when compared to the control group (M =2.5, SD =1.05), there was a strong trend towards a significant difference in scores, t(38) =1.92, p <.068. The external group exhibited the highest level of confidence, followed by the internal group, and then the control group.

The fourth manipulation check measured how lucky participants felt during the miniature golf portion. The average luckiness level varied at a significant level between all three groups. The average luckiness level of the control group was
(M =2.15, SD =.88), that of the internal group was (M =3.50, SD =.95), and that of the external group (M =2.9, SD =.72), N (60), p <.05.

Only one personality trait in the test, extraversion, was shown to have any significant correlation to the belief in luck. The Pearson's correlation coefficient showed a positive correlation between belief in luck and extraversion r =.32, N (60), p < .05.

The Belief in Good Luck Scale measured participants' belief in luck. The average belief in luck score did not vary significantly between any of the three groups. The control group had an average belief in luck score of (M =38.4, SD =9.22), the internal group had average belief in luck score of (M =38.45, SD =11.46), and the external group had an average belief in luck score of (M =35.05, SD =7.90).

This study's main purpose was to determine if the average number of successful putts varied across the groups based on the source of felt luck. The study used the number of successful putts in miniature golf (when the ball ended up where it was supposed to be) as the de-

pendent measure. As predicted, participants performed better when superstitious expressions of luck were activated. The internal source of luck (M =3.35, SD =1.59) performed significantly better than the control group (M =1.80, SD =1.28), N(39), p <.05. The external expression of luck also performed significantly better (M =3.25, SD =1.97) than the control group (M =1.80, SD =1.28), N(39) , p <.05. There were no significant differences between internal (M =3.35, SD =1.59) and external (M =3.25, SD =1.97) expressions of luck. The results indicate that sources of felt luck do bring improved scores (see Figure 1), but this study did not find any differences between internal and external expressions of luck on performance.

4. Discussion

The findings in this study show that participants in both the internal and external luck source groups performed significantly better than the control group participants on miniature golf. However, there were no statistical differences between internal and external superstition in terms of manipulation groups on performance. Because there were no statistical differences between the two luck conditions: external and internal, the results suggest that their respective psychological mechanisms of motivation and illusion of control may hold the same potential to increase performance on a task.

The widely recognized mechanism behind the impact of external expressions of luck is motivation, whereas the recognized mechanism underlying internal superstition is illusion of control (Damisch, Stoberock, & Mussweiler, 2010). This study would suggest that, while the mechanisms that underlie each superstition type diverge, performance results increase by comparable amounts. What factors could contribute to this lack of observed statistical difference?

There are two major explanations for why this study did not find any differences between expressions of luck. First, there may not be a difference between internal and external expressions of felt luck. It could potentially not matter what type of superstition is activated in order to get performance benefits. Second, there may have been methodological errors in the study that failed to bring out differences between expressions of luck. One main limitation in the study was the inconsistent relationship between the researcher and participants in the external superstition group. The reason that the external group could be seen as a limitation to the study is because the participants had a previously unknown, or in some cases known, researcher wishing them luck. The relationship of the researcher to the participant was not a consistent component. This variable was difficult to control for because of the small college the participants were selected from and the random assignment of the participants in the groups. The relationship between the researcher and participants varied notably in the external group. Another limitation to the study is that all participants were all randomly assigned into one of the three conditions, so it was not clear if participants who were randomly placed in the internal group owned a personal lucky charm that they could bring to the study. As a result, the 20 participants assigned to the internal superstition group may not have had a personal lucky item that evoked feelings of luck. Regardless, all participants in the internal group were asked to bring a lucky item, but if they didn't have a lucky item they were asked to bring an object nonetheless. It is likely that some individuals assigned to in the internal group may not have had a lucky object, so they may have brought a non-lucky item to fulfill the requirement of bringing an object to the experiment.
This could have potentially skewed the results of the internal group, so that no differences were found between the internal and external expressions of luck in this study.

This study shows that internal and external expressions of felt luck are both beneficial for improving performance. Both subcomponents of luck are advantageous in performance because they activate psychological mechanisms that create a favorable environment for the participants. Internal expressions of luck are recognized to activate illusion of control, which creates an advantageous sense of ease and control in an unknown environment, which can be beneficial when making a decision or performing a task. External expressions of luck are widely known to activate motivation, and if an individual embodies an external expression of luck they may have an increased motivation to perform better. The psychological mechanisms for internal and external sources of luck create environments that are advantageous for those believing in luck and lead to performance benefits in comparison to individuals who don't activate those psychological elements.

Additionally, this study also found that there is a positive correlation (r =.32) between belief in luck and extraversion. A study done by Neil Lutsky found this same positive correlation between extraversion and belief in luck at r =.305 (N =49). Why could there be a

connection between extraversion and belief in luck? One hypothesis for why extroverts believe in luck more often than introverts is that typical extrovert characteristics include a desire for novel experience with different people, which could allow extraverted individuals more chances to experience luck. Two representative traits of extroverts are that they are outgoing and enjoy unfamiliar situations, so they tend to put themselves in new positions more often than introverts, who tend to shy away from new situations. Because extroverts tend to seek out new situations, and go with the flow, when things work out for they might misattribute luck as the reason why they're successful in certain endeavors instead of their personality traits. Because extroverts are outgoing they have more chances to engage in luck scenarios and contribute these new experience to a positive belief in luck. Another idea is that the possibility of failure doesn't evoke the same anxiety for extroverts as it does in introverts. A further defining trait of extroverts is that they are less likely to experience anxiety over negative feedback or failure compared to introverts. This easygoing demeanor may entice extroverts to have a higher belief in luck as a factor in decision making, because they are not as worried about the outcome compared to introverts. Introverts tend to be planners and make decisions ahead of time, so they factor luck less in their decision. Because introverts don't allow luck or chance to be a part of their options when making decisions, they are less likely to experience luck benefits, so they may not believe in the power of luck. Extroverts are more likely to allow chance outcomes to be involved of their decision making process, so they have a higher chance of experiencing positive luck benefits.

Further research could address the limitation surrounding participants owning a lucky object in this study and/or further explore the correlation between the personality trait of extraversion and the belief in luck. A future study could examine internal expressions of luck in individuals who have a salient belief in their lucky object. This study didn't distinguish between how powerful a participants lucky item was to them, so by identifying this variable and expanding on it regarding performance benefits, researchers can better understand the effects of internal expressions of luck on performance. Further exploration between the correlation of extraversion and belief in luck could be beneficial in discovering why the belief of luck is more powerful in some individuals based on personality traits. If personality traits could help determine belief in luck saliency, then future research could determine if separate personalities respond differently to luck.

In conclusion, the results of this study supported the hypothesis that superstition contributes to improved performance, but there were no significant differences between internal superstition and external expressions of luck. The lack of difference between the two types of luck could either be contributed to methodological errors or there, simply, may not be a difference between the two various types of luck in regards to increasing performance benefits. The results of this study confirm previous research findings. Additionally, this study found that out of the five main personality traits tested, extraversion was the only personality characteristic correlated to the belief in luck.

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The Psychological Effects and Cultural Implications of Female Genital Mutilation: A Literature Review

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ABSTRACT. Female Genital Mutilation (FGM) has been recognized by the World Health Organization as a topic of fierce debate, especially within African cultures. Although there has been a significant amount of focus placed on the physical ramifications of FGM, there has been a paucity of literature that examines the long-lasting psychological effects of the practice. The present study is a literature review of the relevant research addressing the negative psychological effects and cultural implications of the practice of FGM. Utilizing definitions, case studies, comparative studies, and a brief discussion of counseling implications, this paper aims to introduce and explain the main psychological effects that can occur in those who undergo FGM, and to discuss the cultural framework that surrounds the issue. According to this study, the most prevalent and consistently found psychological effects appear to be depression, anxiety-related disorders, Post-Traumatic Stress Disorder (PTSD), and psychosexual dysfunction. Additionally, the most significant cultural implications seem to be religious, familial, and related to social identity.

1. Introduction

For many years, there has been a Human Rights debate centered on the topic of Female Genital Mutilation (FGM), especially within African cultures. According to the World Health Organization (2013), FGM is defined as "procedures that intentionally alter or cause injury to the female genital organs for non-medical reasons", and can be further broken down into four distinct categories. Type I (Clitoridectomy) refers to excision of the prepuce and clitoris, Type II (Excision) refers to removal of the prepuce and clitoris with removal of the labia minora, Type III (Infibulation) refers to excision of the external genitalia with narrowing and stitching of the vaginal orifice, and Type IV refers to otherwise unclassified genital mutilation, including piercing, pricking, cauterizing or cutting of the vulva.

It is estimated that 140 million women are suffering from the ramifications of FGM today, and that at least three million undergo a subtype of the procedure each year (WHO, 2013). FGM is usually performed by an older woman in the community on girls between infancy and age fifteen (WHO, 2013). The practice is often done in secret during the middle of the night without warning the girl (Whitehorn, Ayonrinde, & Maingay, 2002). Anesthetics are not typically used, and the procedure may be done using a range of available objects for the cutting including sharp rocks, broken glass, or razor blades.

Advocates against FGM stress the medical problems that result from the procedure, such as severe infection, recurrent bladder infections, childbirth complications, and infertility, but gloss over the psychological implications of this practice. The mental repercussions that affected women have to deal with can be just as severe, and are frequently intensified by the experience of the excruciating physical pain (Slack, 1988). The practice also has certain cultural implications as they relate to close relationships and social isolation. This article provides an overview of the literature that discusses the psychological effects and the cultural implications of FGM. The review includes definitions, case studies, comparative studies, and a brief discussion of counseling implications related to the psychological effects of FGM. This review is in no way an exhaustive discussion of the topic of FGM as it is discussed within several academic disciplines. My goal in this article is to tell the "story" of FGM as I introduce and explain the main psychological effects that can occur as a result of FGM, and discuss the cultural framework

that surrounds the issue. For the purposes of this paper, I will refer to FGM as an all-encompassing term unless one of the four types is specified in the articles cited.

2. Psychological Effects

Although research is sparse, there have been some studies that focus on possible psychiatric effects of FGM. Although a range of psychological effects have been reported, the most prevalent and consistently found effects are depression, anxiety-related disorders, Post-Traumatic Stress Disorder (PTSD), and psychosexual dysfunction (Hearst, 2013). Interestingly, a common link in the development of these disorders is the experience of flashbacks to the FGM event.

Depression, Anxiety, and PTSD

Long-lasting and excruciating pain is a key contributor to the development of anxiety and depression (Slack, 1988). Because FGM causes young girls such intense pain in a very sensitive area of the body, it carries the potential to cause substantial psychological problems. Because the pain of FGM recurs throughout a female's life as the traumatized area is directly affected during female biological processes such as menstruation, urination, and childbirth, the anxiety and depressive episodes may have a similar persistent effect (Slack, 1988). In fact, the depressed and anxious mood caused by painful memories of undergoing circumcision may continuously lead women to feel worthless, guilty, and incapable, sometimes leading to suicidal ideation (Whitehorn et al., 2002).

A study conducted by Nnodum (2007) from Imo State University which focused on depression, compared the depressive symptoms of 690 circumcised women to 660 uncircumcised women using a researcher-structured questionnaire (Nnodum, 2007). Depressive symptoms were investigated using statements adapted from Beck's Depression Inventory (Beck, Steer, & Brown, 1996), and results suggested that circumcised women had a significantly higher level of depression than uncircumcised women (Nnodum, 2007). Mutilation of the genitalia, the intense pain felt during intercourse, and the inability to achieve sexual gratification are all elements of FGM that predictably lead to feelings of inadequacy and incompleteness that ultimately force a woman into a state of depression (Nnodum, 2007).

Behrendt and Moritz (2005) conducted a 3-month study on a group of 24 uncircumcised and 23 circumcised Muslim women in Senegal. After spending two days building rapport with an interviewer, participants were given a semi-structured interview with diagnostic questions based on the Mini International Neuropsychiatric Interview (Sheehan et al., 1998) and the Traumatic Life Event Questionnaire (E.S. Kubany, unpublished, 1995; Behrendt et al., 2007). The researchers found that 90 percent of the circumcised women had experienced feelings of intense fear, helplessness, horror, and severe pain with 80 percent of circumcised women reporting that they continued to have traumatic re-experiences of the procedure. Compared to only one uncircumcised woman, 80 percent of the circumcised women met the criteria for an anxiety disorder, and 30.4 percent of the circumcised participants in the study were diagnosed with PTSD. This percentage is equivalent to the 30-50 percent rate of PTSD among those who suffer early childhood abuse (Behrendt et al., 2007).

Following the research of Behrendt et al., Suardi, Mishkin, and Henderson (2010) explored a case study of a 19-year-old woman, referred to as "F", from a West African country who underwent Type III FGM at the age of 10. F was originally admitted to a United States pediatric emergency room with symptoms of nausea, loss of appetite, and severe abdominal pain. Further testing did not show any physical medical problems, so she underwent a psychological assessment. She did not qualify for a diagnosis of Depression or Generalized Anxiety Disorder, but according to the UCLA PTSD Index for DSM-IV—TR Adolescent Version (Steinberg, Brymer, Decker, & Pynoos, 2004), F had a score that suggested mild PTSD symptoms. The facts that she went through a traumatic event involving her experience of an actual physical injury with a substantial amount of trauma, and that she continuously re-experiences it, serve to legitimize her diagnosis. In addition, F reported sleep problems due to increased arousal, most likely caused by an increase of anxiety (Suardi et al., 2010). After this assessment, F was able to make the connection between her somatic and psychological symptoms as her reports of abdominal pain coincided with her re-experiences of her circumcision, or even just thinking about returning to her home country. This case study highlights the important connection between the physical and psychological effects of FGM. In F's case, the psychological impact of PTSD actually manifested itself physically as it perpetuated her somatic symptoms.

A study done by Elnashar and Abdelhady (2007)

investigated the psychological effects of FGM, comparing a random sample of 200 circumcised women to 64 uncircumcised women. The data were collected in a questionnaire format utilizing the Symptoms Check List 90 that was developed by Derogatis and colleagues to identify patterns of psychological ill-being (Derogatis, 2000). Results revealed that compared to the uncircumcised women, the circumcised females had significant differences regarding somatization, anxiety, and phobia (Elnashar et al., 2007). It was also found that women suffer from feelings of incompleteness, anxiety, depression, chronic irritability and frigidity as a result of undergoing FGM.

Psychosexual Dysfunction

Psychosexual dysfunction is characterized by the inability to achieve sexual arousal or satisfaction at the appropriate times or in the proper situations. It is most typically the result of mental or emotional issues, the most frequent being depression, anxiety, traumatic sexual experience, guilty feelings, stress, and negative body image (Mount Sinai Hospital, 2013). Most of these factors are associated with FGM, and thus have the capacity to manifest as psychosexual dysfunction in a circumcised woman.

El-Defrawi, Galal Lotfy, Dandaash, Refaat, and Eyada (2001), carried out a study supporting the notion that female genital mutilation can lead to a variety of psychosexual dysfunctions in affected women such as dysmenorrhea, lack of sexual desire, and difficulty reaching orgasm. Both circumcised and uncircumcised women in Egypt were interviewed about their sexual behaviors and attitudes using the Arabic version of the Sexual Behavior Assessment Schedule (El-Dafrawi, 1992). Results showed that significantly more circumcised females (83%) reported a lack of sexual desire than uncircumcised women. Circumcised women were also less pleased by sex, and were less likely to initiate sex with their husbands (El-Defrawi et al., 2001). Not only did they have problems during sex, but some women also developed a phobia of sex, as well as emotional problems as they relate to their relationships with their husbands and the decision of whether or not to circumcise their own daughters.

Other research has focused on determining the main outcome of psychosexual effects due to FGM (Ibrahim, Ahmed, and Mostafa, 2012). Two hundred and twenty married women, both circumcised and uncircumcised, were asked to complete a validated 19-item Female Sexual Function Index (FSFI) questionnaire in an interview setting. They were evaluated psychologically via the symptoms check list developed by Derogatis & colleagues mentioned in a previous section. Finally, each participant was given a gynecological examination (Ibrahim et al., 2012). Of the circumcised participants, 86% had undergone Type I FGM while 14% had undergone Type II. Positive elements of sexual experience such as desire, lubrication, satisfaction, and orgasm, were all reported more frequently within the uncircumcised group than within the circumcised group. Additionally, significant differences were found between the two circumcised groups in their total FSFI scores. The group of women who underwent Type II FGM scored significantly lower than the Type I FGM group in desire, lubrication, satisfaction, pain, and orgasm, and reported higher scores of somatization, depression, phobia, and anxiety as it typically a more invasive procedure.

3. Cultural Implications

Although it is certainly necessary to understand the mental health implications of FGM, it can be useful to examine how the practice is culturally perceived (Hearst & Molnar, 2013). As one can imagine, undergoing FGM and all of the consequences that come along with it would not be something most women would choose to do for personal pleasure. According to the Elnashar et al. (2007) paper mentioned in a previous section, women were most likely to undergo FGM for a traditional or religious reason. The social pressure associated with certain cultural ideologies seem to outweigh the desire to avoid physical suffering. Many girls and women are left to suffer in silence as they feel that there is no acceptable way to express their fears within their culture. Sometimes, they are even told that if they do express their fear, they will cause the death of their mothers and the shame of their families (The Harvard Law Review Association, 1993).

The socio-cultural intent of FGM is thought to be the prevention of sexual promiscuity among young women, and to avoid pregnancy outside of wedlock (UNICEF, 2005). Circumcision becomes a social status marker, communicating a woman's place within the structure of society. It is perceived to be a metaphorical shield of protection for women that prevents them from shaming their families due to the implied chastity of the practice (Gruenbaum, 2005). According to research, however, FGM does not necessarily lower a woman's level of sexual promiscuity from the baseline. In the previously

mentioned study done by Nnodum, results showed that there was no significant difference between the level of sexual promiscuity in circumcised women and uncircumcised women as the level of promiscuity was already very low among uncircumcised women, essentially operating as a floor effect (Nnodum, 2007). This result raises the question, then, of whether or not FGM serves a significant cultural purpose, a question at the core of many arguments against the practice.

In cultures that practice FGM, it is often used as a protective mechanism for a woman's marriageability (Gruenbaum, 2005). Women frequently experience psychological tension when they anticipate the severe pain they must undergo in order to fulfill their social roles as a wife and a mother. Psychosexual dysfunctions that result from FGM certainly have implications for the marriages and relationships of these women (Ibrahim et al., 2013), as suffering from a psychosexual disorder would complicate the process of sexually satisfying a partner. This results from the distractedness by the anxiety that precedes sex, the intense physical pain that results from it, and the feelings of inadequacy and failure that come afterward. Additionally, women who undergo FGM also tend to experience a sense of failure to fulfill their social roles as persistent worry and constant pain interfere with their ability to be a wife and a mother (Whitehorn, 2002). These feelings can further lead to experiences of social isolation for circumcised women.

The issue of FGM also places pressure on social situations, specifically on close relationships. In a self-report study, Youssouf (2013) reported that living in a culture that practices FGM contributes to marital tension, as well as difficulties for women regarding decision-making about their daughters' futures. For instance, Youssouf's father did not want her and her sisters to be circumcised, but her mother was so insistent upon it that they were circumcised in secrecy while her father was away. This created a lot of tension in her parents' marriage, almost leading to their divorce. Whereas her father did not agree with FGM, Youssouf's husband wanted their daughter to undergo the procedure, placing Youssouf in a difficult position between maintaining marital harmony and doing what she felt was right according to her moral beliefs. Either way, she was going to face psychological pressure as a result of this tension. Youssouf's story provides a specific example of how socio-cultural factors are at play in the practice of FGM.

4. Implications for Treatment and Counseling

In the West, there are more clearly defined interventions to treat psychological disorders. Interventions in non-westernized cultures often take completely different forms. In the majority of Muslim cultures, religion is seen as the primary means of maintaining psychological health, and the Islamic quest includes the scrutiny of the human make-up (Baasher, 2001). That is, it requires self-analysis to identify where one falls short of his ideal. This includes the recognition of one's inner weakness that requires religious devotion and useful work to be overcome. Ultimately, the tension between religious underpinnings as a means of mental health maintenance and its support of a practice that is so detrimental to mental health causes a real problem for circumcised women. What are they to do if the religion that is supposed to support their mental health is the same religion that causes them to suffer the psychological trauma in the first place?

FGM is a sensitive topic for both physicians and patients, and there has not been a very clear way to handle the issue in a medical setting. Women who are victims of FGM have had mixed opinions about how they wish to talk about their circumcision with physicians (Hearst et al., 2013). Some women become confused when doctors do not discuss it with them because they are expecting instrumental assistance. On the other hand, some women become frustrated with discussing FGM because they argue that it becomes a topic of fascination for the doctor, causing them to be seen as "specimens" instead of as humans. Thus, there are obvious issues with how to treat the effects that result from FGM. Perhaps this is when the previously mentioned "culturally sensitive" approach would be most effective (Hearst et al., 2013). Women who have come to America after experiencing FGM report wanting their physicians to know that they have undergone the procedure, but they do not want to discuss it further unless there is an anticipated problem that would involve discussion. And when discussion is necessary, it is important that physicians use terminology with which their patients are comfortable. For example, most women who have undergone the procedure do not call it FGM, but prefer the term "circumcision" as it seems more neutral (Hearst et al., 2013).

Evidently, FGM is associated with many long-term psychological, marital, and social problems for the women who undergo the procedure. It is important, then, for health centers, hospitals, mosques, and women's organizations to spread the message about the health impair-

ments associated with FGM in order to protect women in the future. With the goal of helping patients cope with life problems, counselors should also make sure that they are well-informed about the culture from which a patient comes (Nnodum, 2007). This is especially important for counselors working with victims of FGM. It has even been suggested that counselors seek to be a part of promoting public engagements about ending the practice. By doing this, counselors could help women protect their health, their marriages, and their role fulfillment within society.

In conclusion, FGM wears its title as a controversial topic as a result of its combination of culturally imbedded importance and severe psychological consequences. Psychological effects include, but are not limited to: depression, anxiety, PTSD, and psychosexual dysfunction. FGM can also cause feelings of isolation by interfering with marriage relationships, and the ability for a woman to fulfill her social role. Physicians and counselors should be careful to consider the cultural background of an FGM victim so that he can most effectively help her cope with her physical and psychological problems.

5. Suggestions for Future Research

There is clearly a need for intervention and compromise with respect to the practice of FGM on behalf of women. This will require that the focus on the implications of FGM not remain solely on the physical, but also adjust to include the long-lasting psychological damage that FGM can cause for women. As previously mentioned in this paper, there is a lack of psychological research done on the topic of FGM. Because it is such a substantial Human Rights debate, many studies have been focused on more explicit physical danger that could qualify as child abuse. Further psychological studies could help clarify the causes and consequences of the pain and suffering involved with FGM, and serve as a promotion for development of ways to combat its negative effects (Gruenbaum, 2005). Some of the research in this article has highlighted the ways in which psychological effects of FGM perpetuate the physical symptoms. This fact suggests that psychological research could prove beneficial as supporting evidence for research done on physical trauma. Studies that focus on predictive and protective factors (e.g. social situation and parents' beliefs) of FGM could be of great value as they could direct education and awareness to the communities that have the highest prevalence of such factors. That being said, it is also important to keep in mind the cultural variances in the motivations for FGM that are community-specific (UNICEF, 2005).

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Mapping a Novel Hedonic Hotspot in the Orbitofrontal Cortex

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ABSTRACT. Prevailing research suggests a complex hedonic reward network exists in the brain that is coordinated in generating hedonic experience. Previous sites of hedonic hotspots have been identified within subcortical structures such as the nucleus accumbens and the ventral pallidum. However, little is known about the identity and role of other brain substrates that are part of this reward circuit. Here, the role of orbitofrontal cortex (OFC), a region located within the prefrontal cortex known to be involved in sensory experiences of pleasure, was investigated to determine whether activation of this region can independently produce/ampify hedonic impact. Using the affective taste reactivity test with Sprague-Dawley rats, the degree of hedonic amplification in taste reward following (1) opioid or (2) orexin stimulation within the OFC was objectively assesseed. The results show a significant increase in hedonic reactions with either drug microinjection in the rostral portion (+3.50–4.80 mm) of OFC. In contrast, there is also evidence for the existence of a hedonic 'coldspot' within the caudal portion (+2.76–3.50 mm) of OFC, subject to further investigation. Lastly, in agreement with previous work, opioid or orexin stimulation throughout OFC seems to robustly enhance food intake of palatable M&Ms. These results extend the hedonic network to now include a cortical pleasure generator.

1. Introduction

Previous studies have shown that hedonic hotspots exist within specific areas of the brain, most notably the nucleus accumbens and ventral pallidum; opioid-like neurochemical signals microinjected into these areas are able to directly magnify hedonic reactions to food rewards (Berridge, Lawrence, van Ditzhuijzen, Davis, Woods, & Calder, 2006; Peciña & Berridge, 2005; Smith & Berridge, 2005). Nonetheless, there exists evidence of a larger functional circuitry formed by the interaction between multiple hedonic hotspots within the brain (Peciña, Smith, & Berridge, 2006; Richard & Berridge, 2013; Richard, Plawecki, & Berridge, 2013; Smith & Berridge, 2007). Here we attempt to identify and characterize the possible presence of a hedonic hotspot within the orbitofrontal cortex through taste reactivity experiments on Sprague-Dawley (SD) rats.

One of the major goals of affective neuroscience is to identify the brain substrates that are associated with pleasure (Berridge & Kringelbach, 2013). In this vein, neuroimaging studies have found that a large number of brain structures are activated in response to rewarding stimuli (Beaver, Lawrence, van Ditzhuijzen, Davis, Woods, & Calder, 2006; Kringelbach, 2005; Pessiglione, Schmidt, Draganski, Kalisch, Lau, Dolan, & Frith, 2007; Small, Veldhuizen, Felsted, Mak, & McGlone, 2008). Nonetheless, there remains the question of which, if any, of these structures are directly responsible for and thus cause the pleasure associated with the reward, as opposed to being simply correlates of externally generated hedonic activity, due to spreading network activation or as neurological responses to the pleasure event (Berridge, Robinson, & Aldridge, 2009). In order to identify these areas of the brain that are responsible for pleasure causation, research studies specifically look at brain regions that, when manipulated (e.g. pharmacological activation), are able to directly amplify hedonic impact (Castro & Berridge, 2014; Peciña & Berridge, 2005; Smith & Berridge, 2005; Söderpalm & Berridge, 2000; Tindell et al., 2006); this would imply that these brain sites are capable of independently producing or amplifying the experience of pleasure.

In our discussion of hedonic impact, an import-

ant distinction exists between the psychological components of reward of "liking" effects and "wanting" effects. "Liking" refers to sensory pleasure that may be experienced, consciously or unconsciously, as a reaction to hedonic stimuli. The extent of "liking" reactions may be measured objectively through behavioral studies, such as through orofacial expressions in the case of taste arousal. On the other hand, "wanting" refers to incentive salience, a type of incentive motivation that promotes consumption of and approach towards rewards (Berridge et al., 2009). Concordantly, "wanting" effects can be measured using food intake volume as an indicator of internal motivation. Typically, due to their roles in reward acquisition, "liking" and "wanting" co-occur naturally in a brain. A dissociation of their effects is commonly observed when we perform specific investigations into hedonic hotspot regions (Berridge et al., 2009). Hence in these cases, stimulation of specific brain areas may result in enhancement of "wanting" but not "liking". Our investigation therefore is to identify if an area of the orbitofrontal cortex can specifically enhance "liking" when subject to appropriate neurochemical manipulations.

In the aforementioned studies, researchers employ the use of opioid, endocannabinoid, or GABA-benzodiazepine neurotransmitter systems in their investigations (Berridge, Ho, Richard, & DiFeliceantonio, 2010; Mahler et al., 2007; Richard, Plawecki, & Berridge, 2013). These are a few of the known neurochemical systems that are able to enhance "liking" within specific sites in the brain. These specific sites are known as 'hedonic hotspots', which are anatomical subregions within specific brain structures that, when activated, result in an amplification of sensory pleasure sensation (Berridge et al., 2009 Peciña et al., 2006). The current candidate locations of these hotspots include a cubic millimeter (1.0 mm3) region in the rostrodorsal quadrant of the medial nucleus accumbens shell and a 0.8 mm3 region in the posterior ventral pallidum (Berridge et al., 2009; Peciña & Berridge, 2000; Smith & Berridge, 2005; Smith & Berridge, 2007). These hedonic hotspots also demonstrate reciprocal interactions with each other; for instance, an opioid transmission in the NAc hotspot can cause activation in the VP hotspot, vice versa (Peciña et al., 2006; Smith & Berridge, 2007). Thus, it has been proposed that there may exist a network of hedonic hotspots, other than the ones previously identified, throughout the brain that are coordinated in producing hedonic experience (Peciña et al., 2006). In particular, several cortical regions show promise for housing a hotspot, which will be discussed below.

In the prevailing research surrounding hedonic substrates of the brain, the opioid peptide neurotransmitter system is one of the best studied. There exist three major opiate receptor subtypes: μ, delta, and kappa, all of which are present and localized within the medial prefrontal cortex (Steketee, 2003). Previous studies have shown that opioid stimulation increases food intake, which by inference is an enhancement of "wanting" (incentive salience), and causes conditioned place preference effects (DiFeliceantonio, Mabrouk, Kennedy, & Berridge, 2012; Glass, Billington, & Levine, 1999; Grandison & Guidotti, 1977; Mucha & Iversen, 1986; Wise, 1989). Accordingly, microinjections of an opioid agonist (morphine) into the nucleus accumbens shell produces a general increased eating effect in rats. However, in addition to food consumption, opioid stimulation produces selective increases in positive hedonic patterns of behavioral affective reaction elicited by oral sucrose, in a localized region of the nucleus accumbens; this region has hence been defined as a hedonic hotspot (Peciña & Berridge, 2000). Identical results of localized hedonic enhancement were observed when the selective μ-opioid agonist DAMGO ([D-Ala2, N-MePhe4, Gly-ol]-enkephalin) was microinjected into the posterior ventral pallidum, the other identified hedonic hotspot region (Smith & Berridge, 2005). Most recently, Berridge and colleagues have shown that delta and kappa receptor stimulation can similarly enhance hedonic reactions, as long as they are activated within the confines of the respective hotspots (Castro & Berridge, 2014). Conversely, there is also evidence for opioid stimulation that suppresses hedonic reactions, in regions anatomically distinct from the hotspots; these are thereby known as hedonic coldspots (Castro & Berridge, 2014; Pecina & Berridge, 2005; Smith & Berridge, 2005). Hence, the evidence shows that opioid stimulation can induce dissociable effects of "wanting" and/or "liking", the latter occurring in localized regions of specific brain substrates. The presence of localized "liking" effects from opioid stimulation would thereby serve as a reliable indicator for the presence of any currently unidentified hedonic hotspots or coldspots.

A secondary neurotransmitter system that had been implicated in hedonic reward is orexin, which also has receptors expressed in the prefrontal cortex (Marcus et al., 2001). Orexin neurons have projections from the lateral hypothalamus, a brain region regularly implicated in food reward and hunger, to many forebrain targets in-

cluding the nucleus accumbens and ventral pallidum (Baldo, Daniel, Berridge, & Kelley, 2003; Fadel & Deutch, 2002; Harris & Aston-Jones, 2005). A recent study has shown that an orexin hotspot exists within the VP in essentially the same location as the previously identified opioid hotspot (Ho & Berridge, 2013), and related preliminary research suggests that an orexin hotspot in the nucleus accumbens may also exist. In addition, as with the case of opioids, orexin stimulation also appears to enhance food intake in parts of the brain including the lateral hypothalamus, paraventricular nucleus, and nucleus accumbens (Dube, Kalra, Kalra, 1999; Edwards, Abusnana, Sunter, Murphy, Ghatei, & Bloom, 1999; Thorpe & Kotz, 2005).

The primate orbitofrontal cortex (OFC) receives sensory inputs from a number of sensory modalities such as taste, olfaction, vision and somatic sensation (Carmichael & Price, 1995; Critchley & Rolls, 1996; Öngür & Price, 2000; Rolls, 2000). Specifically, it contains the secondary taste cortex, the secondary and tertiary olfactory cortical areas, and the inferior temporal cortical visual areas, which code for the reward value of their respective sensations; the neurons within these areas respond only to food (through taste, smell or sight) when the primate is in a state of hunger. Accordingly, these areas also exhibit a decrease in neuronal response to a food when it is eaten to satiety, while retaining the respective reward values of other foods through a mechanism known as sensory-specific satiety (Rolls et al., 1981; Rolls, 1986). Sensory-specific satiety refers not only to alterations in neural activity, but also entails a decline in the hedonic and motivational value from consuming a food that the subject was previously exposed to, evidenced through taste reactivity and food intake tests in rats as well as in humans (Balleine & Dickinson, 1998; Berridge, 1991; Havermans, Janssen, Giesen, Roefs, & Jansen, 2009). The existence of this mechanism as mediated by the OFC suggests an important and significant role in the affective brain representations of food. Specifically, that the OFC is capable of modifying reward values (hedonic impact) of experienced stimuli. Lesions of the OFC also eliminated the ability of monkeys to modify their behavior to changes in the incentive value of food (Butter, Mishkin, & Rosvold, 1963) and altered food preferences (Baylis & Gaffan, 1991). These findings suggest that OFC activation in the presence of hedonic activity is not merely representative of a simple neurological response, but indicative of a source of hedonic amplification or attenuation. These results are consistent with human neuroimaging studies, which similarly show that OFC activations are associated with subjective pleasantness produced by sensory arousal (Kringelbach, O'Doherty, Rolls, & Andrews, 2003), therefore affirming its involvement and importance in hedonic reward across species.

In terms of anatomy, the orbitofrontal cortex contains significant densities of opioid peptides and its receptors (Lerich, Cote-Vélez, & Méndez, 2007; Steketee, 2003). Concordantly, research studies have shown that bilateral infusions of DAMGO in circumscribed regions of the OFC leads to increased food intake in rats, similar to its action within the aforementioned known opioid hotspots (Mena, Sadeghian, & Baldo, 2011). Likewise, orexin receptors are known to be expressed within the OFC (Marcus et al., 2001). In addition, a recent study has found that the medial orbitofrontal cortex exerts a top-down corticolimbic control of appetitive eating behavior induced by nucleus accumbens microinjections; OFC activation also increased Fos activity within the nucleus accumbens hotspot (Richard & Berridge, 2013). This is congruent with the notion that corticolimbic projections from the orbitofrontal cortex form part of a larger neural network that guides motivation. However, while we know that the orbitofrontal cortex is implicated in appetitive eating behavior, little is known about its role in hedonic "liking" of food reward. Thus, informed by a reliable and significant amount of evidence within the current body of research, we seek to determine the possible existence of a hedonic hotspot within the region of the orbitofrontal cortex.

2. Method

Subjects

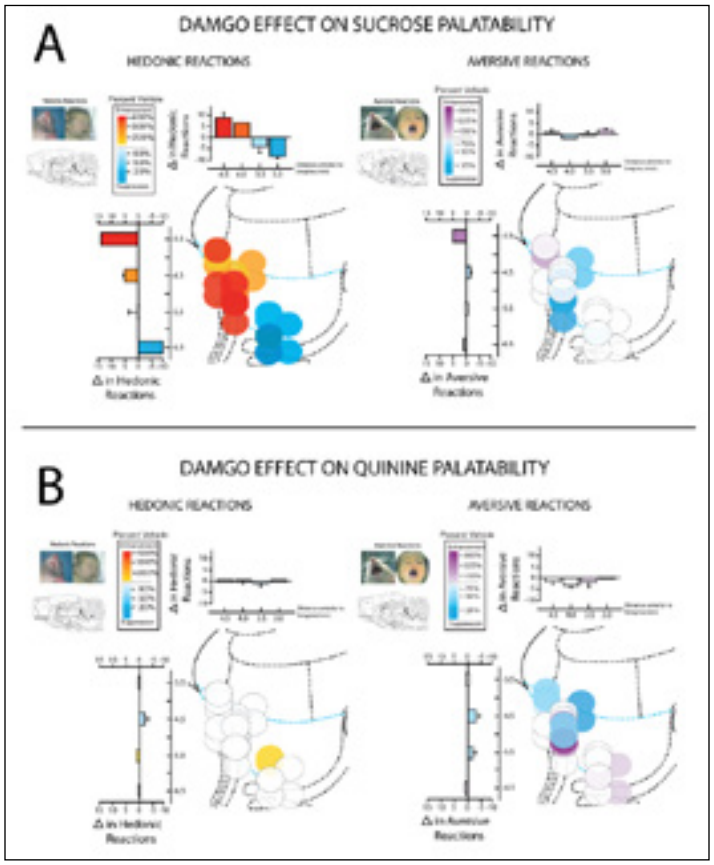
Female Sprauge-Dawley rats (n = 13; 250–350 g at surgery) were housed on a 12 hr light/dark reverse cycle (~21°C) with ad libitum food (Purina Rat Chow) and water (tap water). All experimental procedures were approved by the University Committee on the Use and Care of Animals at the University of Michigan.

Oral Tube and Cranial Cannulation Surgery

All rats were anesthetized with intraperitoneal injections of a mixture of ketamine (80 mg/kg) and xylazine (5 mg/kg), and treated with atropine (0.05 mg/kg) to prevent respiratory distress. Rats also received sub-

cutaneous injections of cefazolin (75 mg/kg) to prevent infection and carprofen (5 mg/kg) for analgesia. After anesthesia induction, polyethylene oral tubing [PE-50] were inserted bilaterally, just lateral to the first maxillary molars and ran subcutaneously along the zygomatic arch to the top of the skull, where they exited through an incision. The tubings were secured with wiring and dental cement.

Figure 1. DAMGO drug microinjection effects on palatability of sucrose or quinine taste stimuli.



Notes. Sagittal slice maps of the orbitofrontal cortex brain region show locations of drug microinjection sites and corresponding effects on palatability of respective taste stimuli. All value comparisons are made against a within-subject vehicle condition. Each color-coded circle represents a distinct microinjection placement site; symbol colors are on a gradient denoting percentage enhancement/suppression of hedonic (left column) or aversive (right column) orofacial reactions to a given taste stimuli (A: sucrose; B: quinine). Histogram bar graphs along the anterior-posterior (3.0–4.5mm) and the dorsal-ventral axes (3.5–6.5mm) denote mean change in number of observed hedonic/aversive reactions within each corresponding level (AP: ±0.3mm; DV: ±0.6mm). DAMGO microinjections in rostral section of orbitofrontal cortex show significant enhancement of hedonic reactions to sucrose but not any other types of reactions.

Rats were then placed in a stereotaxic apparatus (David Kopf Instruments), with the mouth bar set to -3.3 mm below intra-aural zero. Bilateral stainless steel guide cannulae (14 mm, 23 gauge) were aimed 2 mm above points throughout the medial prefrontal cortex (orbitofrontal), between coordinates anteroposterior (AP) +3.24–4.68 mm ahead of bregma, mediolateral (ML) ±1.0–2.0 mm from the midline, and dorsoventral (DV), -5.7–6.0 mm below skull. Cannulae were anchored to the skull using surgical screws and secured with dental cement; stainless steel obturators (28 gauge) were inserted to prevent occlusion of the cannulae. Post-surgery, rats were carefully monitored for 2 h for signs of distress and topical antibiotic was applied to the surgical area to prevent infection. Rats were again administered carprofen 24 h after surgery, and were allowed to recover for at least 7 days before testing.

Habituation and Testing Apparatus

Prior to the first test day, rats were habituated to handling and procedures for 7 days: rats were handled for 10 min per day for 3 days, and then habituated to the testing procedure and apparatus for 1 h each on four additional days. On the 4th day of habituation, rats received 'mock' microinjections (described below) of vehicle before being placed in the testing chambers. On drug test days, each rat received one of three drug microinjections (DAMGO; orexin; vehicle) and were placed immediately in a taste reactivity testing chamber.

The taste reactivity chambers had a transparent floor, under which an angled mirror reflected an image of the rat's ventral face and mouth into a digital video camera. The food intake chamber was transparent (23 × 20 × 45 cm), contained pre-weighed food (~20 g M&Ms), ad libitum water, and granular cob bedding (~2 cm deep).

Intracerebral Microinjections

Drug microinjections were administered bilaterally in a 0.2µl volume on test days spaced at least 48 h apart. On test days, drug solutions were brought to room temperature (~21 °C), inspected to confirm the absence of precipitation, and bilaterally infused at a speed of 0.2 µl/min using a syringe pump attached via PE-20 tubing to stainless steel injectors (16 mm, 29 gauge) which extended 2 mm beyond the end of the guide cannulae into the orbitofrontal cortex. Injectors were left in place for 1 min to allow for drug diffusion, after which obturators were replaced, and rats were immediately placed in one of the taste reactivity testing chambers.

Behavioral Testing of Hedonic Behaviors

Rats were left in their respective taste reactivity testing chambers (described above) for 25 min to allow

for the respective drug to take effect. At 25 min and 30 min post-microinjection, a solution containing sucrose or quinine respectively (0.1 M sucrose or 3x10-3 M quinine) was infused in 1 ml volume over a 1 min period via syringe pump connected to the oral delivery tube. The sucrose mixture was used to elicit positive hedonic reactions while the quinine mixture was used to elicit negative aversive reactions. The taste reactivity behavior of each rat was video-recorded for subsequent analysis.

Behavioral Testing of Unconditioned Motivated Behaviors

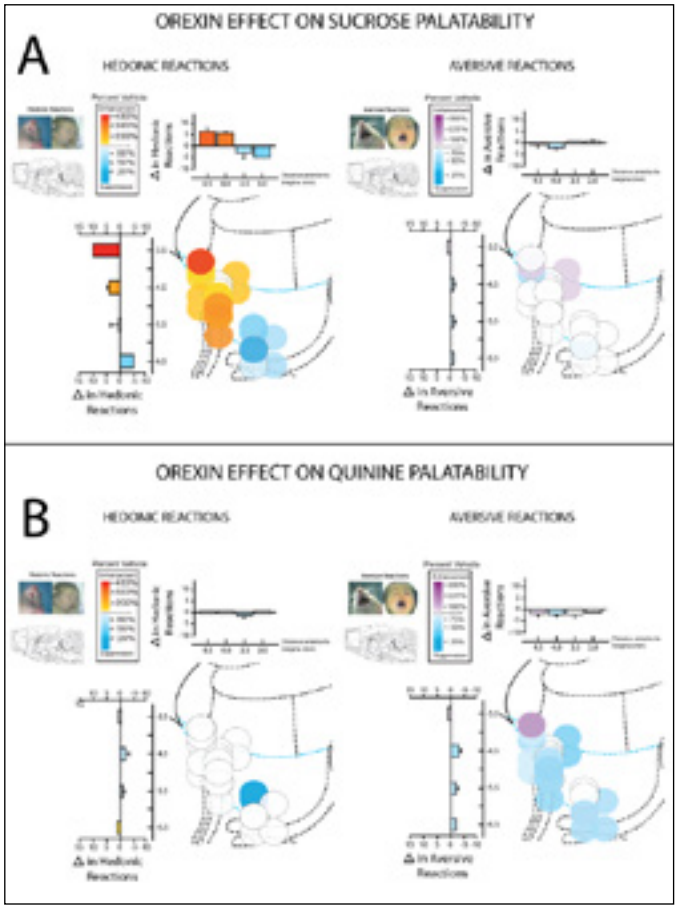
Following the taste reactivity testing, rats were placed in their respective food intake testing chamber (described above). Rats remained in the chamber for 60 min while their eating behavior was video-recorded for later analysis. Immediately following the testing period, the remaining food was removed and weighed to determine volume of consumption.

Behavioral Coding of Videorecorded Behaviors

Hedonic, aversive, and neutral response patterns were scored in slow motion (1/5 - 1/2 actual speed) by a trained observer blind to the drug condition and cannulae placement, using procedures developed to compare hedonic and aversive taste reactions (Berridge, 2000). Hedonic or positive "liking" responses included tongue protrusions, lateral tongue protrusions, and paw licking. Aversive or negative "disliking" responses included gapes, headshakes, face washing, forelimb flails, and chin rubs. Neutral responses include relatively non-valent behaviors or passive dripping of solution out of the mouth, rearing or mouth movements. All video analysis was conducted using Observer software (Noldus Information Technology, 2008). A time bin scoring procedure was used to ensure that taste reactivity components of different relative frequency were balanced in their contributions to the final affective hedonic/aversive totals (Berridge, 2000). Individual totals were calculated for hedonic and aversive categories for each rat by adding all response scores within an affective category for that rat.

Eating patterns were scored in the same manner described above, by a trained observer blind to the drug condition and cannulae placement. The behaviors scored included appetitive behavior (food carrying, food sniffs), eating behavior (consumption), drinking behavior (licking from water spout), general motor behavior (rearing, cage crossing), fearful defensive treading/burying behavior (pushing of bedding) and grooming behavior (Aldridge, Berridge, Herman, & Zimmer, 1993). Individual totals were calculated for each rat with regards to the time they spent exhibiting each type of behavior.

Figure 2. Orexin drug microinjection effects on palatability of sucrose or quinine taste stimuli.



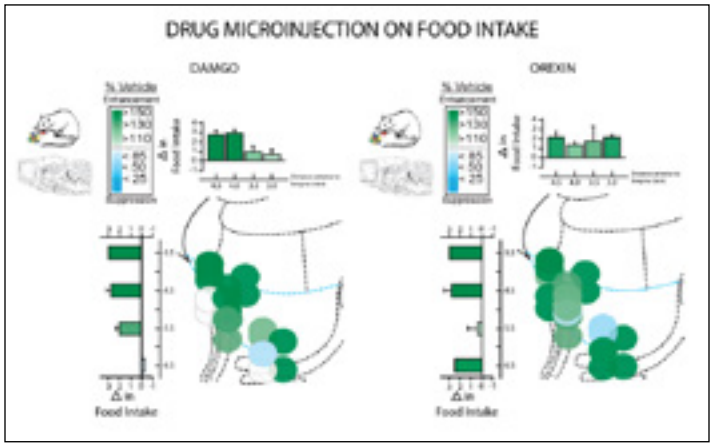
Notes. Sagittal slice maps of the orbitofrontal cortex brain region show locations of drug microinjection sites and corresponding effects on palatability of respective taste stimuli. All value comparisons are made against a within-subject vehicle condition. Each color-coded circle represents a distinct microinjection placement site; symbol colors are on a gradient denoting percentage enhancement/suppression of hedonic (left column) or aversive (right column) orofacial reactions to a given taste stimuli (A: sucrose; B: quinine). Histogram bar graphs along the anterior-posterior (3.0–4.5mm) and the dorsal-ventral axes (3.5–6.5mm) denote mean change in number of observed hedonic/aversive reactions within each corresponding level (AP: ±0.3mm; DV: ±0.6mm). Orexin microinjections in rostral section of orbitofrontal cortex show significant enhancement of hedonic reactions to sucrose but not any other types of reactions.

Histology

After testing was complete, rats were deeply anesthetized with an overdose of sodium pentobarbital, and their brains were removed and fixed in 10% paraformaldehyde for 1–2 days followed by 25% sucrose solution

for at least 3 days. To assess microinjection site locations, brains were sliced at 60 μm on a freezing microtome, and stained with Cresyl violet. Microinjection sites were mapped onto coronal slices from a rat brain atlas (Paxinos & Watson, 2007). Functional effects on appetitive and aversive behaviors were mapped using color-coding to express intensity of changes in motivated behaviors for individual behaviorally tested rats. A site was considered to be in the rostral orbitofrontal cortex if it was located between +3.50 – 4.80 mm AP and caudal if it was located between +2.76 – 3.50 mm AP.

Figure 3. DAMGO and orexin drug microinjection effects on subject food intake.



Notes. Sagittal slice maps of the orbitofrontal cortex brain region show locations of drug microinjection sites and corresponding effects on food intake. All value comparisons are made against a within-subject vehicle condition. Each color-coded circle represents a distinct microinjection placement site; symbol colors are on a gradient denoting percentage enhancement/suppression of food intake volume (left column: DAMGO; right column: orexin). Histogram bar graphs along the anterior-posterior (3.0–4.5mm) and the dorsal-ventral axes (3.5–6.5mm) denote mean change in food intake volume (in grams) within each corresponding level (AP: ±0.3mm; DV: ±0.6mm). DAMGO and orexin microinjections show significant enhancement of food intake irrespective of microinjection location within the orbitofrontal cortex.

3. Results

An alpha level of .05 was used for all statistical tests. Overall, there was no main effect of drug on hedonic reactions to sucrose. However, an anatomical analysis revealed both DAMGO and orexin stimulations caused opposing processes within the rostral or caudal sections of the OFC. When microinjection sites were located within the rostral section of the OFC, the number of positive hedonic orofacial reactions elicited by the taste of sucrose was enhanced two to three-fold for microinjections of opioid agonist or orexin, compared with within-subject control levels measured after vehicle microinjections in the same rats ($F(2,20) = 6.16$, $p < .01$). In comparison, when microinjection sites were located within the caudal section of the OFC, the number of positive hedonic reactions to sucrose were suppressed to less than half that of within-subject control levels ($F(2,6) = 1.47$, $p = .30$).

Specifically, mu receptor stimulation by DAMGO microinjections within the rostral section of the OFC tripled the number of hedonic reactions elicited by the taste of sucrose, compared with vehicle levels in the same rats (average = 312%; $p = .02$). Orexin receptor stimulation by orexin microinjections within the rostral section of the OFC doubled the number of hedonic reactions elicited by the taste of sucrose, compared with vehicle levels in the same rats (average = 216%; $p = .02$). Finally, both drug conditions, after microinjection, elicited a two to three-fold increase in food intake independent of stimulation site within the OFC ($F(2,26) = 3.73$, $p = .04$).

Drug Stimulations Produce Selective Hedonic Enhancement

The effect of drug stimulation on overall (hedonic/aversive; sucrose/quinine) taste reactions between-subjects, with the factor difference being site location (rostral vs. caudal), was marginally significant ($F(10,46) = 1.71$, $p = .11$). In particular, drug stimulation effects between sites significantly altered positive hedonic orofacial reactions elicited by the taste of sucrose ($F(2,26) = 6.29$, $p < .01$). No change was statistically detectable for drug stimulation effects on aversive orofacial reactions to sucrose ($F(2,26) = .44$, $p = .65$), hedonic reactions to quinine ($F(2,26) = .26$, $p = .77$), or aversive reactions to quinine ($F(2,26) = .76$, $p = .48$). The consistency of these non-hedonic reactions protects against the possibility that the increase in hedonic reactions may be attributed to alternative locomotor or sensory explanations.

Unlike the localized changes in hedonic reactions, μ-opioid or orexin stimulation produced a significant increase in food intake ($F(2,26) = 3.73$, $p = .04$; DAMGO: $p = .02$; orexin: $p = .03$) throughout all sites within the orbitofrontal cortex, similar to the anatomically distributed eating effects observed in NAc medial shell (Peciña & Berridge, 2005; Castro & Berridge, 2014).

Localization of Hedonic Enhancement Within Rostral Orbitofrontal Cortex

At sites located within the rostral section of the OFC (+3.50–4.80 mm), the number of positive hedonic orofacial reactions elicited by the taste of sucrose was

more than doubled by DAMGO or orexin microinjections ($F(2,20) = 6.16$, $p < .01$; DAMGO: $p = .02$; orexin: $p = .02$). Food intake levels were also significantly increased following either receptor stimulation ($F(2,20) = 10.29$, $p < .01$; DAMGO: $p < .01$; orexin: $p < .01$).

Localization of Hedonic Suppression Within Caudal Orbitofrontal Cortex

At sites located within the caudal section of the OFC (+2.76–3.50mm), the number of positive hedonic orofacial reactions elicited by the taste of sucrose was cut in half by DAMGO or orexin microinjections ($F(2,6) = 1.47$, $p = .30$). Though the data was not statistically significant, this may be attributed to the low n for the subset of rats with caudally located microinjection sites. Further investigation with more animals would enable a better understanding of the functional properties of this subregion. Regardless, this section reported no enhancement of hedonic reactions to sucrose, thereby suggesting the specific localization of the hotspot to only the rostral section of the OFC.

4. Discussion

Our results point to the existence of a hedonic hotspot within the rostral section of the orbitofrontal cortex. μ-opioid and orexin stimulation within this area generates 200-400% enhancements of hedonic reactions to sweetness. Contrarily, a potential region of suppressive coldspot in the caudal section of the OFC was also discovered; mu and orexin stimulation within this area resulted in a 20-50% suppression of hedonic reactions to sweetness; however, the data is not significant, attributable to the limited number of subject trials. In addition, all drug placements located within the OFC elicited a 150-300% increase in food intake; this data corresponds to similar previous studies of hedonic hotspots (Peciña & Berridge, 2000; Smith & Berridge, 2005; Castro & Berridge, 2014), as well as previous investigations into the OFC (Mena et al., 2011).

The small, selective region of hedonic enhancement within the rostral section contrasts with the lack of enhancement across the rest of the OFC, as the opposing behavioral effects would wash out any localized effect. This data confirms a selective role in hedonic reward for the rostral OFC, which as shown, is capable of independently producing and amplifying hedonic experience. With regards to the psychological components of reward "wanting" and "liking", the OFC displayed an anatomical localization of "liking" function to the aforementioned rostral section; in comparison, "wanting" effects from drug stimulation were detected throughout the OFC.

Our study is limited due to the low number of trial animals; as such, more trials would enable a clearer elucidation and mapping of the anatomical locations of the identified rostral OFC hotspot. Future Fos plume studies would also help to clearly define the actual boundaries of this novel hotspot. Furthermore, as apparent from the statistically insignificant distinction of the caudal OFC coldspot, more subject trials may provide enough data to establish significance in confirming the existence of a coldspot. Conditioned taste aversion experiments would help to further verify that our experimental findings demonstrate an alteration in hedonic value of taste stimuli, rather than simply an alteration of their sensory quality. Though it was shown that opioid or orexin receptor function is sufficient to produce the effects of the hotspot, it would also be insightful to determine their necessity. Finally, further research could also be done to investigate stimulation effects of other opioid receptors (delta, kappa), which had recently been found to also act as hedonic generators (similar to mu and orexin) within the nucleus accumbens hotspot (Castro & Berridge, 2014).

Our findings are consistent with the original expectation of locating an area of hedonic hotspot within the specific region of the orbitofrontal cortex. It is also significant that, unlike previously discovered subcortical brain structures containing hedonic hotspots, the OFC hotspot is uniquely located within the cortical region. The presence of a hedonic generator within the cortical region lays down a foundation for a new, informed understanding of the hedonic circuit as an extensive network that stretches throughout the entire brain; from the parabrachial nucleus of the brainstem pons (Berridge, 1996), to subcortical structures as the nucleus accumbens and ventral pallidum, to this newly discovered hedonic OFC hotspot in the cortex.

With the current understanding of the OFC as a sensory integration nexus involved in affective brain representations and reward processing (Berridge & Kringelbach, 2008; Critchley & Rolls, 1996; Öngür & Price, 2000; Rolls, 2000), our findings support this role of the OFC by establishing it as a brain structure capable of independently generating hedonic pleasure. Multiple neuroimaging studies had implicated the OFC in reward representations elicited by an assortment of sensory cues (De

Araujo, Rolls, Kringelbach, McGlone, & Phillips, 2003; Gottfried, O'Doherty, & Dolan, 2003; Hinton, Parkinson, Holland, Arana, Roberts, & Owen, 2004; Kringelbach et al., 2003); our discovery offers concrete evidence that the neural activity of the OFC does not simply represent a network correlation but rather an active generation of hedonic experience.

The significance of the OFC lies not only in its interpretation of primary sensations, but also in its unique involvement in the representations of the reward value of abstract reinforcers. Human neuroimaging studies point to the OFC as a region activated during social judgment (particularly empathic and forgivability judgments) (Farrow et al., 2001), musical experience (Blood, Zatorre, Bermudez, & Evans, 1999), as well as monetary transaction (Bechara, Damasio, Damasio, & Anderson, 1994). Accordingly, humans with bilateral damage to the OFC region demonstrate a deficiency in their ability to use positive and negative outcomes (incentives) to guide choice behavior (Baxter, Parker, Lindner, Izquierdo, Murray, 2000), and exhibit severe impairments in real-life decision making despite preserved general intellect (Bechara et al., 1994).

Consequently, our findings characterizing the OFC as a hedonic generator paves the way for novel and effective treatments in the field of abnormal brain function. Through appropriate manipulations of brain structures such as the OFC, we may seek to remedy or improve patient outcomes for disorders such as major depression, binge eating, addiction and other behavioral disorders (Covington et al., 2010; Drevets, 2007; Stanfield et al., 2009; Volkow & Fowler, 2000; Woolley, Gorno-Tempini, Seeley, Rankin, Lee, Matthews, & Miller, 2007). Proposed therapeutic strategies can include deep brain stimulation of the OFC in the treatment of anhedonia, as has been shown to be effective with studies targeting the nucleus accumbens (another hedonic hotspot), even for patients with resistant forms of clinical depression (Bewernick et al., 2010; Schlaepfer et al., 2008). Alternatively, optogenetic approaches have also demonstrated promising results in modulating brain activity (selective or inhibitory effects), for the treatment of aforementioned disorders (Diester et al., 2011; Lobo, Nestler, & Covington, 2012).

Successful manipulations of the brain's hedonic centers may be beneficial in treating patients with deficits of positive hedonic impact, such as in major depressive or bipolar disorders. Unrestrained and functionally problematic motivational "wanting", as exemplified in compulsive disorders, eating disorders and addiction, can potentially be curbed by clinical applications informed by a growing understanding of the brain's underlying reward circuitry. The role of the unique cortically-located orbitofrontal cortex in reward circuits can prove useful in informing future therapeutic strategies for cases of psychopathology, paving the way for more effective treatments.

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