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About mSURJ

The McGill Science Undergraduate Research Journal (mSURJ) is a student-run and student-founded scientific journal. Our mission is to encourage, publish, and promote undergraduate research by training students to share their ideas with the research community. mSURJ is the only initiative at McGill to offer this educational component for science students.

mSURJ covers the entire spectrum of scientific disciplines at McGill. Submissions range from research articles to research letters, or everything in-between. Articles are selected on a competitive, peer-review basis. The journal also publishes a limited number of non-peer reviewed interest pieces such as editorials, or feature articles.

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ON THE COVER

Designed by DEBBIE GELTNER

The cover displays the famous 1982 scanning electron microscopy image of plasma cells (Photographers Bruce Wetzel/Harry Schaefer, courtesy National Cancer Institute). A black and white representation of a stabilized dispersion (United States Patent 6946117) shows perforated microstructures suspended in a chemical medium. This system allows the delivery and dispersion of pharmacological agents in the respiratory track.

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Foreword

The word "author" is derived from the Latin verb *augere*, which means to increase and enrich. The author, then, is one who expands on previous thoughts and ideas. Interestingly, the same Latin root also give rise to the word "authority", thereby conferring upon authors a responsibility for their published material.

In scientific writing, this responsibility includes the importance of designing replicable experiments and engaging in thorough data analysis. However, it is not, and indeed must not be, limited only to the methodological features of the empirical study.

Scientific knowledge travels outside specific disciplines to innervate many different fields and hence, the scientific author stands accountable to a wide audience. The scholarly manuscript acts as a vehicle for the transmission of ideas within and between disciplines and, should therefore be clear, effective, and accessible to the uninitiated.

Writing about science is just another facet of practicing science. The rigor and acumen that guided the empirical work should be mirrored in both the substance and the form of a manuscript. As early as 1667, a guide to scientific writing, published by the Royal Society, Britain's oldest scientific society, cautioned against an impenetrable style of writing. Its author famously suggested abandoning the jargon of scholars to favour the vernacular language of craftsmen, farmers, and merchants. The notion of a hermetic science, one that is accessible and accountable only to itself, disrupts the very purpose of scientific communication. The density of writing is neither an indicator of the complexity of a concept nor of the sophistication of its analysis.

This journal is a venture in the world of effective science writing. We not only encourage our contributors to write engaging and non-technical articles – we require it. Our rigorous editorial process strives to be a true experience in scientific writing and serves as a training ground for undergraduates writing academic articles.

Ultimately, we hope to facilitate cooperation among disciplines, and inspire student involvement in the development and dissemination of knowledge.

Arij Riahi
Editor-in-chief
BSc 08, Anatomy & Cell Biology

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We also extend our gratitude to the professors and graduate students who participated in the peer-review process for their time and their expertise. The strength of our editorial process relies on their invaluable input.

Finally, we wish to thank the student contributors, who have worked tirelessly in the face of rigorous demands. Their effort is admirable.

Netrin-1 receptor deficiency protects against psychostimulant-induced behaviours in mice

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Abstract

Psychostimulant drugs, such as cocaine and amphetamine, increase extracellular dopamine in certain brain regions, including those that make up the mesolimbic dopamine system. Dysfunction of this system is implicated in drug addiction. The development of the mesolimbic dopamine system involves netrins, a group of secreted proteins that guide growing axons to their targets. Mice that develop with reduced expression of deleted in colorectal cancer, a netrin-1 receptor, exhibit abnormal dopamine release in response to psychostimulant drugs. Furthermore, these mice show reduced amphetamine-induced locomotor activation when compared to wild-type mice. In this study, our objective is to further examine the drug-induced behaviours of DCC deficient mice. We compared adult DCC deficient and wild-type mice using two behavioural tests. First, we examined the locomotor response of these mice to cocaine. Second, using conditioned place preference, we assessed the rewarding effects of amphetamine. DCC deficient mice showed reduced cocaine-induced locomotor activation and diminished amphetamine-induced reward when compared to wild-type mice. Taken together, this study suggests that DCC deficiency can protect against certain psychostimulant-induced behaviours.

Keywords

DA: *dopamine*
DCC: *deleted in colorectal cancer*
UNC-5: *uncoordinated-5 protein homologue*
TH: *tyrosine hydroxylase*
Nacc: *nucleus accumbens*

Introduction

Psychostimulant drugs, including amphetamine and cocaine, are highly addictive and prone to abuse. Many of the effects of these drugs on humans and rodents are mediated by alterations in dopamine (DA) signaling in the brain. One such change is an acute increase of extracellular DA in the mesolimbic DA system, which is involved in reward and motivation (Ritz and Kuhar, 1993). Activation of this system results in drug-induced behaviours such as locomotor activation and compulsive reward seeking. These behaviours are stereotypical and allow for an operational characterization of addiction that can be studied using laboratory animals (Bozarth, 1986). Sensitization of these behaviours is a characteristic of an addicted state resulting from repeated drug exposure. Importantly, sensitization to the stimulatory effects of psychostimulants is accompanied by changes in the neuronal circuitry of the mesolimbic DA system (Robinson & Kolb, 1999; 2004). Therefore, knowledge of the development of mesolimbic DA circuitry may yield useful information for understanding how this system is altered by psychostimulant drugs.

During brain development, neuronal organization of the mesolimbic DA system relies on both environmental and genetic factors. Alterations to any of these factors can lead to variations in development, connectivity, and neurotransmission of dopaminergic neurons (Lipska et al., 1993; Riddle & Pollock, 2003; Ventura, 2004). Even subtle variations in DA circuitry during development can alter the amount of DA released in the nucleus accumbens (NAcc), the terminal region of mesolimbic DA projections, in response to psychostimulant drugs (Brake, 2004; Di Chiara et al., 2004). This leads to individual variation in the behavioural effects of psychostimulant drugs. However, little is known about what developmental changes can lead to alterations in DA function and to variable responses to psychostimulant drugs. One possibility is that environmental and genetic factors alter the number of growing DA axons that successfully reach their appropriate synaptic targets, leading to variation in dopaminergic circuitry and hence, functioning of the system.

Though the mechanisms underlying the organization of the mesolimbic DA system are poorly understood, recent evidence suggests a role for the netrin family of guidance molecules in the development of this system (Livesey et al., 1997; Lin et al., 2005; Grant et al., 2007). The netrin family, including the mammalian netrin-1, guides growing axons to their appropriate synaptic targets (Dickson, 2002; Manitt & Kennedy, 2002). The effect of netrin-1 is bidirectional; it may attract or repel axons depending on the variety of netrin-1 receptors expressed on the cell surface of the neurite (Barallobre et al., 2005). Binding of netrin-1 to the receptor DCC (deleted in colorectal cancer) causes DCC multimerization, which acts as an attractive signal stimulating neurite growth towards the source of netrin-1 (Stein et al., 2001). If both DCC and another netrin-1 receptor, UNC-5 (uncoordinated-5 protein homologue), are present on the cell surface, binding of netrin-1 results in DCC-UNC-5 heterodimerization, which signals repulsion (Hong et al., 1999). It is therefore postulated that the direction of neurite growth is determined by the relative concentrations of DCC and UNC-5 present on the cell surface. Studies of vertebrate netrin-1 signaling and homologous signaling pathways in C. elegans and D. melanogaster have demonstrated that this signaling pathway plays a fundamental role in nervous system development (Hiramoto, 2000; Barallobre et al., 2005). Interestingly, DCC is highly expressed by midbrain DA neurons during development and in adulthood (Livesey and Hunt, 1997; Lin et al., 2005; Osborne et al., 2005; Grant et al., 2007). UNC-5 is also expressed by these DA neurons in adulthood; however whether they express UNC-5 during development is unknown (Grant et al., 2007). Together, these findings suggest that netrin-1 and its receptors are important for the development and maintenance of the mesolimbic DA system.

To investigate this theory, we used dcc heterozygous knockout mice (as dcc homozygous knockout mice die at birth), which develop with reduced DCC expression (Flores et al., 2005). Though one could hypothesize a compensatory change in UNC-5 expression in response to reduced DCC expression, no such compensatory change exists in adults (Grant et al., 2007). Interestingly, dcc heterozygous mice have reduced DA activity in the NAcc, both at baseline and in response to systemic amphetamine exposure (Grant et al., 2007). In addition, they show altered amphetamine-induced behavioural phenotypes when compared to wild-type

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controls: blunted locomotor activity, an absence of sensitization to this effect after repeated drug exposure, and an absence of sensorimotor gating deficits (Flores et al., 2005; Grant et al., 2007). Drug-free dcc heterozygous animals do not differ from wild-type animals in behavioural testing.

These studies show that netrin-1 signaling plays a role in DA function in the adult mouse. The data also suggests that reduced dcc expression can protect against behavioural effects induced by psychostimulant drugs. In the present study, we aim to further examine the abnormal behavioural responses of dcc heterozygous mice in response to psychostimulant drugs. Using a conditioned place preference paradigm, we assessed cocaine-induced locomotor response and amphetamine-induced reward seeking in both adult dcc heterozygous and wild-type mice. Our results demonstrate that the behavioural phenotype observed in dcc heterozygous mice is not amphetamine-specific. We also demonstrate reduced reward seeking in dcc heterozygous mice.

Methods

Animals

Adult male and cycling female dcc heterozygous C57BL6 mice and wild-type littermates were used for all behavioural experiments. They were obtained from Dr. Suzanne Ackerman (The Jackson Laboratory) and bred at the Douglas Mental Health University Institute animal facility. Pups were weaned at post-natal day 25 and housed with same-sex littermates. Mice were maintained on a constant 12 hour light-dark cycle with ad libitum access to food and water. Only mice between 10 and 13 weeks old were used for behavioural testing. Experiments were controlled for genotype, chamber assignment, and treatment, and were conducted during

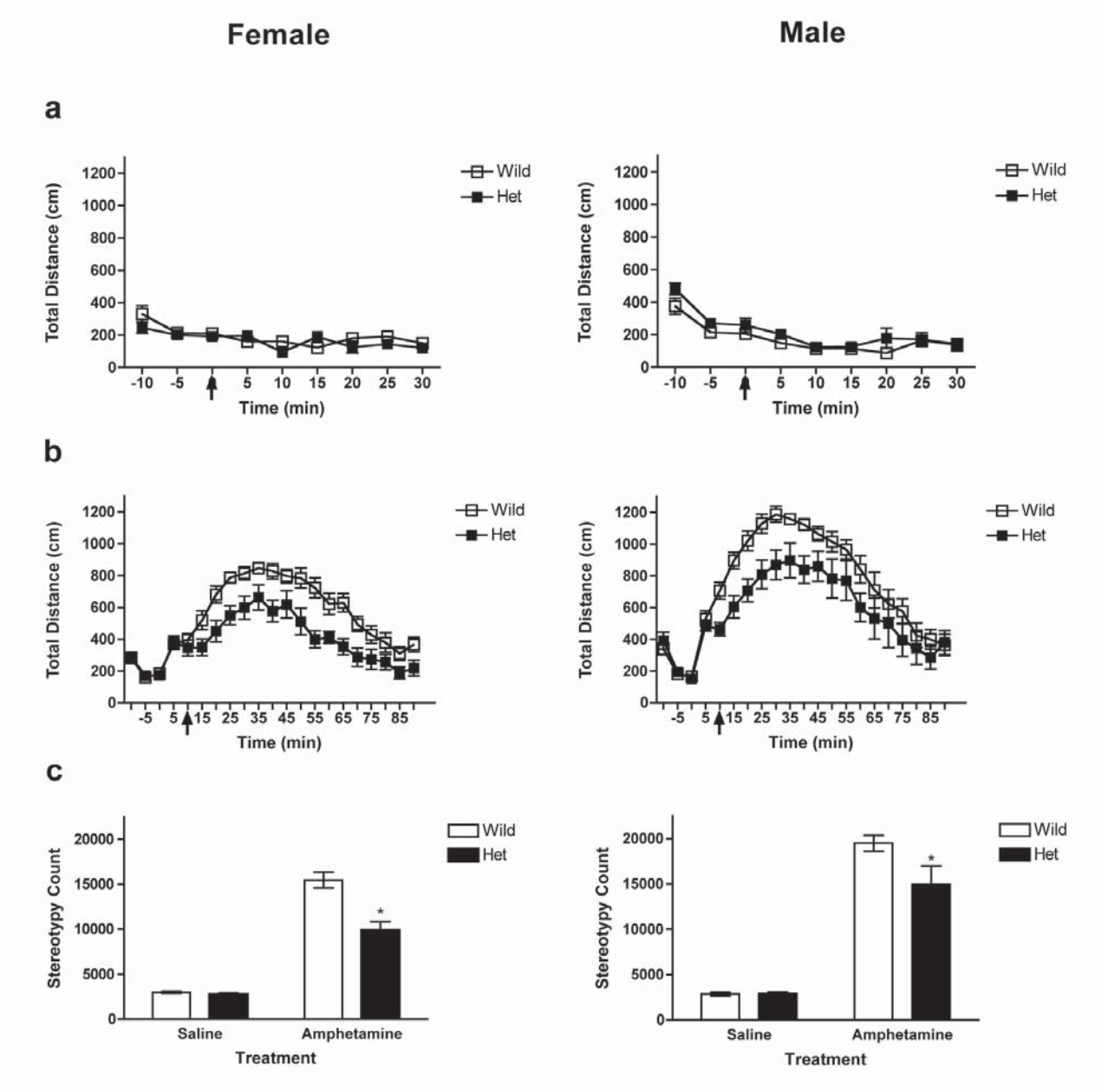

Figure 1: Locomotor response of adult male and female dcc heterozygous mice to an acute injection of amphetamine. Data points represent mean total distance traveled (±standard error means) during a five minute period. Times of injection, at 0 min, are indicated by arrows. (a) No difference in locomotion was seen between dcc heterozygous (Het) and wild-type (Wild) mice following a single injection of 0.9% saline. (b) Following an injection of amphetamine, locomotor activity was blunted in male and female dcc heterozygous mice compared to their wild-type littermates. (c) Stereotypy counts following amphetamine injection, but not a saline injection, were significantly reduced in dcc heterozygous mice compared to wild-type mice.

the light period of the cycle. Only drug-naïve animals were used in this study and separate animals were used for each experiment. All experiments were performed in accordance with the guidelines of the Canadian Council on Animal Care, the Animal Care Committee of the Douglas Mental Health University Institute, and the Concordia University Animal Research Ethics Committee.

Psychostimulant Drugs

d-Amphetamine sulphate (amphetamine; Sigma-Aldrich, Oakville, Ontario, Canada) and cocaine hydrochloride (cocaine; Medisca, Montréal, Québec, Canada) were dissolved in 0.9% saline. All solutions were administered via intra-peritoneal injections.

Locomotor Activity

Apparatus : The locomotor chambers (AccuScan Instruments, Columbus, Ohio, United States) were made of acrylic and modified for use with mice. Activity-detecting infrared sensors were located in one row on the front and back and two rows on the sides. Data was collected using VersaMax Software version 4.0 (AccuScan Instruments). Locomotor activity was expressed as distance traveled as calculated by the VersaMax system using the spacing between infrared beams. Stereotypy was calculated as the number of repeated breaks of the same infrared beam or series of beams.

Procedure : On the first day the mice were habituated for 15 minutes in the locomotor chambers. On the second day, the mice were habituated for 15 minutes in the locomotor chambers, then given an injection of saline and returned to the locomotor chambers for 30 minutes. On the third day, the mice were again habituated for 15 minutes, then injected with either amphetamine or cocaine and subsequently returned to the locomotor chambers for 90 minutes.

Experiments : In the first experiment male mice were injected with amphetamine at a dose of 2.5 mg/kg.This dose was based on a previous study comparing the amphetamine dose-response of adult dcc heterozygous and wild-type mice (Flores et al., 2005). In the second experiment we used female mice with the dose of amphetamine adjusted for females (2.2 mg/kg) to produce equivalent drug concentrations in the brain (Becker et al., 1982). The remaining two experiments used cocaine at doses of 10.0 mg/kg and 20.0 mg/kg and were conducted in female mice. Since the stimulatory effect of cocaine peaks within 10 to 20 minutes post-injection, we used only the first 30 minutes of collected data to measure the effects of cocaine on locomotion and stereotypy (Tilley et al., 2007).

Conditioned Place Preference

Apparatus : The 3-chambered conditioned place preference apparatus (model ENV-013, MED Associates Inc., Georgia, Vermont, United States) were made of acrylic and modified for use with mice. Infrared sensors on the front and back of each compartment were used to detect activity. MED-PsC software (MED Associates Inc.) used breaks in the infrared beams to record the location of the mouse. All three compartments had distinctive visual and tactile cues, creating three different environments. The two side compartments were of equal size, and cues were balanced such that the mice exhibited no pre-

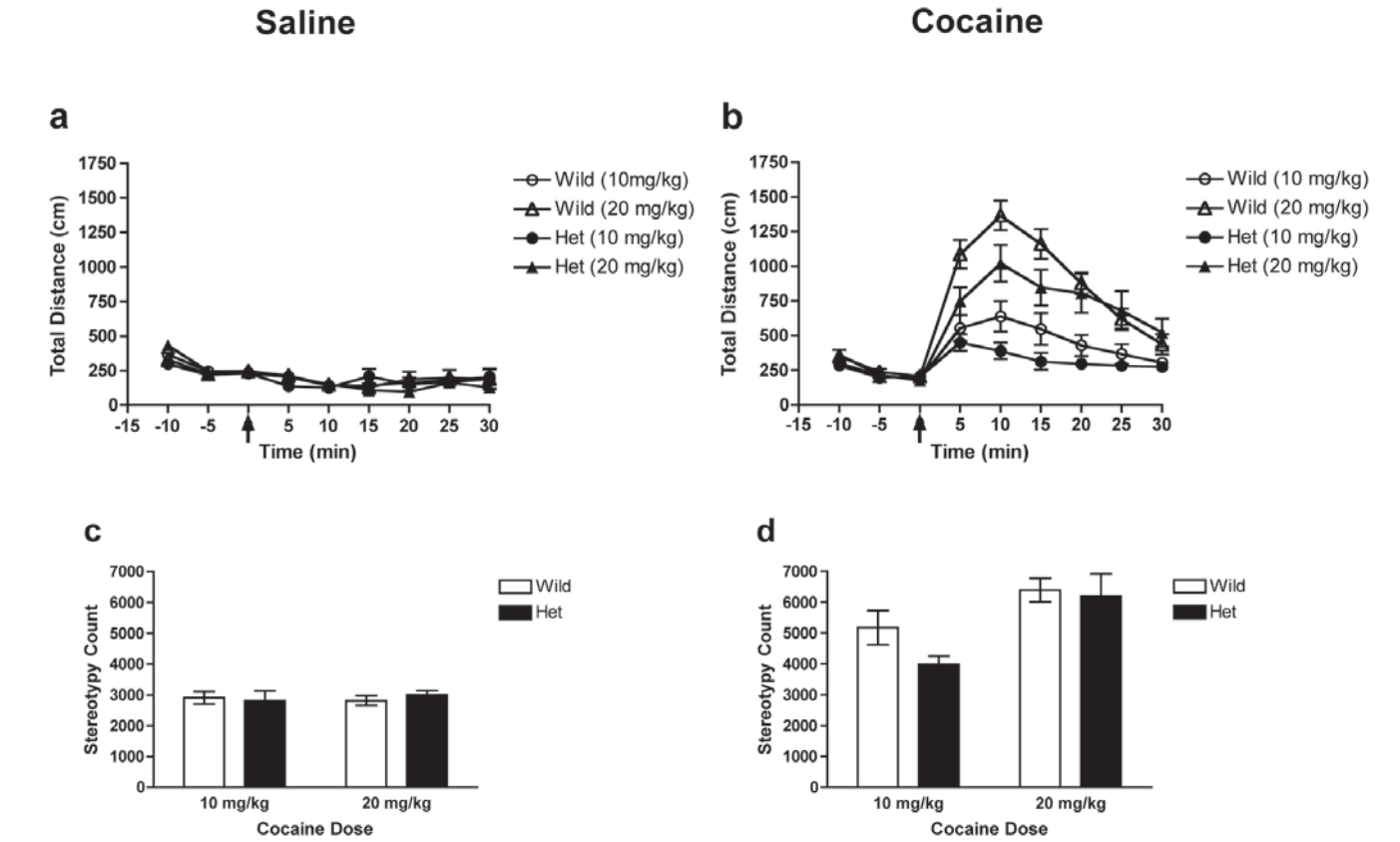

Figure 2: Locomotor response of adult female dcc heterozygous and wild-type mice to injections of cocaine. Data points represent mean total distance traveled (±standard error means) during a five minute period. Times of injection, at 0 min, are indicated by arrows. (a) No difference in locomotion was observed between dcc heterozygous mice (Het) and wild-type mice (Wild) following a single injection of 0.9% saline. (b) Following an injection of cocaine at a dose of 10 mg/kg or 20 mg/kg, dcc heterozygous mice showed significantly reduced locomotor activity compared to wild-type mice. (c) (d) Stereotypy counts following saline and cocaine injections were not significantly different between dcc heterozygous and wild-type mice.

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conditioning preference for either side. The middle compartment was much smaller and used solely as a neutral insertion point on pre-conditioning and post-conditioning days.

Procedure : Testing consisted of three phases: pre-conditioning (day 1), conditioning (days 2, 3, and 4), and post-conditioning (day 5). On day 1, the mice were placed in the middle compartment with free access to all compartments for 30 minutes. This was conducted in four squads between 11:00 am and 1:30 pm, midway between the saline and amphetamine pairings on days 2, 3 and 4. Time spent in each compartment was recorded as the duration of time, in seconds, that an infrared beam in a compartment was broken. For conditioning, mice were randomly chosen to receive amphetamine in one of the side compartments (paired compartment) and saline in the other (unpaired compartment). Compartment-treatment association was balanced such that no particular environment was associated with one treatment more than the other. Likewise, equal treatment was given to both genotypes. Between 9:00 am and 11:30 am on days 2, 3, and 4, mice received saline and were confined to the unpaired compartment for 30 minutes. Four hours later the mice received amphetamine and were confined to the paired compartment for 30 minutes. On day 5, between 11:00 am and 1:30 pm, the mice were again placed in the middle compartment with free access to all compartments for 30 minutes.Time spent in each compartment was recorded.

Experiments : Two experiments were conducted with female mice. One group of mice received an amphetamine dose of 2.2 mg/kg and the other 4.4 mg/kg. These doses were based on a previous study showing conditioned place preference in adult male mice and were adjusted for females (Becker et al., 1982; Budygin et al., 2004).

Statistics

Locomotor Activity: Total distance scores of dcc heterozygous and wild-type mice from single dose amphetamine experiments were compared using two-way repeated measure ANOVAs with genotype as the between-group variable and time as the within-group variable. Interaction between mouse genotype and gender was analyzed using a three-way repeated measure ANOVA with genotype and gender as between-group variables and time as the repeated measure variable. Total distance scores for the multiple dose cocaine experiments were compared using a three-way repeated measure ANOVA with genotype and dose as between-group variables and time as the within-group variable. Stereotypy counts were compared using two-tailed Student's t-tests.

Conditioned Place Preference: Place preference was determined by subtracting the time spent in the unpaired compartment from the time spent in the paired compartment. Place preference scores were analyzed using paired Student's t-tests with the significance level adjusted for multiple comparisons using the Holm-Bonferroni sequentially rejective procedure (Holm, 1979).

Results

DCC heterozygous mice show reduced locomotor activity in response to psychostimulants

We previously reported that adult male dcc heterozygous mice of a 129Sv/BL6 mixed genetic background show blunted locomotor responses to a single injection of amphetamine at 1.5, 2.5, or 4 mg/kg (Flores et al., 2005). To determine if this effect is strain-dependent, dcc heterozygous (n=8) and wild-type (n=11) adult male C57BL6 mice were administered a single injection of amphetamine at 2.5 mg/kg. There was no effect of genotype on locomotion or stereotypy following a single injection of saline (Figure 1a,c). Following the injection of amphetamine, dcc heterozygous mice showed significantly reduced locomotor activity compared to wild-type mice ($F_{1,17}=7.71$, $p=0.013$) (Figure 1b). Student's t-tests revealed that dcc heterozygous mice also showed significantly reduced stereotypy counts ($t_{17}=2.40$, $p=0.028$) (Figure 1c).

To assess for gender differences in the locomotor response of dcc heterozygous mice to amphetamine, we examined locomotor activity in adult cycling female dcc heterozygous (n=10) and wild-type (n=10) mice in response to amphetamine. There was no effect of genotype on locomotion or stereotypy following a single injection of saline (Figure 1a,c). We found that female dcc heterozygous mice showed blunted locomotor activity and reduced stereotypy counts in response to a single injection of amphetamine when compared to female wild-type mice (locomotion: $F_{1,17}=12.22$, $p=0.028$; stereotypy: $t_{17}=4.24$, $p=0.0005$) (Figure 1b,c). These results are consistent with male mice, and though a three-way repeated measures ANOVA revealed a significant effect of gender on locomotion after amphetamine treatment ($F_{1,34}=22.46$, $p<0.0001$), there was no interaction between the effects of genotype and gender ($F_{1,34}=0.3$, $p=0.87$).

Finally, we examined locomotor activity in adult cycling female dcc heterozygous and wild-type mice in response to a high (20 mg/kg) or low (10 mg/kg) dose of cocaine. As expected, there was no difference between the dcc heterozygous and wild-type mice following a single injection of saline (Figure 2a,c). Following a single injection of cocaine the locomotor response of the dcc heterozygous mice (10 mg/kg, n=11; 20 mg/kg, n=9) were blunted in comparison to the wild-type mice (10 mg/kg, n=10; 20 mg/kg, n=9) (Figure 2b). A three-way repeated measures ANOVA revealed significant effects of genotype ($F_{1,35}=3.96$, $p=0.05$) and dose ($F_{1,35}=36.30$, $p<0.0001$), indicating a dose-dependent effect. At either dose, dcc heterozygous mice did not significantly

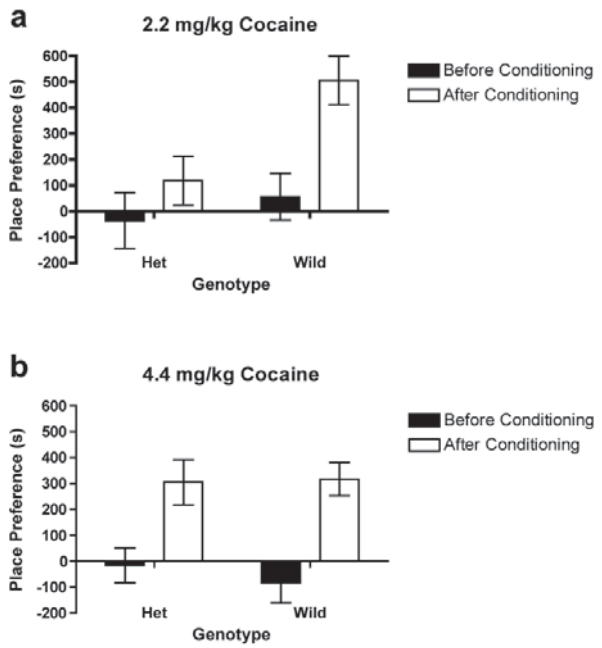

Figure 3: Conditioned place preference for an amphetamine-paired compartment in dcc heterozygous and wild-type mice. Bars represent mean place preference (±standard error means). (a) A low dose of amphetamine induced place preference in wild-type (Wild), but not dcc heterozygous (Het), mice. (b) A high dose of amphetamine induced place preference in both groups.

differ from wild-type mice in stereotypy counts (10 mg/kg, t19=2.00, p=0.06; 20 mg/kg, t16=1.57 p=0.13) (Figure 2d).

In all experiments dcc heterozygous mice showed a blunted locomotor response to the psychostimulant drug administered when compared to their wild-type littermates. They also showed stereotypy counts that were either equivalent to or less than wild-type mice, indicating that the blunted effect on locomotion was not observed due to increased drug-induced stereotypy.

DCC heterozygous mice show reduced sensitivity to the rewarding properties of amphetamine

We determined if amphetamine could induce reward in dcc heterozygous mice. During three days of conditioning adult female dcc heterozygous and wild-type mice (n=10/group) learned to associate one of two distinctive environments with the effects of amphetamine (paired compartment), and the other with saline (unpaired compartment). Preference for the paired compartment during a post-conditioning test, conducted when the mice are in a drug-free state, can be considered as an indication of sensitivity to the rewarding effects of the drug (Tzschentke, 1998). Place preference is expressed as the difference between the times spent in the paired compartment and the unpaired compartment. A positive place preference indicates drug seeking, while a negative place preference indicates drug aversion. Place preference before and after conditioning was compared using paired Student's t-tests with significance level adjusted using the Holm-Bonferroni sequentially rejective procedure (Holm, 1979). At a low dose of 2.2 mg/kg amphetamine, which has previously been shown to induce reward in mice (Budygin et al., 2004), the wild-type mice spent significantly more time in the paired compartment than the unpaired compartment during the post conditioning test (t9=2.88, p=0.018, αadj=0.025). However, dcc heterozygous mice did not show a preference for either compartment (t9=1.3118, p=0.22, αadj=0.05) (Figure 3a). At a higher dose of 4.4 mg/kg both dcc heterozygous and wild-type mice spent significantly more time in the paired compartment (wild-type, t9=3.81, p=0.0042, αadj=0.0125; dcc heterozygotes, t9=3.33, p=0.0088, αadj=0.0167) (Figure 3b).

Discussion

In this study we examined the behavioural phenotype of adult mice that develop with altered netrin-1 signaling. Netrin-1 is a secreted developmental protein involved in directing growing axons towards their synaptic targets (Barallobre et al., 2005). Here, we show that heterozygosity for the netrin-1 receptor dcc protects against the locomotor-activating and rewarding effects of amphetamine and the locomotor-activating effect of cocaine. Given the role of netrin-1 in axon guidance, we believe that altered organization of DA circuitry due to disrupted netrin-1 signaling underlies the observed resistance to these effects of psychostimulants.

A behavioural phenotype in dcc heterozygous mice was first established in mice of 129Sv/BL6 mixed genetic background, which show a blunted locomotor response to amphetamine in a dose-dependent fashion (Flores et al., 2005). Although the current study is an extension of that previous work, here we use C57BL6 strain mice. Since amphetamine-induced behaviours can vary between genetic strains, it is important to determine if the effect of dcc heterozygosity on amphetamine-induced locomotion differs between these two genetic backgrounds. For example, Chen et al. (2007) showed that C57BL6 mice exhibit greater locomotor activation than mice of a 129Sv substrain upon injection of amphetamine. They also showed lower amphetamine-induced striatal DA efflux in 129Sv compared to C57BL6 mice. Here, we demonstrate that dcc heterozygous C57BL6 mice show blunted locomotor activation similar to dcc heterozygous 129Sv/BL6 mice in response to a single injection of amphetamine. Therefore, the blunted amphetamine-induced locomotor activation seen in dcc heterozygous mice is conserved across these genetic strains. It is important to note that while a dose dependent response was observed in 129Sv/BL6 mice, we did not test for dose dependency here. We did, however, find a dose-dependent response to cocaine in C57BL6 mice, which strongly suggests conservation of this component of the dcc heterozygote phenotype as well.

Gender-specific differences in drug-induced locomotor activity have also been observed. For example, Rojas et al. (2007) reported a stronger locomotor response to phencyclidine (PCP) in wild-type C57BL6 female mice compared to male mice. Although they did not observe a gender difference in response to amphetamine, other studies have observed an effect of gender on amphetamine-induced locomotion (Suiciak et al., 2007). This raised the possibility that the behavioural phenotype of dcc heterozygous mice in response to amphetamine may manifest differently in males and females. To address this, we investigated drug-induced locomotor activation in cycling adult female dcc heterozygous and wild-type mice in response to amphetamine. We found that the altered amphetamine-induced locomotor response seen in adult male mice is also seen in adult female mice, indicating conservation of the phenotype across genders in the adult. Despite significantly lower locomotor activation in female mice as compared to males, there was no interaction between sex and genotype. As this behavioural result is consistent between male and female dcc heterozygous mice, female mice were used for further behavioural testing.

The relationship between increased locomotion and DA release in the NAcc is well documented. Locomotor activation in response to amphetamine can be blocked by lesioning the NAcc and by local injection of haloperidol, a neuroleptic DA antagonist (Teitelbaum et al., 1979). In the absence of amphetamine, direct DA injection into the NAcc is sufficient to enhance locomotion (Jenkins and Jackson, 1986). Recent microdialysis data collected in our laboratory showed that blunted amphetamine-induced locomotor activation seen in dcc heterozygous mice is correlated with reduced DA release in the NAcc (Grant et al., 2007). This finding supports the idea that locomotor activation can be used as an indication of the "state of function" of the mesolimbic DA system.

Increased DA signaling in the NAcc is a common psychostimulant effect; however, psychostimulants employ multiple molecular mechanisms to achieve this result. To examine whether dcc heterozygous mice show a blunted locomotor response to a psychostimulant that acts by a different mechanism of action than amphetamine, we investigated their response to cocaine. We found that cocaine reduces locomotor activation in dcc heterozygous mice in a dose-dependent fashion, paralleling the response to amphetamine (Flores et al., 2005). These results suggest that NAcc DA signaling is reduced in dcc heterozygous mice in response to cocaine as well as amphetamine. This provides evidence that the behavioural phenotype of dcc heterozygous mice is conserved across psychostimulants that act via amphetamine-like and cocaine-like mechanisms. Amphetamine-like drugs act mainly by inducing the release of DA from storage vesicles

into the synaptic cleft. Cocaine-like mechanisms prevent the reuptake of DA, thereby increasing the amount of DA in the synaptic cleft (Riddle et al., 2005; Geracitano et al., 2006). It would be interesting to assess the locomotor response of dcc heterozygous mice to additional drugs in both categories as well as to test the cocaine response of dcc heterozygous mice to other aspects of behaviour that are altered in response to cocaine in wild-type mice, such as protection against sensorimotor gating deficits (Doherty et al., 2007). These avenues of study are currently being investigated in our laboratory.

In addition to locomotor activation, psychostimulants have been shown to induce reward in humans as well as mice (Budygin et al., 2004, Tilley et al., 2007). The rewarding properties of a drug can be tested using a conditioned place preference paradigm (Tzschenkte, 1998). In this paradigm, drug administration is repeatedly paired with a compartment that has distinctive visual and tactile features. A second compartment with different visual and features is paired with a control substance, i.e. saline. When allowed to move between the drug-paired compartment and the saline-paired compartment, a mouse that showed no pre-conditioning preference for either compartment will spend more time in the drug-paired compartment after drug treatment. This drug-seeking behaviour is present only when the drug has a rewarding effect for the mouse (Tzschenkte, 1998; Sanchis-Segura & Spanagel, 2006). In dcc heterozygous mice we found no drug seeking behaviour at a dose of amphetamine that elicited drug seeking behaviour in their wild-type littermates. Interestingly, at a higher dose of amphetamine drug-seeking behaviour was observed in both wild-type and dcc heterozygous mice. This raises the possibility that dcc deficiency produces a rightward shift in sensitivity to the rewarding properties of amphetamine in the adult mouse.

Psychostimulant-induced reward, like locomotor activation, is mediated by increased extracellular DA in the NAcc (Sellings and Clarke, 2003; Di Chiara et al., 2004). Interestingly, although DA activity in the NAcc is reduced in dcc heterozygous mice following an amphetamine injection, DA activity and tyrosine hydroxylase (TH) expression in the medial prefrontal cortex (mPFC) are heightened in response to amphetamine, as compared to wild-type mice (Flores et al., 2005; Grant et al., 2007). Given that DA activity in the prefrontal cortex has an inhibitory effect on DA release in the NAcc, it is likely that DA release in the NAcc is reduced as a result of DA hyperactivity in the mPFC (Herve et al., 1981; Deutch et al., 1990; Doherty and Gratton, 1996; Beyer and Steketee, 1999; Ventura et al., 2004, Flores et al., 2005; Grant et al., 2007). This suggests that higher levels of extracellular DA in the mPFC can account for the reduced behavioural responses to cocaine and amphetamine seen in adult dcc heterozygous mice. Dopaminergic projections to the mPFC and NAcc share a common origin, the ventral tegmental area. Indeed, the two pathways, mesolimbic and mesocortical, are commonly referred to together as the mesocorticolimbic DA system (Le Moan & Simon, 1991). As midbrain DA neurons express DCC, and based on our current and previous behavioural and biochemical findings, we hypothesize that the mPFC DA hyperactivity in dcc heterozygous mice is the result of abnormal mesocorticolimbic DA neuronal development, which, in turn, is due to disrupted netrin-1 signaling (Flores et al., 2005; Osborne et al., 2005; Grant et al., 2007).

Due to an incomplete understanding of the biochemical, cellular, and behavioural consequences of DCC deficiency, we cannot be certain as to how DCC deficiency affects the functioning of the mesocorticolimbic DA system. Modulation to aspects of DA function in addition to DA release, such as synthesis, metabolism and signal attenuation, may also contribute to the abnormal DA signaling seen in dcc heterozygous mice. Alterations to brain structures known to innervate the mesocorticolimbic DA system, such as the hippocampus and amygdala, could also contribute to abnormal DA function (Grace et al., 2007). Aberrations in these structures have been observed in developing dcc homozygous mice during embryogenesis, though it is currently unknown if similar defects are present in dcc heterozygotes or if these embryonic aberrations would result in altered structures in the adult (Barallobre et al., 2000). Furthermore, other neurotransmitters known to modulate drug-induced DA activity in NAcc, such as norepinephrine and glutamate, could contribute to abnormal DA function (Ventura et al., 2003; Grace et al., 2007; Rommelfanger and Weinshenker, 2007). However, previous studies appear indicate that the major component of norepinephrine signaling pathway is unaltered in dcc heterozygous mice. Our laboratory has found normal expression of dopamine-beta-hydroxylase, the enzyme that converts DA to norepinephrine, in the mPFC of dcc heterozygous mice, and no difference in amphetamine-induced extracellular norepinephrine in the prefrontal cortex (Grant et al., 2007). Future studies further examining the potential roles of other neurotransmitters and brain structures on DA signaling in dcc heterozygous mice are currently being investigated by our laboratory.

Studies have shown that slight alterations in genetic and environmental factors during brain development may increase or decrease an individual's susceptibility to the effects of psychostimulant drugs as adults (De Wit, 1998; Piazza et al., 1998; Suzuki et al., 2003; Ventura et al., 2004; Miyatake et al., 2006; Reichel et al., 2006; Fujii et al., 2007). Here, we find that altering the expression of DCC leads to a phenotype that appears protected from the rewarding and locomotor-activating effects of the psychostimulant amphetamine. At least one aspect of this phenotype, locomotor activation, is consistently protected across gender, two genetic strains and two mechanisms of drug action, indicating a robust phenotypic expression of the dcc haploid genotype. In addition, these mice appear protected against the rewarding effects of amphetamine. These findings suggest that changes in the expression of the netrin-1 receptors can alter behavioural responses in a very specific manner, which supports our hypothesis that variable expression of netrin-1 receptors can create variations in behaviour between individuals. Due partly to its importance in drug addiction, the development and function of the mesocorticolimbic DA system is an area of intense scrutiny and study in neuroscience. Our findings point to DCC as a critical determinant of mesocorticolimbic DA function and of susceptibility to the effects of psychostimulant drugs.

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Dynamics of the financial market

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Financial mathematics must make use of assumptions in the development of mathematical models that provide predictive power on the behavior of economic markets, as it is impossible to collect data on the market as a whole. As a result, important quantities, such as the risk-measurement of a portfolio, are often inaccurately estimated. The financial market seems to be an erratic, pattern-less system. Indeed, attempts to find patterns, and to explain the processes behind the price movements of an asset, have been largely unsuccessful. This is analogous to the 'Turkey Problem' described by N. Taleb in his book "The Black Swan". To illustrate, a turkey spends its life being fed and raised for slaughter, a fact that is unbeknownst to it. From the point of view of the turkey, life is delicious and predictable, until the day it is killed. For the turkey, its death is a 'black swan event', as it represents something highly unpredictable and catastrophic. This same type of uncertainty is also present in financial markets.

Numerous examples of events with a significant impact on the system demonstrate the failure of the current model can be found. The fate of Long Term Capital Management (LTCM) is one such example. LTCM failed to predict the collapse of the Russian ruble, as it assumed that the Russian government defaulting on its bonds would be a highly unlikely event. Current theory describes these catastrophic events as so highly improbable that their very occurrence is near miraculous, and cannot be predicted. This would be a reasonable theory if there was only one such event, perhaps two, in the course of history. However, one can find plenty of examples of these so-called impossible events by examining historical data. This raises the question; if these events are happening with a higher frequency than expected, is our current model correct? It is important to remember that markets do not obey a model, but rather the model seeks to explain the behavior of markets. As such, we must attempt to construct a model that resembles and predicts our observed data, or the model should be deemed invalid.

Central to the classic financial model is the Gaussian or normal distribution. If a sample of numbers is normally distributed it should cluster around a mean or average value, and the likelihood of an event is increasingly rare as it deviates further from this mean. The occurrence and magnitude of these deviations are described by the variance of the distribution. As such, the normal distribution is described by two natural parameters, the mean and the variance. The normal distribution is followed by many natural systems, and as a result this mathematical model has found applications in many diverse fields from astronomy to population dynamics.

When describing a natural phenomenon, such as the stock market, we seek to produce a model that is able to match an observed pattern. For example, in financial time series, we consider the difference between the natural logarithm of the prices. To illustrate this, consider a time series

$$(P_0, P_1, \ldots, P_{t-1}, P_t, P_{t+1}, \ldots)$$

where the subscript t indicates time intervals. Consider the set of values

$$X(t) = \log(P_{t+1}) - \log(P_t)$$

This is our variable of interest, the unit-free difference in stock prices. It is this variable which classical financial theory assumes to be normally distributed. In other words, we expect the value of a price change over one time interval to be close in value to the mean of the normal distribution, with significant deviations away from the mean being quite rare. If for example we assume our changes to follow a standard normal distribution, which means it has a mean of zero and a variance of one, we would expect the price difference to be zero, so our prices would be constant, and large price fluctuations (whether positive or negative) to be rare. The other important condition that is imposed on this model is that each event is independent. Assuming the price changes follow a standard normal distribution, we can base models on Brownian motion, which is the same concept that describes the movements of particles in fluid. This concept is the foundation for the derivation of the famous Black-Scholes equation, which is used to anticipate market movements by generating a probable value for equity pricing.

Now consider our variable of interest, the change in stock prices, and examine the consequences of assuming this variable to be drawn from the Gaussian curve. Our first assumption is to expect the data to be centered around a mean, which has been shown to be true empirically (Tsay, 2005). However, a problem arises in discussing "outliers" in the data, points that are at least two standard deviations away from the mean. In financial data, one is often presented with these anomalies. For example, consider the stock market crash in 1987, or the internet boom in the 1990s. These events deviated widely from the trend. We can also consider the magnitude of these outlying events. Yet based on the Gaussian distribution model, the odds of a value falling far away from the mean is fairly low. Can the Gaussian model be applied to financial data? Does the normal distribution allow for absolute price movements ten times the average return?

We will consider the weekly closing prices of General Electric as listed by Yahoo! Finance from January 8, 1962 to September 4, 2007. General Electric is an ideal choice to represent the 'average' asset on the market, as its ample data, size and market diversity reflects general market trends. This provides us with 2382 observations which will allows us to use large sample properties. If we assume that the price changes are normally distributed we can find the estimated parameters of the normal distribution. In the case of GE we find the mean = \$0.0002487107 and the variance = \$0.002518528.

Within this data set I have identified eight statistically impossible events. These events represent large price changes. While one such event, such as the 1987 stock crash, may be accepted as a statistical anomaly, the occurrence of eight such events over a time period of 45 years is statistically improbable and contradicts the prediction of our model. We therefore suspect that the returns are not normally distributed. The very existence of these outliers shows that there are observations that cannot be explained by the current theory. It seems that these events occur frequently and generally have lasting impact on the markets. Shouldn't a model that claims to understand the dynamics of price changes take these events into account?

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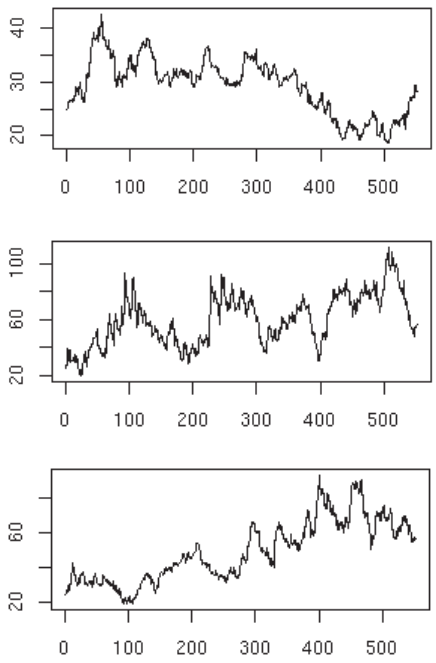
The second essential assumption made is that price movements are independent of each other. Intuitively, one would think that this is not true at all. In fact, consider an individual purchasing stock. The purchase is made based on the stock's past performance, particularly its recent performance. By standardizing our sample (simply subtracting the mean and dividing by the variance) we can assess whether these price movements are independent by analyzing increasing or decreasing streaks in the data. If price changes are independent, there should not be any noticeable streaks in the data (large consecutive positive or negative price movements). With this in mind, we find that indeed our data reflect the presence of increasing and decreasing streaks, which are improbable under the classic Gaussian model.

An alternative theory has been explored. Benoit Mandelbrot has developed the area of fractal finance in order to explain these issues that other models have dismissed. He demonstrates that this fractal view of the market, which in some ways is actually an elegant generalization of the Brownian motion concept, fits the observed data more closely than the Gaussian model (Mandelbrot 2004). Simply put, fractals are mathematical objects that look the same when viewed at both low and high resolution. In the case of stock prices, increasing the resolution means looking at smaller time scales. To understand this, consider the following three graphs of



GE stock returns. It is impossible to distinguish between the graphs with time scales of days, weeks and months.

This fractal property coupled with a random element seems to be a better model for price changes. The Multifractal Model of Asset Returns (MMAR) was introduced by Mandelbrot. X(t) is a compound process, such that

$$X(t) = B_H[\theta(t)]$$

Where $B_H(t)$ is a fractional Brownian motion operator with self-affinity index H ,and $\theta(t)$ is the stochastic trading time. While the components of this model reflect complex mathematics, we can still understand how it works.

The self-affinity index H accounts for the observation that no matter what time scale you consider, the system looks the same. This allows the model to compensate for streaks in the data, something which our classical model fails to do. For example, large values of H at some given time result in persistent trading (trading in the same direction), while low values of H indicate very little movement. The stochastic trade time $\theta(t)$, is called a multi-fractal process. This captures the fluctuations in the observed volatility of the data. In other words, this allows the model to take into account what has happened in the past, while allowing for extreme price changes, extremely improbable in the classical model. In refining a model in this fashion, a particular question arises; is our new model more general than the previous model? Alternatively, can we, under certain restrictive conditions, obtain our classical model from our fractal model? As we cannot derive the classical model from the fractal model, if the fractal model is indeed legitimate, the classical model cannot be right, and thus has no real value in describing financial markets.

To conclude, we have explored the current view of the financial markets, assuming that a simple Brownian engine drives the observed price changes in the financial market. We utilized real data and tested the assumptions of this model to see whether the actual data followed the proposed model. Specifically, we identified a set of events occurring relatively frequently which would be impossible based on the classical model. We also noticed the presence of trends in the price changes which provide evidence against the independence of these returns. This in turn implied that our model should incorporate some sort of memory of the past, so we proceeded to incorporate the property of time scale invariance. With this in mind, we presented the MMAR model, which reflected these observations. Under the current mode of thought, models like the Black-Scholes pricing equation underestimate the potential of large increases or decreases in value. These are not merely potential events, but have already occurred, including the collapse of the Russian ruble, the collapse of the subprime mortgage market or Long Term Capital Management, among other financial disasters in history. While we cannot be certain that markets obey the MMAR model, at the very least we can affirm that it is a more accurate representation of financial returns, rendering the classical model obsolete.

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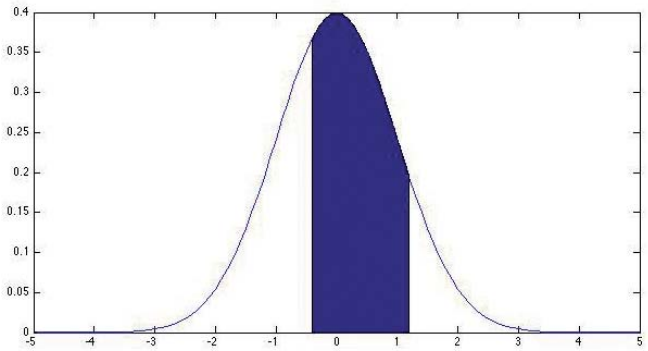

Specialized morphology for a non-specialized diet: Liem's paradox in an African cichlid fish

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Abstract
Cichlid fishes of the East African Great Lakes represent some of the most diverse vertebrate faunas in the world, and trophic specialization, the specific adaptation of feeding structures to one type of prey, is often used to explain the coexistence of these closely related species. However, Liem's Paradox suggests that organisms with specialized phenotypes may act primarily as generalist feeders in nature, which can create a mismatch between diet and morphology. Our goal was to study the diet of a widespread African cichlid, Astatoreochromis alluaudi, over the course of 1 year to test the hypothesis that the molluskvore-like morphology of this species is not an appropriate indicator of diet choice. Lake Saka, in Uganda, was sampled monthly throughout 2006, and stomach content analyses were performed on preserved specimens using established techniques to identify the relative importance of various prey items in the diet of A. alluaudi. Stomach content analyses indicated an omnivorous diet in all months, consisting mostly of insects, fish, and plant matter, whereas snails accounted for only a small portion of their overall diet. Although trophic morphology in this species is a plastic trait, specimens from Lake Saka exhibit a molluscivore-like morphology. Our data suggests that the morphology of this generalist feeder may have developed to exploit non-favoured resources, a clear example of Liem's Paradox. This study emphasizes the importance of examining both stomach contents and trophic morphology before inferring the feeding ecology of a species.

Keywords
Trophic specialization, African cichlids, Molluscivore, Omnivore, Morphology.

Introduction

Of all the freshwater fish species in the world, the cichlid fishes of East Africa are arguably the most diverse, well-known, and well-studied group (McKaye and Marsh 1983). Over 600 species, half of which are endemic to the area, were found in one watershed in the Lake Victoria basin alone (Alphen et al. 2003). It has long been hypothesized that specialization in feeding (trophic) morphology and diets of these fishes is an important factor contributing to their radiation within these watersheds (Liem 1991). Furthermore, trophic specialization has been used to explain the coexistence of such a large number of closely related organisms and the existence of the multitude of endemic species present in any given lake (Fryer and Iles 1972, Barlow 2000). For example, cichlids from the African Great Lakes are known to include piscivores (Mbabazi et al. 2004), paedophages (fish egg and embryo-eaters), insectivores (Hoogerhoud 1987), crustacean-eaters, molluscivores (Greenwood 1964), lepidophores (scale-eaters, Hori 1993), herbivores, epilithic and ephiphytic algae-eaters, phytoplanktivores (Katunzi et al. 2003), zooplanktivores (Goldschmidt et al. 1993), and detritivores (Greenwood 1959). Astatoreochromis alluaudi is a widespread East-African cichlid (Hoogerhoud 1984, Huysseune 1995) that has two distinct and well described pharyngeal jaw morphologies, which vary depending on diet. Cichlid fishes possess a second pair of jaws, which aid in prey processing, located deep in their buccal cavity known as pharyngeal, or throat jaws. Large-jawed, hypertrophied morphs of A. alluaudi have been associated with a snail-eating, molluscivorous diet, whereas small-jawed, non-hypertrophied morphs are believed to feed on softer food items such as insects and plant material (Smits et al. 1996). Studies have shown that this morphological differentiation is a plastic trait dependent on juvenile prey choice, irrespective of paternal phenotype (Greenwood 1964, Hoogerhoud 1986).

Although it is a widespread cichlid in East Africa, A. alluaudi has been most extensively studied in Lake Victoria, where it consumes primarily snails (Witte 1981), and as a result, it is largely assumed that this species is a molluscivore (Greenwood 1964). However, A. alluaudi has also been reported to feed on various prey resources, and is known to prefer insect prey if it has access to this resource (Slootweg et al. 1994). Since jaw morphology in A. alluaudi is a developmental trait that apparently canalizes early in an individual's lifetime, a fish exposed to hard-bodied food items during its early life stages will develop molariform morphology even if it routinely feeds on softer prey items (Smits, 1996). Similarly, in times of resource scarcity, even highly specialized fishes have been observed to switch to an omnivorous diet in order to meet their energy requirements (McKaye and Marsh 1983). Thus, it is possible that seasonal fluctuations in resource abundances at a site may influence jaw morphology and diet in A. alluaudi, particularly if there are certain periods of time during which the cichlid must consume snails in order to survive even if it feeds on other prey types preferentially the majority of the year.

In Lake Saka, a crater lake located in western Uganda, morphological analyses performed on A. alluaudi specimens (L.J. Chapman unpubl. data) revealed that this particular population has relatively well developed hypertrophied jaws not characteristic of A. alluaudi that are fully insectivorous (Figure 1). However, many cichlid fishes with specialized feeding structures have been reported as generalist feeders in field studies, questioning the accuracy of studies focusing solely on morphology as a predictive tool.

The general goal of our study was to describe variation in the diet of A. alluaudi from Lake Saka, Uganda to explore whether morphology is a good indicator of feeding habits in this species. To meet this goal, we analyzed stomach contents of fish collected monthly in 2006 from Lake Saka, Uganda. We believe that this study has important implications for ecological studies of fish, which often categorize species based on their trophic structures without rigorously examining their feeding ecology.

Materials and Methods

Lake Saka (0°40'N, 30°15'E, Figure 2) is a crater lake located near Fort Portal, Uganda. This region of East Africa is characterized by two wet and two dry seasons per year. In Lake Saka, surface dissolved oxygen varies from 6 mg l-1

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to supersaturation (greater than 12 mg l-1) throughout the year, often exceeding 15 mg l-1 in the surface waters. This is thought to reflect enhanced phytoplankton productivity over the past 3 decades (Crisman et al. 2001).Water column anoxia does not seem to occur in the northern section of the lake where A. alluaudi were collected (approximately 2 m depth) but have been reported in the deeper areas of the lake, particularly in the southern section where there is a very small but deep crater (>12 m) (Crisman et al. 2001). Specimens were collected bimonthly from the northern part of Lake Saka, using baited metal minnow traps set between 10:00 am and 2:00 pm from January to December 2006. Due to issues concerning site access, no samples were collected during March. Fish were euthanized with MS222, preserved in 10% formalin, and transported back to McGill University for stomach content analysis.

Fishes of varying standard lengths, as measured from the tip of the snout to the start of the caudal fin rays, were chosen to obtain a representative sample of the population; and the two samples were combined within each month to provide a larger and more representative monthly sample. In total, 138 fishes with non-empty stomachs were examined. For most months, all fishes were sampled; however, for months with large numbers of collected fish, we subsampled 10 fish with non-empty stomachs. Sample sizes for each month ranged from a minimum of 5 stomachs (February) to 19 stomachs (November), but on average, 12 non-empty stomachs were analyzed per month. Fish stomachs were dissected out, and stomach fullness was visually assessed using the following criteria: level 1, empty stomach; level 2, 1-25% full; level 3, 26-50% full; level 4, 51-75% full; level 5, 76-100% full. Stomach contents were observed under a dissecting microscope (LEICA MS5), and food items were separated and identified taxonomically to order level resolution when possible (McCafferty 1981). Food items were sorted into one of the following categories: fish (mature/juvenile individuals and fish larvae), gastropods, insects (primarily Ephemeroptera and Diptera), plant matter (mostly algae and macrophytes), oligochaetes, and zooplankton.

We quantified each item by using a modified version of the points method (Hynes 1950). First, the percentage volume of each food item was visually assessed by estimating how much it contributed to the total amount of food found in a given stomach. Each food item found in an individual fish was allotted a number of points based on its relative percentage rounded to the nearest 10 percent. To take into account the percent fullness of each stomach, these points were then multiplied following fullness criteria: level 2, by 0.25, level 3; by 0.5, level 4; by 0.75 and level 5; by 1. Finally for each sample, the total number of points per category was calculated and divided by the total number of non-empty stomachs in the sample.

We used linear regression on the points results to evaluate whether the mean monthly points for fish, insects, plant matter, or gastropods correlated with seasonal rainfall. This was done in two ways: we used the monthly rainfall and the 2-month running average, which takes the accumulated rainfall from the previous month into consideration.

Results

Over the course of the year, A. alluaudi consumed a variety of prey items in varying amounts (Figure 3). Overall, fish formed the most abundant category, and comprised nearly half of the annual diet of A. alluaudi. The fish remains found in the stomachs appeared to be cichlids, but no further analyses have been done to identify the species of cichlid. The plant matter food category consisted principally of algae and macrophytes. Debris consisted primarily of small rocks or sandy particles; which were probably taken unintentionally while feeding on insects or plants (Hyslop 1980). Both plants and insects appeared to be important resources in the diet of A. alluaudi and were often the second or third dominant food types encountered each month (Figure 4).

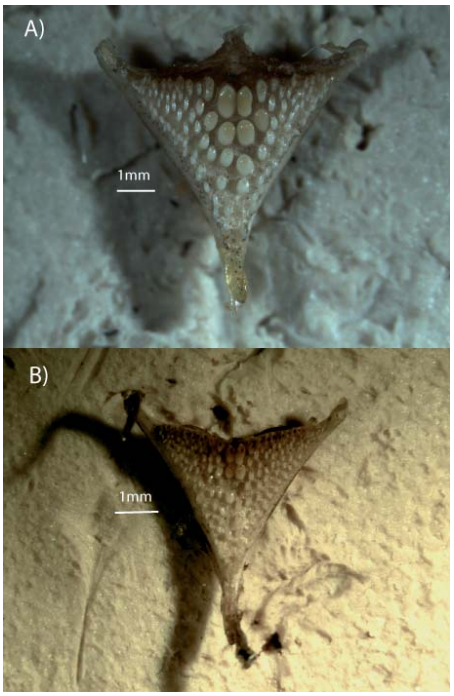

Figure 1: Dorsal view comparison of the lower pharyngeal jaw of A. alluaudi specimens from (A) Lake Saka and (B) Lake Nabugabo. Both fish specimens have standard lengths of 63.95 ± 2.00mm. Lake Nabugabo is a well-studied site where snails are absent, and A. alluaudi routinely feeds on insects and fish.

Although gastropods were present in the stomachs consistently throughout the year, they did not seem to form a major component of the diet of A. alluaudi, and in most months, they ranked as the third or fourth most abundant prey (Figure 4). Overall, gastropods contributed less than 5% of the annual diet of A. alluaudi (Figure 3). Oligochaetes and zooplankton only contributed to 10% of the overall diet (Figure 3). Some stomachs were found to have exclusively zooplankton or worms, which eliminates the possibility that A. alluaudi unintentionally fed on these resources. Linear regression analyses indicated no significant relationship between four of the important food categories and rainfall: fish ($F=0.340$, $p=0.574$), insects ($F=1.025$, $p=0.338$), plant matter ($F=0.063$, $p=0.807$), and gastropods ($F=1.257$, $p=0.291$). However, our analysis of running rainfall average indicated a weak correlation with percentage piscivory per month ($R2=0.301$, $p=0.08$).

Discussion

Feeding specialization and Liem's paradox

Our results provide evidence suggesting that A. alluaudi from Lake Saka are generalist feeders, consuming mostly other small cichlids, insects, and plant matter. Over the year of study, we found that only a very small portion of their diet was comprised of snails. This finding was unexpected based on our morphological studies which demonstrate that this population has prominent pharyngeal jaws usually associated with

a more, although not exclusively molluskivorous diet.

The lack of a tight correspondence between diet and morphology found in our study has been observed in other cichlid species (Katunzi 1983) as well as in Darwin's finches (Tebbich et al. 2004), and is often referred to as Liem's paradox (Liem 1980). In essence, Liem's theory suggests that specialized phenotypes are retained to exploit less-favored food sources rather than favored resources. This adaptation is possible if the specialized phenotype is still efficient in processing other resources allowing individuals to feed on various foods, especially when the favored prey resources are limited. In the case of A. alluaudi, large jaws may be used to feed on snails when fishes or insects are rare.

The likelihood of specialized feeders being able to efficiently switch between resources was believed to be small, especially when the resources required different feeding techniques to be eaten (Barel 1983). However, studies of Darwin's finches have reported these apparently specialized birds switching both prey base and feeding technique in response to fluctuating resource abundances (Schluter 1982, Tebbich et al. 2004). In the case of aquatic species, it was long hypothesized that biting and sucking feeding techniques were only compatible to a certain extent because of anatomical demands (Barel 1983). However, recent studies refute this presumed trade-off (Van Wassenbergh et al. 2007), and have shown that some fish that seem morphologically adapted to be biters are as effective in sucking prey as are sucking specialists (Bouton et al. 1998, Van Wassenbergh et al. 2007). The sucking technique is an effective way of consuming most insect larvae such as Diptera larvae, which is a major food source of A. alluaudi. Since insects comprise 18% of A. alluaudi's overall diet, we assume that morphological specialization in A. alluaudi does not constrain its ability to feed on insect larvae, a potentially favoured resource. Flexible omnivory might allow A. alluaudi to better coexist with other species by reducing niche overlap and direct interspecific competition. Omnivory may also be adaptive from an optimal foraging perspective. According to optimal foraging theory the caloric value obtained from a potential prey item must exceed the time and energy costs of catching and processing a given prey (Robinson and Wilson 1998). In other words, calorie-rich food may not be consumed by certain species if there are lesser-quality but easily-handled

Figure 2: Map of Uganda showing the location of Lake Saka.

foods available. It is reasonable to believe that the energy payoff as well as the handling time differs for all the prey items consumed by A. alluaudi, and that these foraging considerations influence a fishes diet, especially in a spatially and temporally variable habitat. However, specific details concerning the energetic quality of possible prey in this system have not been documented and were beyond the scope of this study.

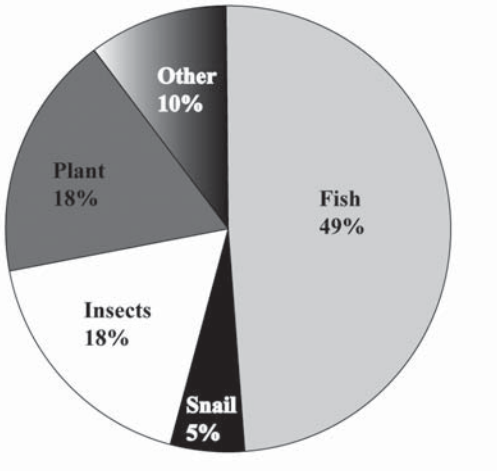

Figure 3: Average diet composition of A. alluaudi in Lake Saka over all months.

In proposing that A. alluaudi in Lake Saka are very omnivorous and that mollusks comprise a small component of their diet, we assume that our analysis of diet was relatively unbiased. The analysis of stomach contents as a means to describe seasonal variation in an organism's diet or dietary comparison between species is a standard and widely used practice in fish ecology (Hynes 1950). However the appropriate method often depends on the organism of study, the mechanical digestion apparatus, and the types of prey consumed. We selected the points method for characterizing the diet of A. alluaudi since the food experiences a high degree of mechanical processing in the pharyngeal jaws before entering the stomach. It takes into account the stomach fullness, but has been criticized for its subjectivity (Hyslop 1980). Regardless of the method used, overrepresentation or underrepresentation of some items may be induced by different digestion rates. For example, crustaceans and fish remains, principally scales take longer to be digested and thus tend to be overrepresented in analyses (Van Wassenbergh et al. 2007). The abundance of scales found in A. alluaudi stomachs could be a reflection of this bias; however, we believe that these potential limitations would not have altered the main results of this study.

Hypertrophied morphology, architectonic interdependency and dissolved oxygen

Another plausible explanation for the retention of molluscivorous morphology in A. alluaudi resides in the concept of architectonic interdependency. The cranial musculo-skeletal system of fishes is extremely complex and necessary for various vital functions such as capturing and processing prey, gill ventilation, protection to vital organs, and locomotion. Thus, many of these functions are interdependent through architectonic relations, and an adaptive response to one structure might consequently affect another. The large volume occupied by the specialized pharyngeal jaws of A. alluaudi in Lake Saka has multiple direct and indirect effects on other head structures, such as gill size. Dissolved oxygen (DO) levels in the shallower waters of the hyper-eutrophied Lake Saka are high, and

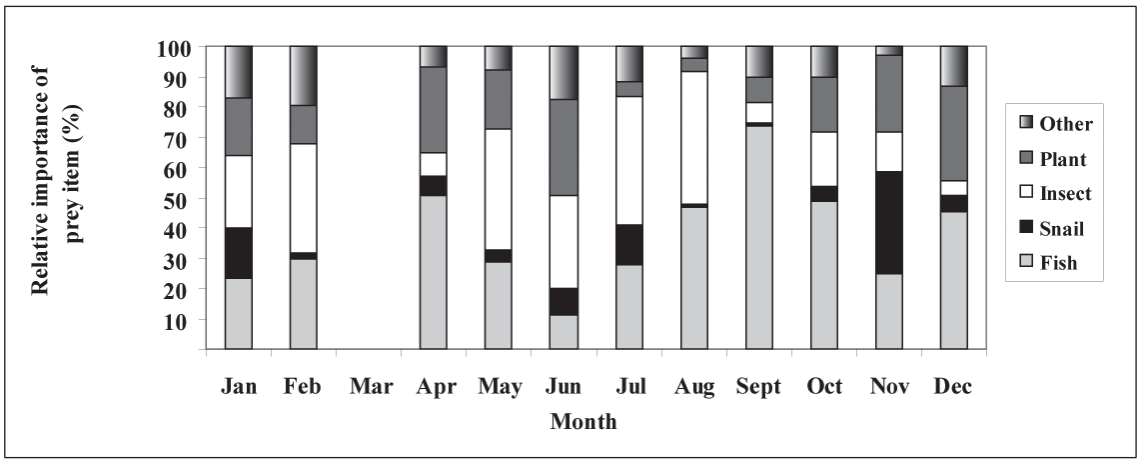

Figure 4: Percent importance as determined by the points method of major food categories in the stomachs of A. alluaudi in 2006.

the lake almost never experiences anoxic conditions in the surface waters (Crisman et al. 2001). The specimens collected in this study were from shallow waters, where oxygen content in the upper waters tends to be supersaturated during the day. Many haplochromine species known to have mollusk-associated pharyngeal jaws are, in fact, limited to water less than 12 m deep (Greenwood, 1960; Witte, 1981). One plausible explanation for this lies in their morphocranial architecture: very large pharyngeal jaws constrain larger gill sizes that develop in low DO. The relationship between gill size in fishes and dissolved oxygen levels has been well studied for several species of African fish (Chapman et al. 1995, Schaack and Chapman 2003, Chapman et al. 2006) . Since A. alluaudi populations from Lake Saka are not constrained by low DO concentrations and are characterized by a relatively small total gill surface area (Chapman et al. 2007) there is no evidence for a spatial trade-off which would constrain jaw development.

Seasonal variation in resource availability

The feeding behavior of A. alluaudi did not show any clear patterns correlated directly with rainfall variation. However, fish prey appeared to be more abundant in the diet of A. alluaudi during rainy months, particularly small larval and juvenile fish. Reardon and Chapman (2008) have found that spawning peaks in the cichlid species from this region are associated with seasonal peaks in precipitation. Hence, during wet seasons, juvenile fish density may be particularly high, offering an easy and rich food source for piscivorous species. More detailed studies of the relationship between seasonal dietary change and prey availability will be required to fully explore this idea.

Conclusion

Although cichlids are among the best-studied families of freshwater fishes in the world, there is still much that is unknown about the ecology of these organisms. East African cichlids from the Great Lakes are often used as a model system to study evolutionary questions concerning speciation (Fryer 2001, Alphen et al. 2003), coexistence (Fryer and Iles 1972, Ribbink 1991, Genner et al. 1999b), and the origin of ecological specialization (Bouton et al. 1997, Genner et al. 1999a, Mbabazi et al. 2004). However, many species endemic to the Lake Victoria watershed have yet to be described, and the role of trophic specialization in promoting the coexistence of closely-related species has been increasingly called into question (Genner et al. 1999b, Katunzi et al. 2003). This study provides evidence to suggest that A. alluaudi from Lake Saka is a generalist feeder whose diet is principally composed of insects, fish, and plant matter. This result is unexpected based on earlier studies of A. alluaudi jaw morphology from Lake Saka, which are not characteristic of insect-eaters. Based on this morphology, we anticipated snails to be an important food source for this population. However, Astaroerochromis alluaudi seems to be another case of Liem's paradox, where when favoured prey are rare fishes develop a specialized morphology to exploit a non-favoured resource. These results highlight the importance of combining dietary analyses with morphological studies in order to fully understand the feeding ecology of a species.

Acknowledgements

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Using CloudSat data to look at the cloud and precipitation structure in the Arctic

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Abstract

Clouds and precipitation are fundamental determinates of the Arctic climate. Despite the presence of clouds and storm systems, very few precipitations reach the ground. In fact, polar precipitation patterns are characterized by frequent sublimation of the water particles during their descent. This discrepancy between cloud coverage and storm systems and the amount of precipitation on the ground is not explained because relatively little is known about these features due partly to the lack of available data. Recently, CloudSat, a polar-orbiting satellite, was launched in order to provide unique information about the cloud and precipitation structure of the Arctic, which cannot be measured using current weather instruments. The data is used in the context of the Storm Studies in the Arctic, a field experiment that started in Fall 2007 in the Canadian Arctic, aims at describing the extreme weather occurring over Baffin Island and Iqaluit. The present study focuses on the Southern Baffin Island using data from CloudSat for the area covered by the Storm Studies in the Arctic field experiment. We analyzed 37 passes of CloudSat over Iqaluit and examined cloud altitude and type, environmental conditions like temperature and relative humidity, as well as the precipitation type, size, and rate. Our analysis shows that the cloud structure is characterized by formations that are mostly stratiform, composed of one to two cloud-layers at low- and mid-level, and mostly thin individual clouds. A low cloud base and a large cloud thickness seem to be requirements for precipitation over the region. Although these results do not give satisfying answers regarding what triggers sublimation , they provide general information about the macrostructure of the clouds. This is a first step in understanding why in the Arctic very little precipitation reaches the ground. We believe further information about cloud microstructure can be garnered using data from CloudSat. Our data therefore provides recommendation for the Storm Studies in the Arctic experiment that started after this study was conducted

Keywords

Cloud, precipitation, Arctic, water cycle, climate.

Introduction

CloudSat, a satellite launched in April 2006, will provide data concerning cloud properties not available from current weather instruments. The region that CloudSat examines overlaps with the area covered by the Storm Studies in the Arctic (STAR) field experiment in the Canadian Arctic, whose main focus is the extreme weather occurring over Baffin Island and Iqaluit. Many storm systems pass over this region, and their structure is often altered by the variable surface, ranging from ice-covered ocean to one to two kilometer high terrain. No systematic study of the evolving clouds and precipitation over this region has ever been conducted; hence CloudSat, in combination with data from other satellites in the same orbital configuration (the A-train), provides unprecedented information on these features. Our project will assess these data in order to determine whether these precipitation fields or the patterns lead to extreme weather events ,using a wide variety of field observations, including surface-based Doppler radar, ground-based remote sensing, enhanced upper air soundings, special precipitation measurements, and research aircraft flights into storms.

CloudSat's mission is to provide observations regarding cloud abundance, distribution, structure, and radiative properties. In combination with four other satellites in a polar-orbiting configuration approximately 705 km above the Earth's surface known as the A-train, CloudSat will track cloud patterns using the very first millimeter-wavelength radar. With roughly 1000X greater sensitivity than existing weather radars, the Cloud Profiling Radar (CPR) is a nadir-looking radar which measures the power backscattered by clouds as a function of distance from the radar. The CPR's vertical resolution is 500 m while its cross-track and along-track (horizontal) resolutions are 1.4 km and 2.5 km respectively (Stephens, 2002).

The main objective of STAR is to study the nature of cloud and precipitation associated with extreme events. This field-based project is concerned with the documentation, understanding and prediction of meteorological and related hazards in the Arctic. It invokes a wide variety of field observations including surface-based Doppler radar, ground-based remote sensing, enhanced upper air soundings, special precipitation measurements, and research aircraft flights into the storms.

Methods and objectives

Analysis was performed over Iqaluit, located at the southern tip of Baffin Island, since it is here that the first field experiment of the STAR project was conducted. By looking at the cloud and precipitation fields as well as the occurrence of precipitation over the region, we hope to answer the following questions: (i) what cloud structure leads to precipitation, and (ii) when light precipitation occurs, is this linked with low-level clouds or multi-layered clouds, clouds with large vertical extent, or mean-surface evaporation and/or sublimation?

To perform the analysis, we used the CloudSat data derived from profiles taken over a 100-km radius centered at Iqaluit (63° 45'N; 68°31'W) between August 31st and December 31st, 2006. That period was chosen to match the forthcoming STAR field season that took place during the fall season of 2007. All of the profiles extracted during the analysis are compared with surface observations of temperature, pressure, relative humidity and precipitation type from Environment Canada taken at Iqaluit from the YFB weather station.

CloudSat data were analyzed for the following parameters: ice effective radius, ice water content, liquid water content, pressure, temperature, specific humidity, and radar reflectivity. Also, the cloud mask assigns a value between 20 and 40 when a cloud is detected with increasing certainty; the cloud echo value is a number attributed to the detected cloud formation which can either be low-, mid-, high- or multi-level cloud. All parameters are expressed in two dimensions; altitudinal height and along-track, the latitude-longitude coordinates of the profile.

The cloud mask is a representation of the cloud coverage

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while the cloud echo specifies the cloud type (Haynes & Stephens, 2007). The radar reflectivity is the ratio of the returned signal to the incident signal of the targeted atmospheric particles and hence indirectly represents the precipitation rate. Reflectivity values greater than 0 dBZ indicate that precipitation is occurring, with light rain or snow having reflectivity of roughly 5 dBZ. The ice effective radius corresponds to the mean size of a population of ice crystals (Wysek, 2005); the ice crystals are the particles responsible for snow formation. Lastly, the ice or liquid water content represents the quantity of water present in the atmosphere in either ice or liquid phase.

The relative humidity (RH) values were calculated from the ratio of the environment vapor pressure to the saturation vapor pressure:

$$RH^i = \frac{e}{e_s^i} \times 100$$

where *e* is the vapor pressure and e_s^i is the saturation vapor pressure with respect to ice. The environment vapor pressure was extracted using the specific humidity values, where specific humidity is the mass ratio of water to air present in a given volume.

$$e = \frac{p}{1 + \varepsilon/r}$$

where *p* is the pressure and ε=0.61298. The mixing ratio r is calculated from:

$$r = \frac{q}{1 - q}$$

where *q* is the specific humidity. The pressure and temperature values were used to calculate the saturation vapor pressure using the Magnus-Teten formulation (Murray, 1967):

$$e_s^i = 6.11 \times 10^{9.5T/(265.5+T)}$$

where *T* represents temperature.

Figure 2 and Figure 3 are examples of these cloud and precipitation structure parameters, respectively, retrieved and plotted for one pass over Iqaluit. All of the parameters retrieved for each chosen pass were plotted similarly to facilitate analysis. Each event was examined from the corresponding figure and tabulated using criteria such as cloud base and top height, cloud thickness (or vertical extent), and cloud and sub-cloud temperature with respect to relative humidity. Plots of the parameters after classification following these criteria were made to determine cloud and precipitation structure.

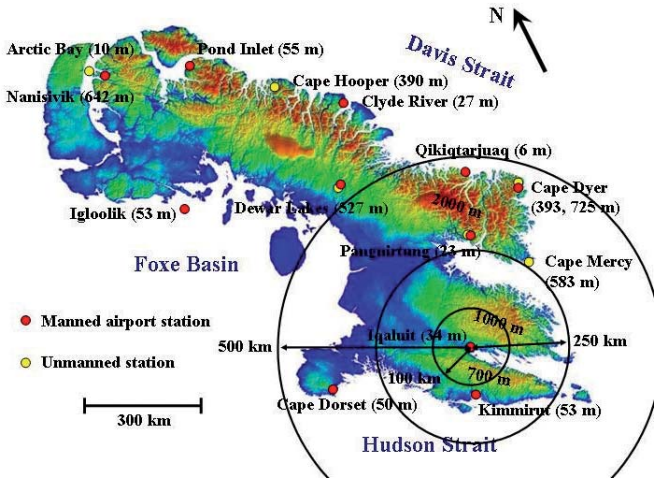

Figure 1: Baffin Island

Results and Discussion

During the 16-day return cycle of the satellite, there were 37 passes over the delimited region. Of these, 18 presented cloud features without precipitation, two showed precipitation in the formation process followed by sublimation, and four displayed clouds with precipitation at the surface. Accordingly, these events were classified in four categories: no cloud, no precipitation, sublimation and precipitation. Unfortunately, the analysis was limited by the amount of data collected since the satellite, launched just over a year ago, has not yet covered a fall season.

Using the four categories of classification, the structure of the clouds over Iqaluit was characterized to help determine a mechanism for precipitation over the Arctic. Cloud formations were mostly stratiform, composed of one to two cloud layers at low- and mid-level, indicating that cloud-base height is usually below 2 km. Also, individual clouds were mostly thin, with a thickness usually smaller than 2 km.

When examining individual precipitation events, it appears as though the resulting precipitation has the following characteristics: the cloud-base height is lower than 1 km and the cloud thickness is greater than 3.5 km, the sub-cloud environment has a temperature higher than -10°C and a relative humidity higher than 90%, and the ice water content is of 250 mg/m3 or more (or liquid equivalent). The first four characteristics are illustrated in Figure 4.

According to previous studies, a low cloud base and a large cloud vertical extent are requirements for clouds to produce precipitation in the Arctic (Stewart et al., 2004). This conclusion clearly matches our analysis. Also, the sub-cloud environment, which is determined by the relative humidity and temperature thresholds, is consistent with observations previously performed in the Arctic (Stewart & Burford, 2002). The latter statement can be understood physically from the fact that a warm and close-to-saturation environment will promote precipitation to form and fall to the ground. It is also usual to assume that relative humidity values higher than 87% will guarantee cloud formation (Wang & Rossow, 1995). The temperature factor has similarly been shown to relate to the saturation vapor pressure, and also to the growth rate of ice crystals (Rogers & Yau, 1989). Therefore, a colder atmosphere is more saturated with the same amount of water vapor, but allows fewer ice crystals to grow to snow particle size. To support this fact, it is common to look at the ice water content parameter that determines the amount of water present in the atmosphere to allow ice crystals to reach precipitation size from aggregation or accretion.

We were not able to elaborate on the differences between the events where precipitating particles reach the ground and the events where the particles undergo sublimation during their descent. Hence, the macrostructure of the clouds is likely not the underlying cause of sublimation. We did, however, observe that all precipitation events that displayed reflectivity values associated with precipitation had particle radii reaching 100 μm. We propose that this value is a threshold for particles to begin falling. Based on this information, we raise the question of whether the ice water (liquid) content remains sufficient to overcome sublimation throughout falling. Looking at the maximum value of ice water content for each event, we notice that precipitation events have an ice water content of 250 mg/m3 or more (or liquid equivalent) while sublimation events show an ice water content of 150 mg/m^3 or less (or liquid equivalent). The ice water (liquid) content might be the factor that would determine if the falling particles will reach the ground.

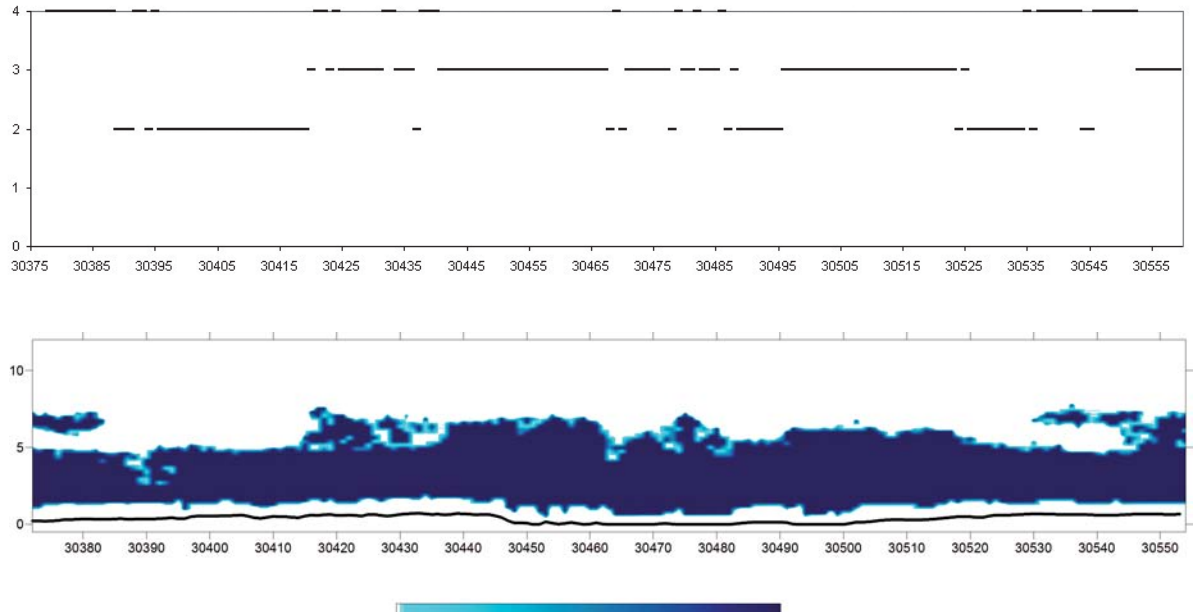

Figure 2: Example of vertical profiles of precipitation structure from CloudSat's orbit number 2183 over Iqaluit (October 25, 2006 at 03:00 LT). The horizontal axis represents the profile number of the pass which is similar to the horizontal distance. The vertical axis represents the height from the ground up to 8 km. The topography is indicated by the black horizontal line at around 0 km in height.

After analyzing the structure of clouds and the occurrence of precipitation over Iqaluit, one question remains: Why is light precipitation or sublimation so frequent in the Arctic? From our preliminary analysis and results, we suggest two reasons: (i) the ice effective radius is usually too small (threshold is ~100 μm), and (ii) the ice or liquid water content is usually too low (threshold is ~250 mg/m3. Further investigation of the cloud microstructure is required to properly explain the frequency of sublimation events in the Arctic.

Conclusion

From the 37 passes of CloudSat analyzed during this study, it is possible to draw a general outline of the cloud structures and occurrence of precipitation over Iqaluit. Our research is the first one to examine these phenomena using CloudSat data. Our preliminary results provide insight into the characteristics and processes leading to clouds and precipitation in the Southern Baffin Island and Iqaluit region, as well as to the extreme weather events that sometimes occur. Further information from the A-train formation added to the ongoing STAR project will enable a more complete study of the cloud physics in the Artic in a near future. Furthermore, the information presented in the current study has been used by the autumn 2007 STAR field experiment centered in this region to test hypotheses concerning storm evolution. During this campaign, the vertical profiles of many atmospheric properties have been measured with the help of dropsondes and other instruments onboard aircraft in the vicinity of Iqaluit, Pangnirtung, and other locations around Southern Baffin Island. The current project has therefore acquired unique in-situ information in tandem with that of CloudSat and the A-train, and the analysis of this information will contribute to our understanding of clouds and precipitation over the Northern Polar regions.

Acknowledgements

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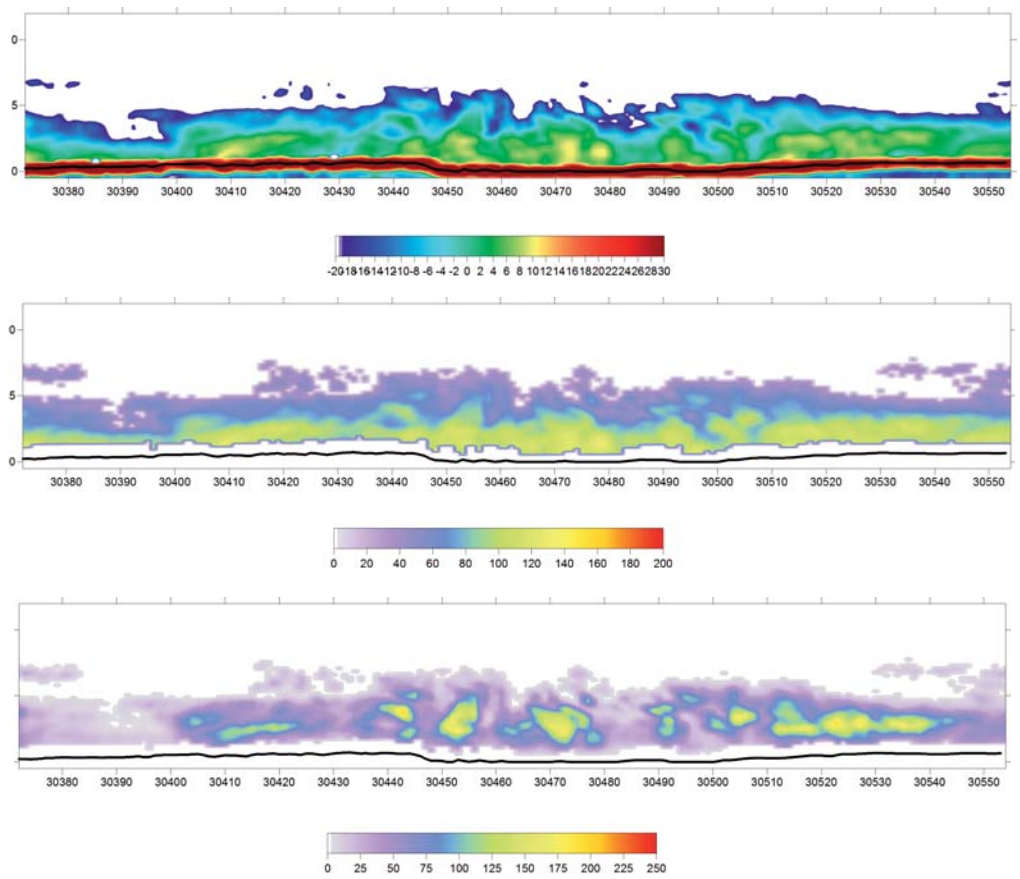

Figure 3: Example of vertical profiles of cloud environment properties generated for the CloudSat orbit number 2183 corresponding to October 25, 2006 at 03:00 LT for Iqaluit. The horizontal axis represents the profile number of the corresponding CloudSat pass which is similar to the horizontal distance along the profile. The vertical axis represents the height from the ground up to 8 km.

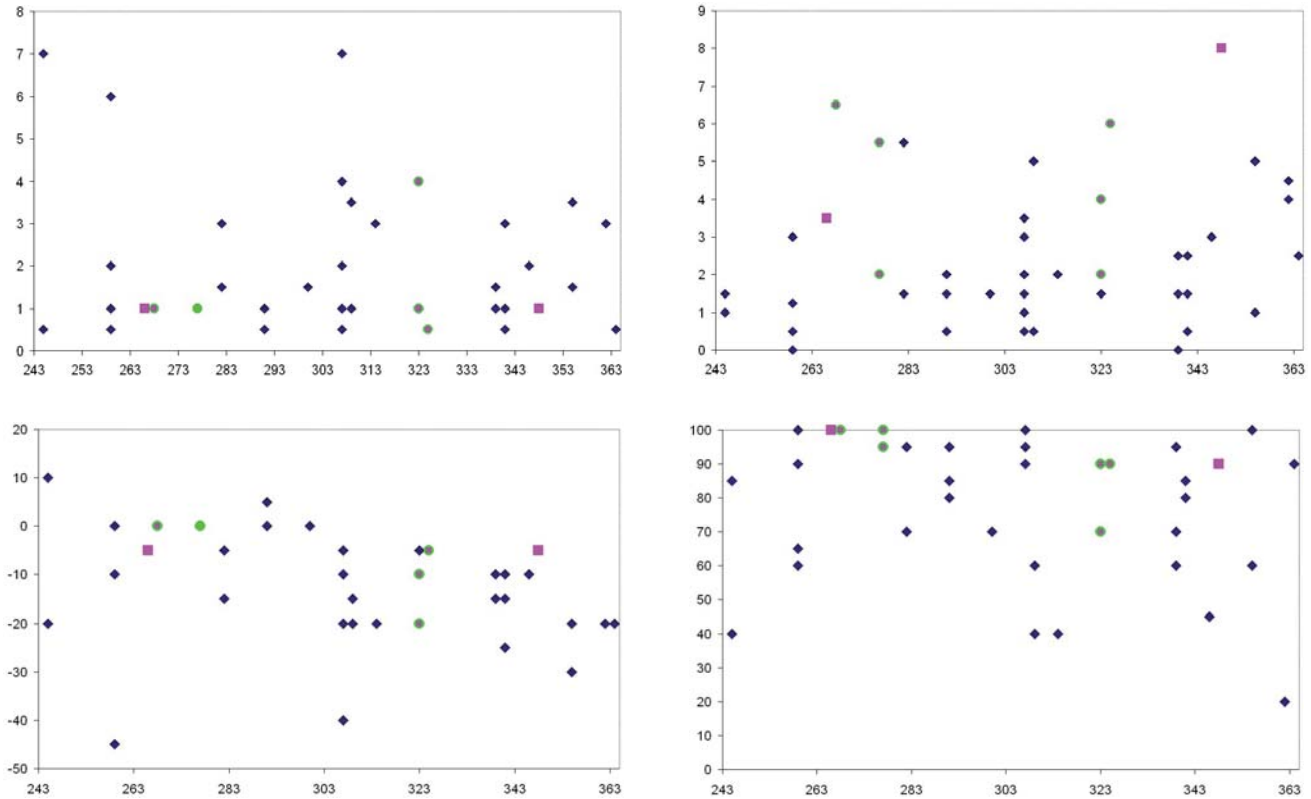

Figure 4: Cloud structure over Iqaluit during the autumnal period. The horizontal axis represents the events from August 31st (day 243) to December 31st, 2006 (day 365). The events are classified as follows: cloud formation without precipitation (blue diamonds), with sublimation (magenta squares) and with precipitation (green circles).

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Viability of renewable technologies from marine derived energy as global sources of electricity

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Abstract

Due to dwindling natural resources and continually increasing energy demands, renewable energy may be the solution to the world's future energy needs. The oceans represent a large reservoir of energy and marine derived renewable energy may in turn represent a significant source of global electricity. In particular, the marine renewables reviewed in this study are ocean thermal energy conversion and wave energy. Unlike more mainstream renewables, little research has been undertaken to determine the capabilities of these technologies. However, the authors believe that these technologies have the potential to contribute significantly to the global energy market. Global potential maps for each technology were constructed using analysis of data sets provided by the International Research Institute for Climate and Society (Columbia University) and a Geographic Information System. These show the best viability of OTEC to be concentrated around the Equator, where the vertical ocean temperature gradients are at least 20oC/km, and that the most suitable areas for wave power are concentrated between the latitudes of roughly 40 to 60o N and S, where surface wind speeds average at least 8 m/s. Given these areas, gross potential outputs were calculated to be 605 TW for OTEC and 3368 TW for wave power, 1% of which is still greater than the global electricity demand (13.2 PWh, or 1.51 TW of power, in 2000 (EIA, 2007)). These results are promising, but they do not reflect technological, sociological and economical limitations. The environmental impacts of these technologies may range from local effects on ecosystems and biodiversity to long-term global climate and oceanic implications. Compared to modern non-renewable energy sources, these technologies have no significant greenhouse gas emissions. Given the globally significant potential outputs and limited environmental impacts of OTEC and wave energy, it is clear that marine renewable energy technologies are viable as future sources of electricity.

Keywords

Renewable energy sources, ocean thermal energy conversion (OTEC), wave power, ocean thermal gradient, global electricity demand.

Introduction

As oil prices soar and we are confronted with climate change and declining natural resources, renewable energy sources are being turned to as a potential means of meeting the world's ever-increasing energy demand. Currently, the global electricity market is largely dominated by fossil fuels (EIA, 2007). In the USA, fossil fuels produce 74.4% of the total electricity, whereas renewable resources other than hydroelectric power account for only 2.1% (Figure 1). A previously overlooked reservoir, the ocean, is however emerging for harvesting vast quantities of renewable energy. Roughly 50% of the world's population lives 200 km from a coast (Table 1) (EIA, 2007) and consumes more than 50% of the world's energy and electricity. Marine derived technologies, if viable as energy sources, are ideally situated to provide for the regions that are in the greatest need.

This study focuses on two particular up-and-coming marine derived renewable energies: ocean thermal energy conversion (OTEC) and wave power. Marine-generated energy cannot directly provide for fuel or transportation. Thus, for the purposes of this study, these technologies will be considered to provide electricity rather than energy. Unlike well-studied renewable resources such as solar and wind power, relatively little is understood about marine derived renewable energy, and most of the technologies in existence are only in the prototype or experimental stage. There exist very few operational power plants globally. Due to the small scale of the prototypes and short timescales of their experimental phases, the long-term practicality and environmental impacts of these power plants are hypothetical at best.

The purpose of this study is to ascertain whether or not marine derived renewable energy could contribute significantly to the global electricity demand, thereby providing insight into the ultimate worth of investing in the development of this energy for large-scale operation.

Techniques and Technology

Two end-member types of OTEC devices exist: an open system and a closed system (Pelc and Fujita, 2002; Heydt, 1993). The closed system involves heating of a fluid with a low boiling point, usually ammonia (Pelc and Fujita, 2002) or propane (Heydt, 1993), with relatively warm water from the surface. The production of vapour at this stage turns a turbine, generating energy. Cold sea water pumped from depths is then used to cool and recondense the vapour, creating a convection cell in the working fluid container. The fluid is then reheated and passed through the turbine in a continuous cycle.

The open system OTEC device does not contain a working fluid. Instead, it pumps the warm surface water into a vacuum chamber, flash evaporating the water and propelling it through a turbine. Cold water is then used to cool and recondense the water vapour. Fresh water is released from the system as a by-product.

Many different techniques have been developed to harness energy from waves (Baddour, 2004). Among the most promising is Ocean Power Delivery LMD's Pelamis Sea Snake design (OPD, 2007). The Pelamis Sea Snake measures about 150m in length and is composed of four articulated sections. The device is oriented with its length parallel to the incoming direction of the waves. Hydraulic rams are positioned in each of the joints which resist the motion induced by the waves. The rams pump high pressure oil through hydraulic pumps, which in turn generate power. Each of the joints has a capacity of about 250 kW, adding up to a total capacity of 750 kW per Sea Snake. In order for the Pelamis to produce significant energy, the device must be used in concert with several others, forming a "wave farm" (OPD, 2007; Thorburn and Leijon, 2006). A typical wave farm for the Pelamis includes about 40 devices set in a honeycomb type of layout occupying about 1 square kilometer and has a potential of about 30 MW[1] (OPD, 2007).

Methodology

Global maps of suitable locations for wave and OTEC power stations were created using the International Research Institute for Climate and Society's IRI/LDEO database (IRI, 2007). Data analyses was carried out using this database, imported into a Geographic Information System (GIS) program, and subsequently coupled with GPW global population data (GPW, 2007) and per capita energy/electricity consumption data from the year 2000 (EIA, 2007). Whereas electricity could presumably be transported to areas further than 200 km inland, this study only evaluates coastal regions as these would be regions most

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immediately affected by marine energy.
Mean annual ocean temperatures were obtained from the LEVITUS World Ocean Atlas 1994 (IRI, 2007). Ocean areas ideally suited for the utilization of OTEC technology were determined on the basis of an ideal thermal gradient of 20oC over ocean

	Within 200km from coast	Globally
Population	2.94 billion	5.96 billion
Energy Consumption	64.7 PWh (7.29 TW)	116 PWh (13.3 TW)
Electricity Usage	7,500 TWh (0.856 TW)	13.2 PWh (1.51 TW)

Table 1: Population (GPW, 2007), energy and electricity (EIA, 2007) usage from the year 2000.

depths of 1000m (Tanner, 1995; Pelc and Fujita, 2002; Heydt, 1993; Nihous, 2005; Lennard, 1995). Wind speed at the air-sea interface was used as a proxy for wave height and period. Surface waves are created as a result of the friction between wind and the ocean surface, and Clément et al. (2002) claim that waves with periods of 7-10 s and amplitudes of ~2 m are at the threshold of producing a significant energy flux. According to Baddour (2004), these can be obtained in areas where wind speeds reach an average of 15-20 knots (7.5-10 m/s); thus, 8 m/s was chosen as a suitable minimum average for areas where wave energy could be harnessed. Wind speed data were taken from the DASILVA Atlas of Surface Marine Data 1994 (IRI, 2007). Data were provided as monthly meridional and zonal[2] wind speed means; using IRI, monthly mean scalar wind speed values were calculated and averaged to yield an annual mean.

Global populations within coastal regions directly adjacent to OTEC- and wave-power-suitable waters were calculated, as well as along coasts within a 500km radius from these regions. Energy and electricity consumption within these regions was also calculated on a per country, per coastal city and per capita basis. Direct comparison of these values was used to calculate the potential yield of marine-derived energy technologies, placing the viability of these technologies into context and giving an indication of how they fit into the global energy market.

The arbitrary simplification was made in which only one OTEC platform could operate for a square kilometer of ocean, and Lennard (1995) estimates that the low-end potential electricity output for an OTEC installation is 5 MW. A Pelamis Sea Snake wave farm approximately one square kilometer in size is anticipated to have a 30 MW capacity (OPD, 2007).

The ocean areas suitable for marine derived energy technologies were evaluated in 5 discrete areas up to 1000 km offshore, as well as within the entire area that could be potentially harnessed for these energies. The entire potential area is the most extreme end member case for coverage. The scale of the different increments was chosen considering the resolution of the datasets that were used to construct the maps, as well as the feasibility of transport of electricity over long distances.

In order to calculate the global potential power generation of OTEC and wave technologies, per-kilometer power capacities were multiplied by the areas viable for utilization of each respective energy technology. In addition, the areas required to meet the electricity demands of various coastal populations (i.e., within 200km of a coast, within selected coastal cities, etc) were calculated and compared to the areas actually viable for each energy technology. In the context of the global population and electricity demand from the year 2000, a number of coastal cities of high and low electricity demand (EIA, 2007) are presented later in the study.

Results

Suitable ocean areas for OTEC (where the thermal gradient over 1000m depth is at least 20oC) straddle the Equator in tropical regions up to ~20oN and ~30oS (Figure 2). This ideal gradient is limited to ocean waters beyond continental shelves and platforms, and does not include coral reefs or areas where there is significant upwelling of water from depth.

The most advantageous waters for installation of wave power plants (where surface winds average 8 m/s) are in mid-range latitudes, from roughly 30o-60o N and 40o-60o S (Figure 2). Anomalously windy regions extend north above Scandinavia towards Svalbard along the northern North Atlantic storm track, south along some coasts of Antarctica, and in more tropical areas such as northern South America, southern Madagascar, the Horn of Africa and the east coast of China. It is important to note that these potential areas can be reduced by negative local atmospheric and surface oceanographic parameters, such as storm frequency and seasonal ice cover. Suitable ocean areas for installation of OTEC and wave power plants are presented in Table 2, as well as the potential electrical outputs for each technology within various distance increments from shore. Table 3 shows that it would be possible to provide a significant amount of electricity from the ocean for several coastal cities with the highest and lowest electricity demands globally.

Environmental Consequences

There has been no published research focusing on the large-scale ecological consequences of offshore renewable energy development (Gill, 2005) as these technologies exist only in prototype phases. Studies of impacts from fishing, marine dredging and other activities were used to evaluate the possible environmental impacts of OTEC and wave power (Gill, 2005). However, these proposed impacts have not been demonstrated on real electrical plants and their long-term consequences remain uncertain.

OTEC plants incur minor environmental impacts compared to traditional power plants (Pelc & Fujita, 2002). The only

Distance from shore (km)	Ocean area suitable for OTEC (km^2)	Potential power assuming maximum coverage by OTEC (TW)	Ocean area suitable for wave energy (km^2)	Potential power assuming maximum coverage by Wave Power (TW)
Total area	121.1	605	112.3	3368
0-100	15.2	76	5.2	156
0-200	32.4	162	10.9	327
0-500	71.6	358	30.4	912
0-1000	103.6	518	63.1	1892

Table 2: Maximum potential electricity production by OTEC and Wave Power installations. Suitable OTEC areas are located where there exists a 20oC thermal gradient over 1000m depth; suitable wave areas located where average surface wind speeds reach 8 m/s. OTEC installations assumed to produce 5 MW/km2 of electricity; wave installations assumed to produce 30 MW/km2.

significant CO2 emission would occur during construction (Matsuno, 1998) and operation phase. Approximately four years are required to offset the CO2 emissions associated with their construction compared to non-renewable plants (Vega, 2003). The CO2 released during operation, related to the outgassing of sequestered carbon into the atmosphere (Pelc & Fujita, 2002), represents less than one percent of the amount released by a fuel oil plant (700 grams/kWh, from Vega, 2003).

Other impacts from an OTEC station may include a change in the thermal structure of the ocean, the release of toxic chemicals and the entrainment of small organisms by intake pipes (Pelc & Fujita, 2002). With time, the continual mix of cold and warm water could lead to a change in the overall temperature gradient (Avery, 1994) resulting in mortality in coral and fish communities and damaging the local ecology (DiChristina, 1995). Furthermore, the addition of deep nutrient rich water could impact the shallow low-nutrient ecosystems typical in tropical regions. The release of toxic working fluids (such as ammonia and propane) would occur only in the case of mechanical problems, breakdowns, and storms, and would damage local marine ecosystems. Entrainment of marine species is proportional to the amount of water pumped into the system and would be less significant for smaller installations. OTEC devices can provide cold water for mariculture and air-conditioning as well as desalinated fresh water, reducing energy demand for air-conditioning and minimizing emissions due to the transportation of fresh water to islands.

Wave power technologies interact with both near-shore

1 Values for potential capacity are given in power, thus TW and not TWh
2 Meridional refers to a vector parallel to latitude; zonal refers to a vector parallel to longitude

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Cities of highest electricity usage	Total electricity consumed by city (TWh)	OTEC			WAVE			OTEC and WAVE	
		Area suitable for OTEC within 0-100 km from coast (km^2)	Area suitable for OTEC within 0-200 km from coast (km^2)	Area required to provide total electricity (km^2)	Area suitable for WAVE within 0-100 km from coast (km^2)	Area suitable for WAVE within 0-200 km from coast (km^2)	Area required to provide total electricity (km^2)	Percentage of city's electricity demand provided by OTEC and Wave within 0-100km from coast (TWh)	Percentage of city's electricity demand provided by OTEC and Wave within 0-200km from coast (TWh)
Tokyo	184				5000	43900	698	**1300**	**11600**
Paris	69.5					580	264		**150**
London	65.4					5200	248		**1400**
Taipei	48.9	1300	20200	1110	17200	89100	186	**4600**	**24400**
Nagoya	37.4				1800	23700	142	**480**	**6200**
Sydney	32.6					10300	124		**2700**
Hong Kong	31.8				6400	25900	121	**1700**	**6800**
Yokohama	23.3				7200	46500	88.4	**1900**	**12300**
Rio de Janeiro	19.9		2600	452					**110**
Manchester	16.3				57	6400	62.0	15	**1700**
Lome	0.0592	6500	41400	1.35				**290**	**1800**
Monrovia	0.0461	5100	39100	1.05				**220**	**1700**
Ambon	0.0421	13700	71300	0.959				**600**	**3100**
Port-au-Prince	0.0324	7200	52700	0.737		4400	0.123	**320**	**3500**
Freetown	0.0261		18200	0.595					**800**
Belmopan	0.00306		16500	0.0697					**730**
Malabo	0.00143	7900	35800	0.0327				**350**	**1600**

Table 2: Maximum potential electricity production by OTEC and Wave Power installations. Suitable OTEC areas are located where there exists a 20oC thermal gradient over 1000m depth; suitable wave areas located where average surface wind speeds reach 8 m/s. OTEC installations assumed to produce 5 MW/km2 of electricity; wave installations assumed to produce 30 MW/km2.

activities and marine species. By extracting energy from waves, they weaken the force of waves, which while helpful in reducing erosion of a sensitive coast, could alternatively reduce food supply for benthic populations and harm species that rely on suspension to carry their larvae (Pelc & Fujita, 2002).

Large wave farms near the shore could negatively interact with local fisheries and recreational areas. Underwater noise would increase and affect marine mammal and fish species which communicate or navigate sonically. Grease or fluids in contact with sea water could pollute the surrounding marine ecosystem.

In order to minimize the environmental impacts of OTEC and wave power plants, several measures can be taken such as avoiding important fishing, recreational and sensitive areas, using biodegradable and non-toxic hydraulic fluids where possible, minimizing installation size, locating wave farms at least 2 km offshore, spacing their individual generators 150m apart and avoiding high discharges of cold water in shallow warm water.

Discussion

The calculated potential capacities yield a maximum end member (every square kilometer suitable for the techniques) of 3368 TW for wave power and 605 TW for OTEC. The low end member scenario, building installations solely within 100 km of the shore, yields outputs (156 TW for wave; 76 TW for OTEC) several orders of magnitude larger than the global electricity demand (1.51 TW in 2000) (EIA, 2007). Even a small fraction of the potential of these techniques could still amount to a very significant source of power; for example, 1% of the low end member marine potential, 2.32 TW, is still greater than the global electricity demand.

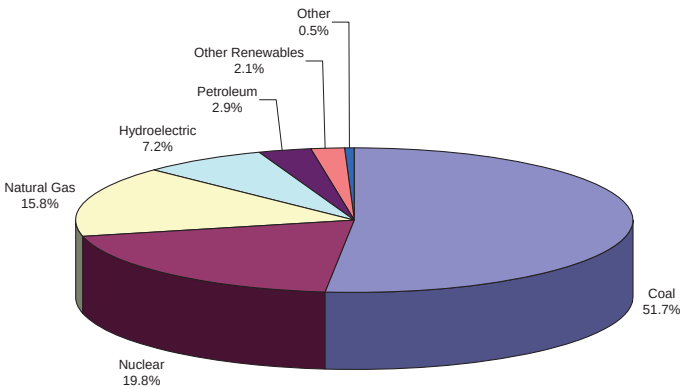

Figure 1: USA Electricity Generation in 2000 (EIA, 2007)

Table 3 shows that the electricity needs of many coastal cities could be met by installations placed within 100 km of the shore, and a larger number could be provided for by plants installed within 200 km. Meeting the energy needs for smaller cities is completely feasible within a few km2, but an unrealistically large area of the ocean would be necessary to provide for the larger cities. It is encouraging, however, that even a fraction of the area covered by these stations may still contribute significantly to these electricity needs.

With insignificant direct environmental impacts and positive by-products such as fresh water, food through mariculture and air conditioning, OTEC power is a promising energy alternative. However, it is important to keep in mind that this technology has the potential to modify the ocean thermal structure with time and could release toxic fluids into the ocean. It is difficult to model the effect of a large scale OTEC emplacement on the marine thermal gradient. Nonetheless, any variations in the gradient would affect not only water temperatures, but also other factors such as marine currents, nutrient distribution, and the interactions between the ocean and the atmosphere, resulting in changes in global climate and ecosystems both in the ocean and on land. The negative impacts of wave power appear to be inconsequential.

Compared to emissions from transportation and conventional fossil fuel energy, are the environmental impacts of marine renewables less harmful? Would these impacts with time bring similar problems to those incurred from new technologies such as wind farms affecting birds and local climate? Indirect ecological effects are especially important because they are the ones usually forgotten. Placement of a marine renewable energy plant could have an effect on biodiversity, food availability, and species competition, predation and reproduction. These could have wider effects than expected on the marine fauna and change the present ecology of the ocean. There are no certainties about the long term impacts of these technologies on the environment.

All the possible sources of error need to be considered before the potential energy outputs can be fully taken into account. The initial estimates for the energy output by each station may be incorrect, thus changing their overall capacities. The original data used to construct the maps in this report may also provide error and change the map results. While accurate to a certain extent, using wind as a proxy for wave power is imprecise. Another source of imprecision is the data collected from INGRID, which is provided at a coarser resolution than the maps constructed on GIS. These and other variables can affect the calculated potentials of these technologies and thus the values obtained in this paper could be either over- or underestimated. Nevertheless, the exact quantities stated in this paper

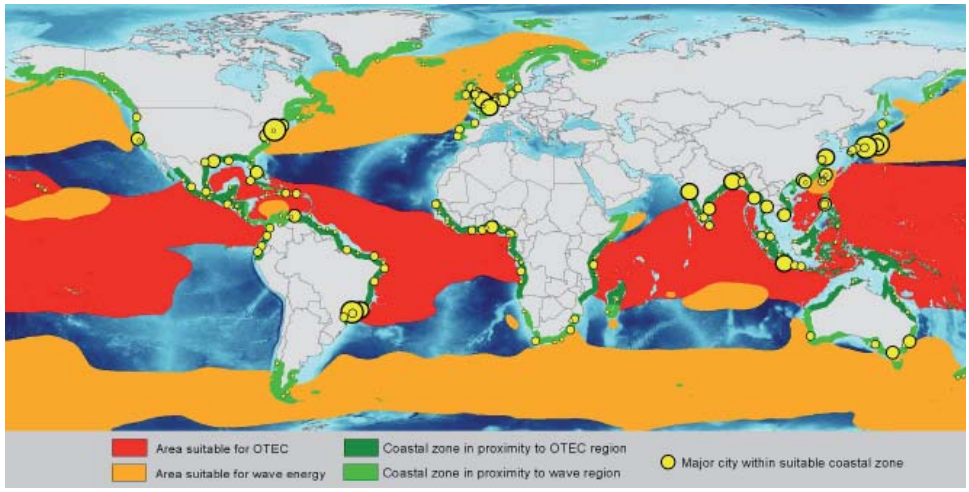

Figure 2: Areas suitable for OTEC (where marine thermal gradients are 20oC/km and greater) and wave-derived energy (where average wind speeds reach a minimum of 8 m/s), and major cities within coastal regions situated in proximity to the areas suitable for these technologies. Sizes of yellow circles are proportional to city populations.

are of less importance than the demonstration that these technologies have the potential to produce a significant amount of electricity.

Conclusion

The calculated values indicate that marine energy sources have the potential to become a significant source of power (2.32 TW at 1% exploitation of lowest end member) for the future global market. Furthermore, the techniques would be highly advantageous for certain isolated areas, such as island nations, and could be used to power less accessible regions that do not have sufficient natural resources to cover their own energy needs. However, the potentials of each technique differ to an extent. Both OTEC and wave power are feasible as global sources of energy, but OTEC may have limited effectiveness due to its potential effects on the thermal gradient of the oceans. Hence, it may be most useful only in areas near the Equator where its by-products can be fully exploited without affecting a large amount of water.

Wave power is thus the technique with the most global potential discussed in this paper. Its environmental impacts are, as far as it can be determined, insignificant. It is relatively simple to design and can be installed anywhere with the required wave heights and periods. Through a combination of both OTEC and wave power, marine derived energy could potentially provide most of the electricity needs of the world. Thus, even if these technologies are not implemented to work at their full capacities, they could still provide a significant amount of renewable electricity for most of the globe.

Marine energies could have the effect of reducing the utilization of fossil fuel and non-renewable energy as well as reducing greenhouse emissions. Future investment in marine renewable technology must be undertaken in order to answer several questions that cannot be addressed without actual full-sized installations, such as confirmed outputs of these plants and their long term impacts on their environments and ecosystems. By establishing that marine derived renewable energy technologies have the potential to produce measures of electricity comparable to the current electricity demand, this study validates such future investment and provides an optimistic perspective on the future global energy market.

Acknowledgements

The authors would like to gratefully acknowledge Professors Bernhard Lehner and Bruno Tremblay for their limitless encouragement and support of this project. We would especially like to thank Professor Lehner for his GIS assistance and expertise.

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Fabrication of optical fiber probes for scanning near-field optical microscopy

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Abstract
Many areas of cell biology have remained unexplored due to the limitations of conventional optical microscopy for image structures smaller than the diffraction limit of light. Scanning near-field optical microscopy (SNOM) is an emerging technique which allows sub-diffraction limit optical resolution and hence access to nanoscale structures such as those in biological cells. Since the success of a scanning probe microscopy techniques depend on the tip, we present a method for the fabrication of SNOM aperture probes and antenna probes capable of high resolution imaging of delicate liquid based samples such as neurons. The procedure includes thinning and tapering optical fibers by chemical etching, followed by the deposition of a thin aluminium film, and micromaching using focused ion beam (FIB). Tip geometry, antenna resonances, excitation conditions, and field localizations have been examined. The probes have a resonant frequency of 18-28 kHz, a Q of 200-400 in air and up to 100 in water, and a spring constant of on the orders of 140 N/m. Successful optical and topographical imaging up to a resolution of 250 nm was achieved with the probes. Together with adjustments in equipment and instrumentation, the probe's optical and mechanical properties allow for a low imaging force and high tip sensitivity which are necessary conditions for the imaging of biological cells.

Introduction

The invention of the optical microscope at the end of the 16th century gave way to a whole new perspective of the world. Used in a diverse range studies, from biology to material sciences, conventional optical microscopy remains the most widespread imaging technique due to its simplicity, versatility, and non-invasive nature. The main disadvantage of the technique, however, remains its limited spatial resolution as predicted by Maxwell's equations. For standard microscopes setups, the equation yields a maximal optical resolution of ~ l /2 or around 250 nm for visible light (Paelser, 1996). With advances in science and technology, there has been a growing need to image at the nanoscale. In particular, there has been an interest in studying the features and mechanisms of biological cells which have thus far been unexplored.

In the last few decades, the development of high resolution microscopy techniques has sought to address this issue. Notably, three methods developed have been the most successful at surpassing the diffraction limit. In the 1970's, E. Ash and G. Nicholls were the first to surpass the limit by using the properties of evanescent waves for scanning in the near-field of a sample; the technique was called scanning near-field optical microscopy (SNOM), an idea first proposed by Edward Hutchinson Synge in the 1930's (Paelser, 1996). More recently, Stefan Hell developed stimulated emission depletion microscopy (STED), capable of attaining resolutions of tens of nanometers using the saturation of fluorescence inhibition by stimulated emission (Hell, 2003). Finally, Eric Betzig and his colleagues created a technique called photoactivated localization microscopy (PALM), where fluorescent molecules are activated one-at-a-time and an image is assembled from their positions (Betzig, 2006).

While all three microscopy techniques can be used to examine biological cells, SNOM is the only one that allows simultaneous mechanical measurements. A SNOM setup involves an atomic force microscope (AFM) with a modified cantilever probe that allows both optical and topographical imaging. For the latter, the probe scans the surface and deflects according to the changes in the interaction between the tip and the sample. Therefore, applied to imaging cells, it can optically resolve the locations of tagged proteins while determining how they influence the mechanical properties of the cell.

As with any scanning probe microscopy technique, the probe or the tip is the critical part of the instrument. Among the most successful SNOM probes are optical fibers tapered by chemical etching which employ sub-wavelength apertures (Paelser, 1996). These probes must be able to efficiently deliver light to the tip and properly image a sample at a high resolution. Additionally, recent research has shown that optical antennae, which are nanostructures resonant at optical frequencies, produce very well confined high electric fields. The combination of the sub-wavelength aperture with the optical antennae allows for a spatial resolution down to tens of nanometers (Taminiau, 2007).

Imaging biological cells presents several challenges and therefore any microscopy techniques needs to consider them. The soft and delicate cells necessitate a low imaging force so as not to destroy the samples. Also, since the sample is maintained in a liquid environment, special steps must be taken to ensure sufficient force sensitivity in the AFM. Hence, we are interested in characterizing and systematizing the fabrication process of SNOM probes that enhance the optical resolution and permit the imaging of biological samples.

SNOM Principle

Transmission mode scanning near-field optical microscopy (SNOM) relies on the efficiency of the probe to deliver light to the tip (Betzig, 1991), a schematic illustration of the SNOM principle is shown (Figure 1). In optical fibers, the probe is separated into three regions (Hecht, 2000). The first consists of a regular optical fiber waveguide where the difference in

Medium	Depth (•m)	Amp (mV)	Drive Amp (mV)	Drive Amp/Amp
air	0	107.927	81.35	0.8
water	230	101.318	100	1.0
water	280	98.694	220	2.2
water	330	94.727	350	3.7
water	380	95.337	700	7.3
water	430	94.055	900	9.6

Table 1: Drive efficiency of the SNOM probe as indicated by the ratio between the drive amplitude and the amplitude of the cantilever tunes

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index of refraction between the core and the cladding allow for the total internal reflection of light. When the fiber tapers off and reaches the diameter of the glass core, a separate waveguide appears. Light reflects off the metal coating, which forms a conical metal-coated dielectric waveguide. As we approach the tip, only a single mode among the multiple modes remains. In this final region, light no longer propagates and the field is evanescent, escaping through the sub-wavelength aperture and exponentially decaying from the tip (Betzig, 1992). By placing the sample surface in the near-field of the SNOM probe ($< l/50$) (Betzig et al. , 1991), it is possible to surpass the diffraction limit. The spatial resolution depends on the size of the sub-wavelength aperture, which only illuminates the area directly below. Aperture probes are capable of imaging fluorescence, as well as metal structures and dielectrics.

A metal nanostructure resonant at optical frequencies acts as a quarter-wave antenna when placed next to the aperture of a SNOM probe (Taminiau, 2007). The local electric field at the edge of the aperture drives the antenna by providing an alternating current in the direction of the antenna (Taminiau, 2007). The electrons then move back and forth within the structure and create a standing wave of a quarter of the wavelength (node-antinode). In the far-field, the metallic protrusion acts like a dipole antenna but in the near-field, localizing the electric field distribution . Since the aluminium coating has a short penetration depth, optically, it behaves similarly like a perfect electrical conductor (Taminiau, 2007). In order for the electric field at the tip to have a maximum strength, the length of the antenna must be a multiple of $< \lambda/4$, with the thickness considered negligible compared the length. Since the antenna will be used only in the near-field, it is sufficient to note that the highly localized field formed at the antenna tip allows for the excitation of molecules in fluorescence imaging. The antenna hence becomes the main component in the imaging process (Figure 2). The resolution is not determined by the aperture size, but by the geometry and field localization of the antenna. In addition, the polarization of light which determines the field distribution at the edge of the aperture must be accounted for. From Figure 3, it is evident that the signal has maximal strength when the polarization of the light is aligned with the aperture and antenna (Figure 3b) and minimal strength when the polarization is completely misaligned (Figure 3c).

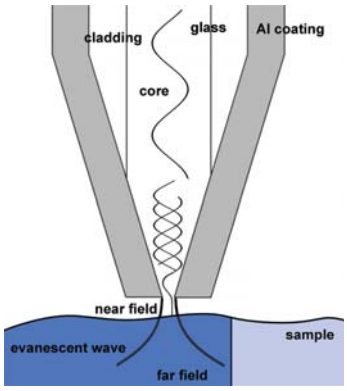


Figure 1: SNOM aperture probe. The transmission of light from the fiber to the surface of the sample and the three regions of the fiber tip.

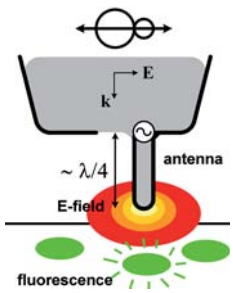


Figure 2: Fluorescence imaging with an antenna probe. The localized electric field at the tip of the antenna excites the molecules which fluoresce.

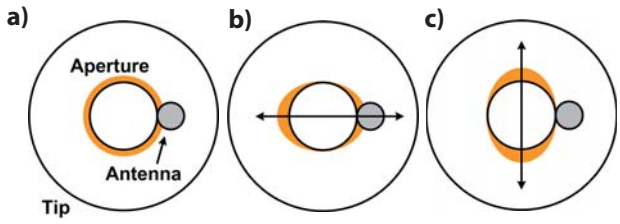


Figure 3: Polarization of light with respect to the aperture-antenna alignment (as indicated by the arrow), where a) no polarization, b) polarization aligned, c) polarization misaligned. The signal is greatest in b) where the polarization is aligned, and lowest in c) where it is completely misaligned, whereas in a) the distribution is even.

Experimental Setup

The setup of the transmission mode SNOM with a bent fiber probe is shown in Figure 4. It consists of a modified commercial atomic force microscope (Asylum Research) with an inverted optical microscope (Olympus), and a laser setup for optical imaging. An AFM uses a microcantilever to scan the surface of a sample. The piezo oscillates the fiber probe

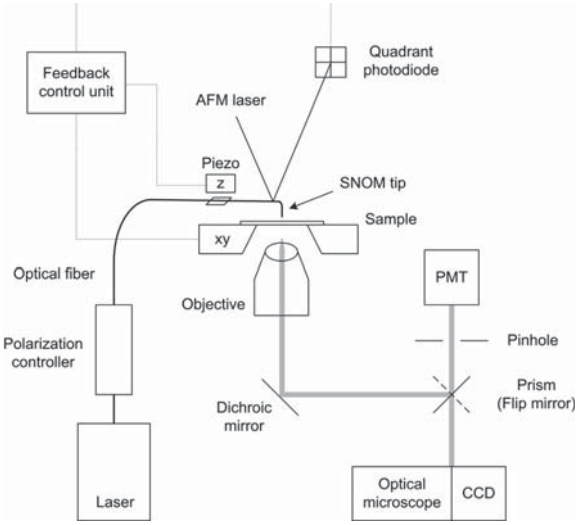
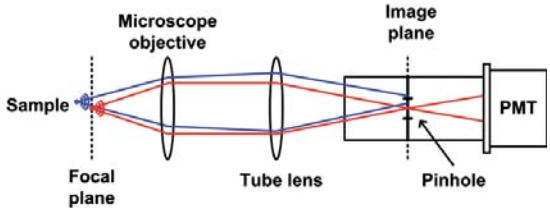


Figure 4 : Transmission mode SNOM setup schematic



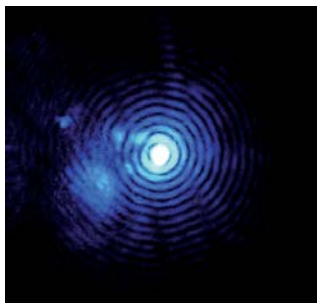


Figure 5 : a) Confocal pinhole setup with b) the diffraction pattern produced by the tip observed after the pinhole

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in the z-direction (up-down) at a specified frequency, while the scanner moves the sample in the xy-directions. The cantilever deflects according to the various forces between the tip and the sample, and the deflection is measured by a laser reflecting off of the cantilever into a photodiode. There are several imaging modes that can be used in SNOM. In tapping mode AFM, the drive frequency is constant and changes in the oscillation amplitude are measured. Since it alternates between having the tip in contact with the surface for high resolution and lifted off to avoid dragging, this mode overcomes the difficulties encountered by other AFM scanning methods, including friction, adhesion, and electrostatic forces. Another relevant mode is the non-contact mode where changes in the resonant frequency of the cantilever as it interacts with a sample are used to maintain a small tip-sample separation (Morita et al, 2002). In both cases, measurements from the laser beam deflection sensor are used to plot the topography of the sample.

SNOM employs a modified cantilever probe. While the surface is scanned, the sample is locally illuminated by the fiber tip which is coupled to the laser (488 nm Argon-ion). The AFM distance is regulated by laser deflection for topographical imaging and for maintaining the sample in the near-field. Optical features are captured by a simple confocal microscope setup with a pinhole and a photomultiplier tube (PMT). The sample can also be observed through the eyepiece or captured by the charged-coupled device (CCD) camera.

As illustrated in the confocal pinhole setup (Figure 5a), the light from the sample is first collimated by the microscope objective and then focused into the pinhole by the tube lens. The confocal pinhole allows the photomultiplier tube (PMT) to capture a thin section of the sample at the focal plane, rejecting out-of-focus features as well as background noise. This facilitates the focalization of the image and ensuring that the sample is in the near-field of the tip. The tip is brought into the focal plane and centered into the pinhole by observing the image of the laser through the aperture. Figure 5b is a photograph taken at the position of the PMT showing the diffraction pattern produced by the tip.

Probe Fabrication

Chemical Etching

The fabrication of SNOM probes requires the tapering of optical fibers to form a tip. Three procedures were employed: tube etching, selective etching, and meniscus etching. The methods can also be employed individually or successively, in various combinations, to achieve the optimal taper angle and tip geometry (Taminiau, 2007; Mononobe, 1996).

First, in tube etching, the fiber (FS-SN-3224a/b, 125 µm), with its arcylate jacket left on, is dipped into 48-50% aqueous hydrofluoric acid (HF) solution. The acid diffuses through the jacket towards the end of the fiber, etching inward and upward producing smooth tapers with sharp tips. Due to the protection from the jacket, temperature fluctuations and vibrations have little influence on the fiber, making the process very practical. However, the geometry of the tip is not consistent and often non-circular.

The second etching technique relies on the selective etching of the core and cladding of highly germanium doped fibers (PSC and FDC). The tip forms at the end of the fiber, which must be cleaved prior to immersion in a buffered HF acid solution (48-50% HF and 40% NH4F). The geometry of the tip is determined by the NH4F:HF:H20 volume ratio and the cladding to core etch rate ratio (Mononobe, 1996), allowing for the production of a consistently circular tip (Figure 6).

The third technique, the meniscus etching procedure developed and patented by Turner, involves dipping the fiber

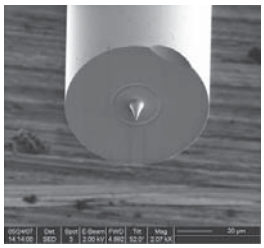

Figure 6 : Selectively etched tip (SEM)

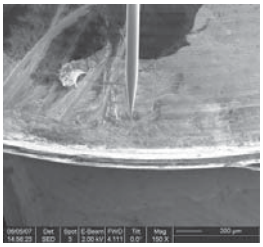

Figure 7 : Meniscus etched tip (SEM)

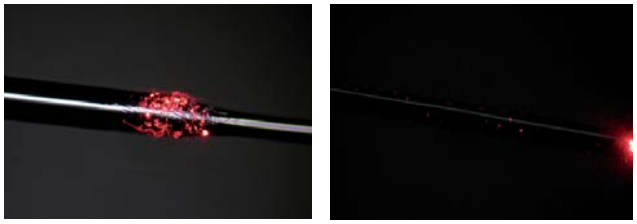

Figure 9 : Photographs of a tip coupled to a laser as viewed from under an optical microscope. We observe a)imperfections in the coating due to uneven etching at the meniscus of a thinned fiber and b) small pinholes along the taper of the fiber from dust particles in the solutions and a large pinhole at the tip.

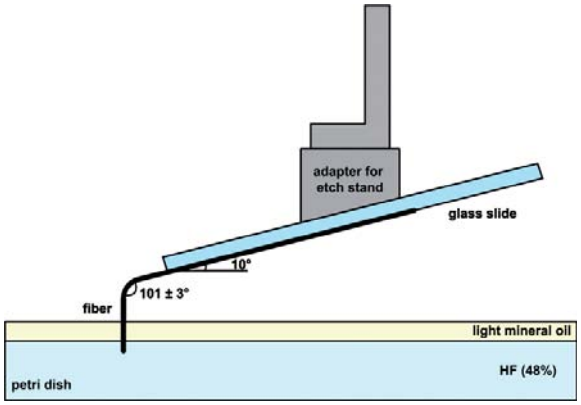

Figure 8 : Setup for etching bent fibers.

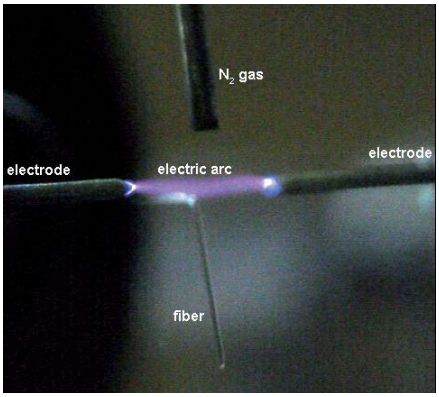

Figure 10 : Electric arc bending of optical fiber

into a solution of HF acid covered with an organic overlayer. Tapering occurs at the meniscus between the HF and the organic layer and it is usually ~ 300µm long (Figure 7). The acid etches completely through the fiber, eliminating the amount inside the solution; hence, the process is self-terminating (Turner, 1984). The angle of the taper is dependent on the nature of the overlayer and the stability of the solution. For instance, mechanical vibrations can lead to the production of rough fiber at the meniscus. Placing the etch stand on a cement block supported by rubber and wood blocks helps reduce these effects and produces smoother tips.

Since it is difficult to control the length of the fiber past the bend while bending it, an adapter for the etch stand is fabricated to taper already bent fibers. In Figure 8, we see the glass slide attached to the adapter at an angle of 10°. The fibers are placed perpendicularly on the slide and dipped into the solution. The length of the fiber that will remain past the bend is determined by the height of the slide and its distance to the bend. This allows us to achieve smaller and more accurate dimensions than obtained by bending etched fibers. This technique is not useful for selective etching, since the tip is formed at the cleaved end of the fiber, regardless of the amount submerged in the solution.

In addition to the formation of the taper, meniscus etching is also used to reduce the diameter of fibers. The entire length of fiber which will be used is submerged in the solution for a predetermined duration (based on the desired remaining diameter). Another etch, either meniscus or selective, is necessary to taper the tip.

Deposition of Aluminium Thin Film

Once the optical fiber is tapered, a metal coating is applied to create a conical metal coated waveguide, confining the light in the taper to the eventual aperture. A thin film of aluminium is deposited onto the SNOM probe in high vacuum (10-7 mbar) using a thermal evaporator 12. Aluminium has a short optical penetration depth, making it the ideal metal to coat the tip (Taminiau, 2007). For an even metal coat, the fibers are mounted onto a carousel attached to a motor rotating at 45° with respect to the direction of evaporation. The thickness of the aluminium coating varies between 150 nm and 500 nm, with a rate of deposition of 3-8 nm/s. While early techniques in SNOM aperture formation consisted of shadowing the tip during evaporation (Betzig, 1991), those techniques produce irregularly shaped apertures with large Al grains protruding past the tip. Instead, we allow the tips to be completely covered and create the aperture by FIB milling. It is important to check for pinholes by coupling the fiber to a laser and inspecting it under a microscope to ensure that the light will be properly transmitted to the tip. In Figure 9, various types of defects in the coating after meniscus etching are illustrated. The imperfections along the

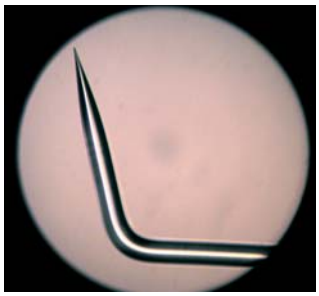

Figure 11 : Bent fiber, 105.6° (10x magnification)

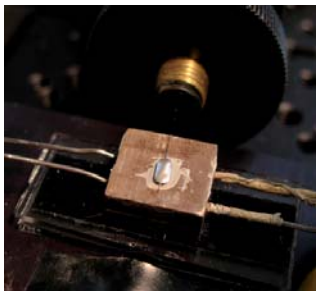

Figure 12 : Silver epoxy curing on heater plate

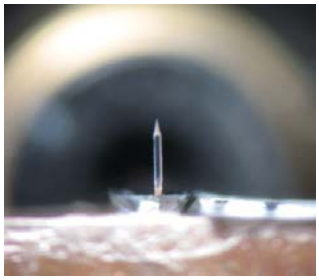

Figure 13 : Bent etched fiber perpendicular to chip

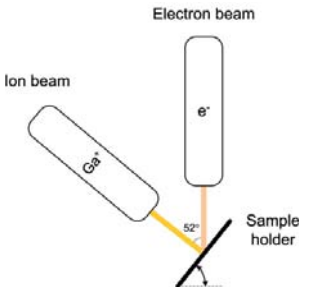

Figure 14 : Diagram of the dual beam SEM and FIB

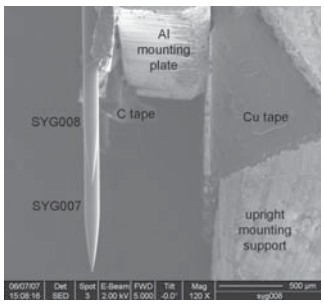

Figure 15 : FIB setup for upright fibers (SEM)

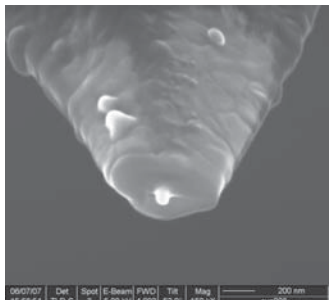

Figure 16 : Antenna on tube etched tip (SEM)

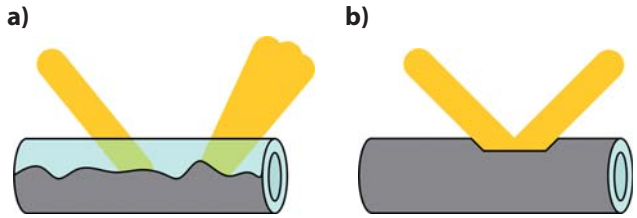

Figure 17 : Laser for AFM optical beam deflection a) passing through the fiber and bouncing off the bottom and b) bouncing off the reflective mirror surface

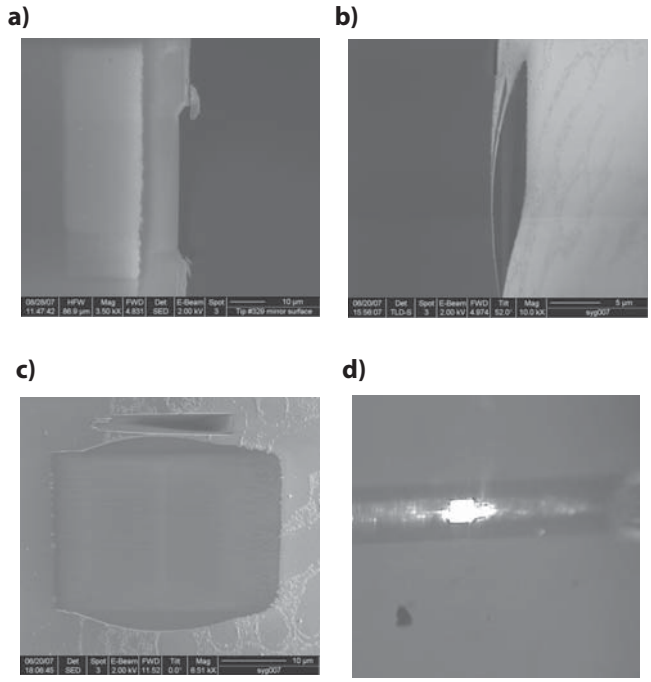

Figure 18 : Reflective surface a) lateral view b) side view c) top view d) top view with laser beam. a) – c) are taken with the SEM and d) is taken with a camera in the AFM.

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length of the thinned fiber in Figure 9a)-b) are located far from the taper and the tip, and will therefore not disturb the transmission efficiency. However, the large pinhole at the tip of the fiber in Figure 9b is critical and renders the tip useless for SNOM. The small defects in the coating can be reduced by rigorously cleaning the fiber with filtered water. To eliminate large pinholes, all contact with the tip must be avoided.

Fiber Bending

For use in the AFM, the fibers are bent at an angle of 101° ± 3°, such that when mounted on the microscope, the tip lies perpendicularly to the plane of the samples. The two techniques most commonly used to bend fibers consist of CO2 laser heating and electric arc melting (Taylor, 1997). We employ a high frequency electric arc which heats and softens the fiber, which is then bent by the nitrogen (N2) gas flow and the force of gravity pulling down the tip (Figure 10). The exact angle of 101° is progressively obtained using successive trials with a measurement of the angle made after each trial. The bent fiber is photographed under an optical microscope and the angle is determined using image processing and analysis software. An intermediate step is shown in figure 11, where the desired angle is reached in less intermediate steps and with greater reproducibility by tilting the stage. This also results in a bend that is more even and consistently round because there is no need to reposition the fiber after successive bends.

Fiber Glueing

The bent fiber is glued onto a V-grooved cantilever chip (Figure 12) to be used in the AFM tip holder. We adjusted the position of the fiber so it to lies flat in the grove, extending 1mm past the edge and remains perpendicular to the plane of the chip. The right angle formed by the fiber and the cantilever chip is verified and corrected using photographs of the chip face on, such as in Figure 13. Silver epoxy is then delicately applied to the surface of the chip and cured until the glue hardens using a heater plate consisting of a current running through resistors.

Focused Ion Beam Milling

Micromachining the fiber tips using focused ion beam (FIB) (FIE DB235) milling allows for great versatility in the fabrication of structures. Figure 14 is a diagram of the system which includes a dual beam scanning electron microscope (SEM) and FIB separated by a 52° angle allowing for simultaneous SEM imaging and micromachining using gallium ions (Ga+). The sample holder tilts, allowing for cuts to be made with the ion beam.

Aperture and antenna formation

The formation of the aperture and the shaping of the tip require successive cuts made with the ion beam. The aperture is obtained by slicing off the tip of the fiber a couple hundreds of nanometers at a time until we observe a difference in contrast, usually indicative of the glass surface. The size of the aperture depends on the amount of the tip removed. For the fabrication of antenna probes, the tip is made by cutting the fiber on one side until we find the glass aperture. Then we remove the material on the other side to create a slice of metal on the edge of the aperture with a width corresponding to the intended diameter of our antenna. The probe is then rotated and mounted on the upright sample holder. The ridge is cut from both sides to carve the shape of the antenna (Taminiau et al., 2007). The setup created for this purpose is shown in Figure 15. The fibers are taped to a thin aluminium mounting plate with carbon tape, making it easy to manipulate them without modifying the orientation or disturbing the tip. This process is iterated until the desired geometry is obtained. An example of an antenna structure fabricated using the FIB is shown in Figure 16. Typical slice sizes are approximately 1 µm (L) x 0.3 µm (H) x 0.2 µm (W) with an ion-beam current of 10 pA for

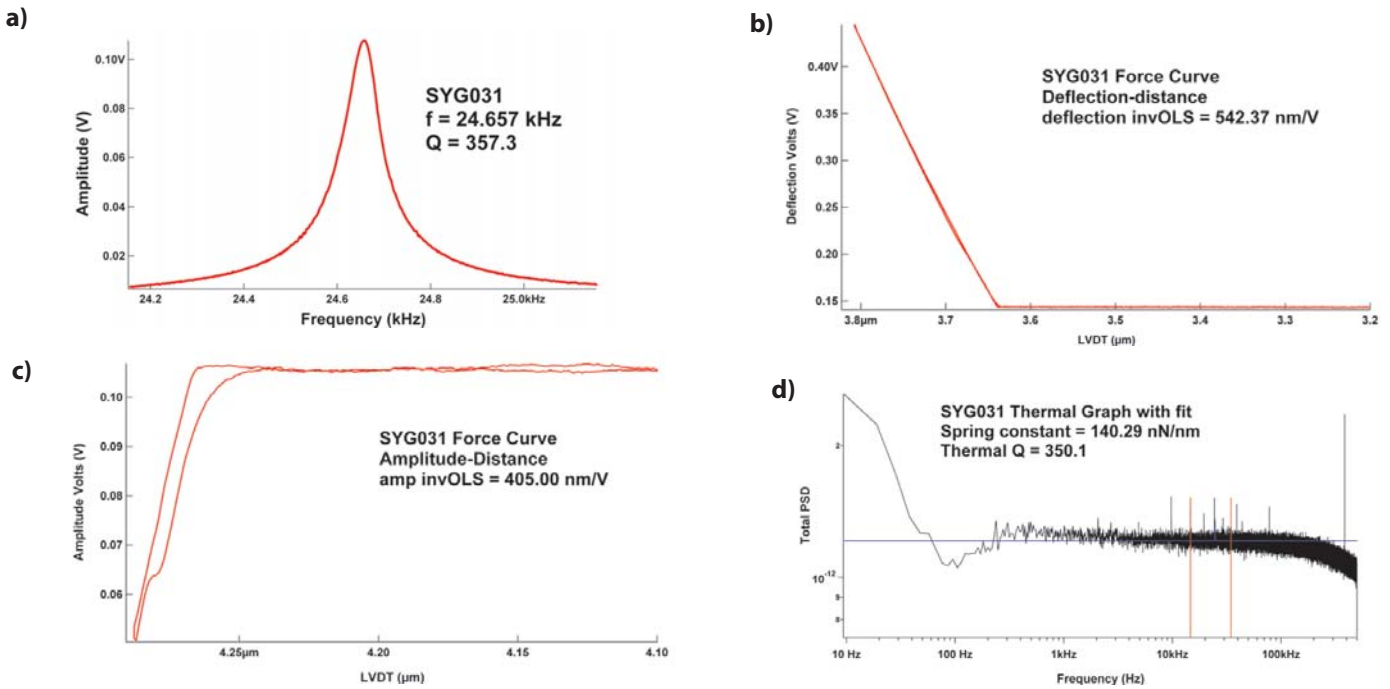

Figure 19: Graphs used in the measurement of the spring constant of tip (meniscus - selective etch) using the AFM: a)The cantilever tune, b) deflection-distance force curve, c) amplitude-distance force curve and d) thermal graph.

up to one minute.

Reflective surface

The initial method of reflecting the AFM laser beam for optical deflection consisted of bouncing the light off the bottom of a metal coated fiber (Figure 17a). However, any aberrations in the fiber and the coating can effect the shape of the beam returned to the detector, reducing the quality and the sensitivity of the beam. To solve the problem, a rectangle the size of the laser beam, 30µm x 60 µm, is cut from the top of the fiber using FIB milling. Once the fiber is coated with aluminium, the surface forms a flat mirror that returns the laser beam without changing its shape, as illustrated by Figure 17b.

Each cut is approximately 30 µm x 10 µm x 2 µm with a beam current of 5000 pA for an average of 10 minutes. Figure 18 is a collection of images of the reflective surface from various angles. As illustrated in Figure 18c, the surface is indeed flat and the laser can bounce off the mirror easily (Figure 18d).

Results and Discussion

Two sets of fiber probes were produced to test mechanical properties. The optical properties were studied using aperture probes provided by J. Ledue. The fibers in both sets were etched after being bent, using the tilted glass slide and etch stand adapter technique. In the first set, the full diameter of 125 µm of the fiber was preserved and the taper was formed by meniscus etch. We then explored the trade-off between size and our ability to fabricate them. For the second set, the fibers were first thinned to 60 µm by meniscus etch before tapering the tip. Due to the smaller dimensions, the thinner fibers were expected to have a better spring constant. There were no significant obstacles encountered due to size during the fabrication process.

Spring Constant

The spring constant of a tip can be measured using the thermal method which is included in the AFM operating software (Figure 19). This procedure calculates the constant using optical lever sensitivity and force-distance calibrations. Specifically, the cantilever tune gives the resonance frequency and the Q factor of the tip. The inverse optical lever sensitivity (invOLS) is obtained from the slopes of the deflection-distance and the amplitude-distance force curves. Finally, the spring constant is calculated from a thermal graph by comparing the peak to the background through Fourier analysis. The measured value of 140 N/nm, with an error of 10%, is comparable to theoretical spring constant of 137 N/m given the dimensions of the fiber. The spring constants for the limited number of fibers tested were of this order of magnitude.

Resonance Characteristics

The standard manufacturer's clip for the AFM tip holder consists of a rigid piece of stainless steel which cannot not be used for the SNOM probes. Compared to traditional AFM cantilevers, the SNOM tips require an opening for the tail of the fiber carrying light to the tip, which the original clip did not have, hence, several alternatives were tested. First, the probes were held in place by a thin sheet of copper bent into shape and anchored down with one screw. Although it was easily mounted and left room for the tail, this first clip did not provide sufficient pressure on the probe, so a second clip (Figure 20) was fabricated. The clip is made from 0.25 mm thick stainless steel foil, a tradeoff between the rigidity to clamp down the chip and the flexibility to allow the chip to oscillate in tapping mode AFM. The clip is held in place by two screws, giving the tip more stability and applying a more even pressure on the chip. In addition, the clamping force is easily adjustable for optimal drive efficiency in air and water by turning the screws.

To verify the effectiveness of the new clip, we examined the resonance curve of a tip held by each clip. Figure 21 shows the two cantilever tunes of a tip in air, obtained by the AFM which scans the range of frequency and records the amplitude of the oscillation as detected by the laser. We observe that the Q factor of the tip with the new clip is almost twice as great as that with the old clip. Since tapping mode AFM measures changes in amplitude of the oscillating cantilever caused by tip-sample forces, a higher Q (steeper resonance curve) means a larger change in amplitude for a given force. Thus, a higher Q improves the sensitivity of tapping mode AFM to force.

It is important to study the resonance characteristics of the SNOM probes in liquid, since it constitutes the medium in which many biological samples must be kept. We performed tests on a glass coverslip inside a sample holder containing water. The graph in Figure 22 shows the resonance curve of a tip as it slowly moves deeper from air into water. By continuously sweeping the drive frequency through the cantilever resonance peak, we are able to observe the exact moment when the tip touches the water, from the abrupt change in the appearance of the curve. We approximate the amount of fiber in contact with liquid at this point to be the length of the taper (~230 µm), where the meniscus forms and which was determined by meniscus etching. There is a first drop in Q factor and shift in resonance frequency, as shown by the red and orange curves. Figure 23 shows the Q factor as a function of the relative depth of the tip, where the reference point is when the tip first touches water. In addition, the drive effi-

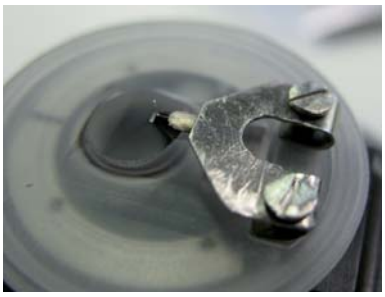

Figure 20 : New clip for the AFM tip holder

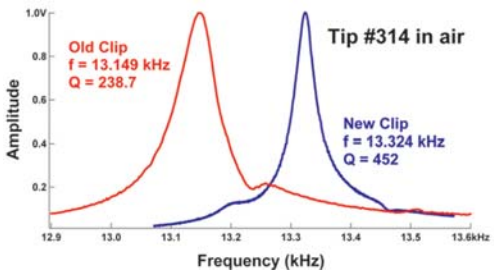

Figure 21 : Resonance curves of tip (courtesy of J. Ledue) with the new clip and the old clip.

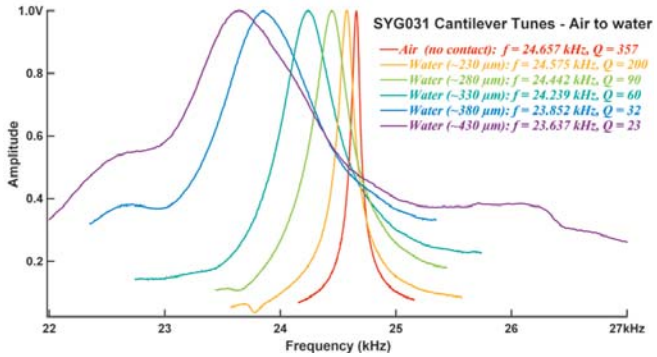

Figure 22 : Resonance curves of a tip (meniscus – selective etch) as it is lowered into the liquid from air. The curves have been normalized for the purpose of comparison. The original amplitude value is not relevant since it depends on the drive amplitude.

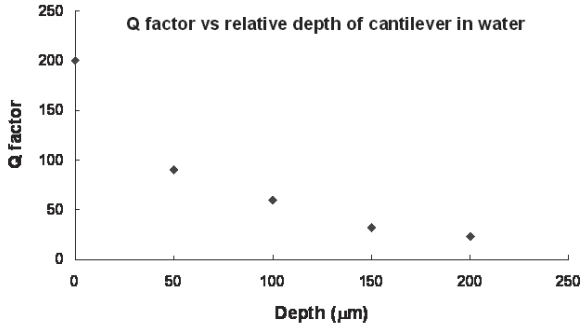

Figure 23 : Q factor vs. relative submerged depth of cantilever in water. The depth is measured from the point where the tip first touches water, so the first measurement in water is at 0μm with a Q of 200.

ciency, as indicated by the ratio between the amplitude and the drive amplitude is also worse as determined in Table 1. Afterward, the AFM head is lowered by 50 μm at a time until we no longer observe a change. The progressive decrease in Q factor, resonance frequency, and drive efficiency is consistent throughout the process. This is due to an increase in effective mass of the cantilever, as well as the drag force of the liquid which is affected by the depth of the tip in water. Nevertheless, the results show that it is possible to obtain good Q factors (60-90) in liquid compared to commercial cantilevers whose Q in water is around 10.

Imaging

The SNOM tips are capable of successfully imaging topographical features. Figure 24 is a three-dimensional image of the height trace of a sub-micron metal triangles calibration sample, imaged by a regular aperture-less probe. The image shows the most clearly defined features are displayed through the use of a slow scanning rate, 0.3Hz in this case.

The probes also allowed for fluorescence imaging (Figure 25). The microcontact printed stripes of the protein poly-l-lysine were imaged with fluorescent dyes FITC and Alexa 546, respectively, using an aperture probe (courtesy of J. Ledue and the Montreal Neurological Institute). In Figure 25a, the rectangular boxes of a darker shade represent areas bleached by the high intensity laser in previous scans. The round spots in both Figure 25a and b are most likely dust particles on the sample since they do not emit fluorescence. Finally, it has been verified from other scans that the diagonal features in Figure 25a and the dots in Figure 25b are indeed on the sample, which is an indication that our tips are able to pick up contrasts. The smallest features that appear in Figure 25b are measured to be on the orders of 250 nm, which is comparable to the diffraction limit of light.

Conclusion

We have determined and characterized the steps in the procedure for the fabrication of SNOM aperture probes and antenna probes and showed that the tapering of the optical fiber by chemical etching yields sharp, symmetric tips. The metal coating and subsequent FIB milling are effective methods for creating the aperture and antenna probes. As well, the techniques for mounting tips in the microscope, approaching the sample and imaging topography and fluorescence have been established.

Notably, we achieved high Q factors for the SNOM probes in liquid, as a result of modifications to the instrument such as insertion of a new clip. The ability to determine probe sensitivity by controlling the depth of the tip in liquid leads to the potential for very high probe sensitivity compared to that of commercial cantilevers in liquid. This makes the probes amenable to non-contact AFM imaging (Morita, Wiesendanger & Meyer, 2002), a technique which has the potential to provide lower forces than tapping mode AFM.

Finally, combined with the preliminary results regarding the aperture size and antenna shape, the SNOM probes should allow for unique combined optical and topographical imaging of biological samples, paving the way for a greater understanding of physical and biological mechanisms within cells.

Acknowledgments

I would like to thank Jeffrey LeDue and Professor Peter Grütter and as well as many other faculty and staff members in the Physics Department at McGill University for their collaboration, guidance, and assistance with the project. I would also like to acknowledge the support of Ms. Marie-Hélène Bernier and the central facilities of the École Polytechnique and the Université de Montréal funded by CFI, NSERC and NanoQuebec for SEM and FIB training and access to microfabrication laboratory. As well, the Montreal Neurological Institute has played a vital role by providing us with biologi-

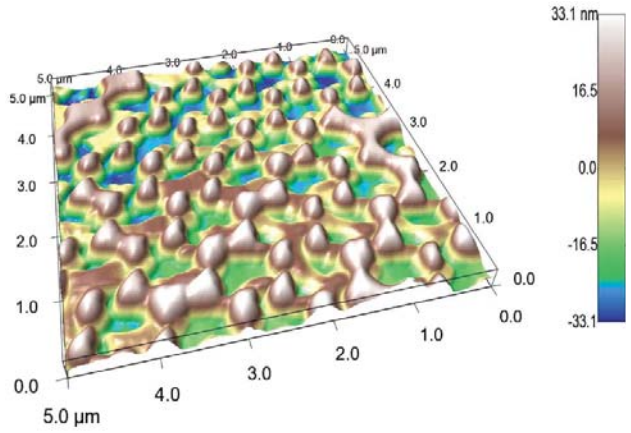

Figure 24 : Height trace of metal triangles with tip (meniscus etch).

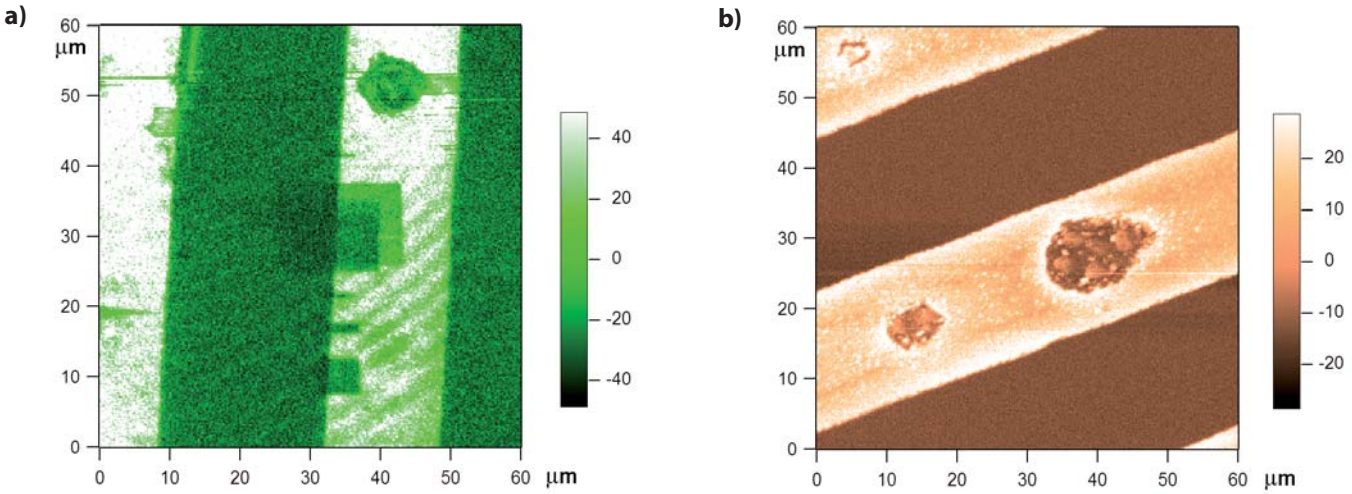

Figure 25 : The protein poly-l-lysine (20µm stripes) with fluorescent dyes a) FITC and b) Alexa 546 (AFM images courtesy of J. Ledue).

cal samples to image. Finally, I would like to thank my family and friends who have constantly supported my interests in science and my passion for research.

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The poppy seed defense: scientifically sound?

Maya Kaczorowski

Introduction

Since the infamous Seinfeld episode, there has been much doubt cast upon the relationship between the effects of poppy seed ingestion and testing positive for opiates. In the episode, Elaine fails a drug test after eating a poppy seed bagel for breakfast. A number of quasi-scientific sources, such as the TV show MythBusters, and several published scientific articles have since confirmed the effect of poppy seed consumption on positive drug testing. However, the amount of poppy seeds necessary for a positive result remains unknown. Additionally, the range of concentrations of morphine and codeine in poppy seeds makes it hard to determine a legitimate threshold value for the concentration of morphine in order to test positive, as there is currently no method that is able to conclusively distinguish poppy seed enthusiasts from drug users based on urine samples.

Testing subjects

Detection of opiates in urine, concentration of morphine and codeine in poppy seeds, and effects of excess poppy seed consumption

Scientists rarely use blood samples when testing for morphine and codeine concentrations, as urine samples are both quicker and more practical (Moeller, Manfred, Hammer, and Engle, 2004). Usually, both morphine and codeine are present in urine and blood after either injecting opiates such as heroin or smoking opium. In tests conducted by C. Meadway, George, and Braithwaite in 1998, participants who consumed large amounts of poppy seeds from either one or two poppy seed rolls had high concentrations of normorphine, morphine, thebaine, norcodeine, and codeine in their urine sample. Notably, the concentration of norcodeine in the participants' urine was exceptionally high, at almost twice the concentration of morphine.

In their study, the concentrations of morphine and codeine peaked in all participants at around the two-hour mark, and, using a cutoff concentration of 300 ng/mL, all participants tested positive for opiates two hours after consumption. However, another study found that participants still tested negative one hour after consumption of a very large amount of poppy seeds, equivalent to those in one fifth of a poppy seed cake, although a number of subjects continued to test positive for up to twenty-four hours post-ingestion (Pettitt, Dyszel and Hood in 1987). This suggests it takes the body some time to process the poppy seeds in the digestive tract. While Hayes, Krasselt and Mueggler (1987) found that their subjects tested negative after 48 hours, Bonicamp and Santana (1998) found that both of their participants still tested positive after the same time period. These time lapse differences can be partially accounted for by the amount of poppy seeds ingested, the participants' body mass index variability, and laboratory techniques used that could impact the concentration of opiates detected in the urine and blood samples.

The concentration of morphine and codeine in different strands of the seeds is also variable. For black poppy seeds, gas chromatography was used to find that the morphine concentration was 17-294 µg/g, and the codeine concentration was 3-14 µg/g, depending on the source and variety of the poppy seeds (L.W. Hayes, Krasselt and Mueggler 1987). Moeller, Manfred, Hammer, and Engle (2004) compared the seeds' morphine content by country of origin. Seeds available through a retail store in Oregon had the highest morphine content at 294 mg/kg. Comparable values were found for seeds from Spain (at 251 mg/kg) and Australia (at 200 mg/kg). In this study, samples from Turkey had the lowest content, with some samples containing less than 0.5 mg/kg of morphine. Since the range of concentrations of morphine reported here was based on testing several more varieties of poppy seeds than in other experiments, it is reasonable to suggest that their values are close to the actual range of opiate concentrations

The effects of ingesting a large amount of poppy seeds on physical and psychological health are unknown. In at least one documented case, a patient with a prescription for methadone had an increased concentration of methadone in his urine upon consumption of poppy seeds (Narcessian and Jung Yoon 1997). These results imply that a high dose of poppy seeds could have negative physiological effects for medicated subjects. In another experiment, communicative and interactive tests (such as walking a straight line or reciting a sentence) were used to determine if participants seemed to be under the influence of opiates after consuming poppy seeds; neither a police officer nor a physician found any evidence suggesting drug usage (Moeller, Manfred, Hammer, and Engle, 2004). However, the same experiment reported that the individual who consumed the largest quantity of poppy seed cake in relation to their body weight "reported a light drug effect including drowsiness with reduced pupil reaction time."

Cutoff rates and by-products identifiable in a subject's urine

Determining whether a subject is a poppy seed enthusiast or a drug user

Many of the above experiments used a threshold concentration of 300 ng/mL, defining higher concentrations as positive tests. However, the United States has recently changed its threshold value to 4000 ng/mL for morphine and 2000 ng/mL for codeine in acknowledgement of the poppy seed defense (Meadway, George, and Braithwaite, 1998). The International Olympic Committee (IOC) has set their threshold for morphine at 1000 ng/mL, and has agreed to re-examine any disputed case upon request (Yonamine, Rodrigues Garcia, and de Moraes Moreau, 2004). Others argue that changing the original threshold value is unnecessary, as a very large quantity of poppy seeds, more than what might be contained in a bagel, would need to be consumed to have a urinary morphine concentration greater than 300 ng/mL. Conversely, Meadway, George, and Braithwaite (1998) found that the maximum concentration of morphine in urine samples after ingesting only 4.69 g of poppy seeds, about one slice of poppy seed cake, was 302 ng/mL. Because of these controversies, scientists have searched for a chemical by-product of either an opiate drug or poppy seeds that would help to distinguish them. Initially, thebaine, a component of poppy seeds, was suggested as a differentiating compound, but was soon discarded because of high between-individual variability in the concentration of thebaine (Moeller, Manfred, Hammer, and Engle, 2004). In an experiment by Meadway, George, and

Braithwaite (1998), only half of the participants tested positive for thebaine after a small dose of poppy seeds while all tested positive after a larger dose. A further complication arises in that if thebaine was used as an indicator, a participant could potentially mask their opiate use by consuming poppy seeds prior to testing, to be subsequently vindicated by the presence of thebaine. In 1998, two scientists from New York proposed the presence of 6-Monoacetylmorphine (6-MAM) to differentiate between an opiate drug user and a poppy seed eater, claiming that 6-MAM was not detected in their participants' urine samples after they had eaten poppy seed bagels (Mulé and Casella, 1998). Although Meadway, George, and Braithwaite (1998) agreed with these findings, they are quick to point out limitations; for example, 6-MAM is present in relatively low concentrations, so the method of detection must be very sensitive. Additionally, they found its half-life to be of only 30 minutes, indicating that the sample would need to be tested promptly after drug use to test positive for 6-MAM. In spite of these results, members of the U.S. Department of Defense and the Department of Health and Human Services, Buddha, Shimomura, and Smith (1999), set out to determine appropriate cutoff values for morphine, codeine, and 6-MAM required to confirm heroin usage. It found was that 1113 samples out of 422 237 were positive for opiates, but of those, only 17 showed a detectable amount of 6-MAM. Among the participants who did not test positive for 6-MAM, it was never stated how many were heroin users, and how many were simply heavy poppy seed eaters, making the results of this study of limited practical significance.

Conclusion

It is currently impossible to accurately determine the cause of a positive urinary test for opiates. Because the amount of poppy seeds ingested, the concentration of morphine and codeine in different varieties of poppy seeds, the subject's body mass index, and laboratory techniques used can all vary, it is recommended to follow the IOC's example and examine each case individually to determine the nature of a positive test for opiates. Until a valid and reliable differentiator is identified, however, the poppy seed defense will continue to be used. Even though the international community has begun to accept poppy seed consumption as a legitimate defense for a positive opiate test, it is recommended to avoid poppy seeds for up to 48 hours before a scheduled drug examination.

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Food temptations spontaneously elicit compensatory beliefs in dieters

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Abstract

Through various self-regulatory strategies, individuals attempt to strike a balance between the satisfaction of immediate desires and fulfillment of long term goals. One such strategy is described by the compensatory beliefs model, which suggests that individuals rationalize their surrender to an immediate desire or temptation. This model is indirectly supported by earlier studies where compensatory beliefs were induced by the experimental context. The current pilot study examines whether compensatory beliefs can be self-initiated i.e. are spontaneously generated as a response to temptation. We recruited ten female McGill students currently on a weight loss diet and assigned them randomly to a temptation and a control group. We presented all participants with a choice between two identical cookies, differently described for the temptation and control groups: for the temptation condition one cookie was labeled as high in fat and sugar and the other as low in fat and sugar while for the control condition both cookies were labeled as low in fat and sugar. Participants listed compensatory thoughts in both a closed and an open response format. Our pilot data show that dieters indeed spontaneously generate compensatory beliefs in response to temptation. Compensatory beliefs should be considered a factor in unsuccessful self-regulation and more specifically in failed dieting attempts.

Keywords

Compensatory beliefs, self-regulation, weight-loss, diet.

Introduction

When personal goals conflict with natural desires and tendencies, goal-adherence is undeniably challenging (Baumeister, Heatherton & Tice, 1994; Rabiau, Knäuper & Miquelon, 2006). Individuals continuously face temptations which are situations where one is pulled in the direction of a particularly alluring choice that satisfies an immediate desire but conflicts with another goal. For example, many people hold goals of achieving and maintaining a healthy, active life and thin figure, but also enjoy unhealthy, sweet, and fatty foods (Rabiau et al., 2006). As a result, an internal conflict arises because individuals know they must resist the temptation if they want to attain their long-term goal. Successful self-regulatory efforts to resist a temptation are those that successfully transcend immediate pleasure for the purpose of adhering to long-term goals (Vohs & Baumeister, 2004). In contrast, compensatory beliefs (CBs) are rationales that are used as justifications for giving in to immediate pleasures (Knäuper et al., 2004; Rabiau et al., 2006), thereby interfering with the attainment of a long-term goal.

The compensatory beliefs model (Rabiau et al., 2006) builds on existing theories of self-regulation, and extends them by describing a cognitive strategy that individuals may use to deal with temptations. Specifically, when individuals are faced with temptations, an internal conflict arises between their desire to satisfy immediate goals (partake in unhealthy behaviours) and fulfill explicit long-term goals (staying healthy). This internal conflict and discomfort may arise while contemplating giving in to the temptation or after having given in to it (cf. Festinger, 1957; cf. Giner-Sorolla, 2001). The internal conflict activates compensatory beliefs by catalyzing the conviction that the negative effects of a desired behaviour can be compensated for, or "neutralized", by the positive effects of another behaviour.

A compensatory belief requires the creation of an intention to perform the compensatory behaviour needed to reduce the internal conflict. In the compensatory beliefs model, intention is equivalent to Gollwitzer's concept of goal intention (Gollwitzer, 1999; Gollwitzer & Brandstätter, 1997), which is described as a feeling of commitment to achieve the goal. Additionally, for a compensatory behaviour to be achieved, individuals must make a concrete, detailed plan of how they will compensate for the unfavourable or unhealthy behaviour in question, and must have a certain amount of self-regulatory capacity to implement the plan (Baumeister et al., 1994; Webb & Sheeran, 2003). The less self-regulatory strength the individual has in a given tempting situation, the greater is the likelihood that compensatory beliefs will be generated, and the less likely he or she is to act upon them by engaging in compensatory behaviours. (Rabiau et al., 2006).

There are two main sources of self-regulation failure: under-regulation and misregulation (Baumeister et al., 1994, 2004). Under-regulation refers to a failure to exert control over oneself, often due to depleted self-regulatory capacity (Baumeister et al., 1994). Misregulation occurs when people monitor and control their behaviour, but in a counterproductive way; for example when their behaviours have different effects on goals than they originally intended. Using compensatory beliefs to regulate temptations can result in both under-regulation and misregulation (Rabiau et al., 2006). Under-regulation typically occurs when compensatory beliefs are activated (because a temptation cannot be resisted), but then the compensatory behaviour itself is not implemented. Misregulation occurs when people successfully manage to implement the compensatory behaviour but the behaviour does not effectively compensate for the unfavourable effects of the tempting behavior. For example, individuals may never make it to the gym to burn off "extra" calories, or even if they do, they may believe that the exercise fully compensates for the extra calories, when in fact they do not. Evidently, compensatory beliefs and behaviours may be unhealthy components of a self-regulatory strategy.

Earlier research on eating behaviours by Lowe (1982) and Urbszat et al. (Urbszat, Herman & Polivy, 2002) can be interpreted as supporting the recently proposed compensatory beliefs model. These authors' findings suggest that individuals strategically use anticipated compensation to regulate temptations. In specific, their experiments demonstrated that restrained eaters (individuals who chronically restrict food intake for the purpose of weight control), actually increased food consumption when anticipating future food depriva-

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tion (Herman & Polivy, 1980). This effect has been called the "last supper effect" (Eldredge, Agras & Arnow, 1994), and has been interpreted to mean that dieters feel justified to overeat in anticipation of a compensating diet (Lowe, 1982; Urbszat, Herman & Polivy, 2002). However, the compensatory belief that future food deprivation will compensate for the present food consumption in these experiments was not self-initiated, but rather created by the experimental context, in that participants were instructed to go on a diet the following day. As such, the studies did not provide direct evidence that people self-initiate compensatory beliefs as a strategy for dealing with temptations.

The objective of the present pilot study thus was to provide initial evidence that compensatory beliefs are indeed elicited as a reaction to temptations, by directly examining dieters' cognitive processes in a situation where they were tempted to consume high calorie food.

Method

Overview

We presented participants with two cookies. Though identical in appearance and content, one was described as vanilla-flavoured and the other as almond-flavoured. In the temptation condition, one cookie was labeled as low in fat and sugar, the other as high in fat and sugar. In the control condition, both cookies were labeled as low in fat and sugar. Which flavor was assigned to which cookie was randomly determined for each session. Participants were asked to describe in writing their thoughts while deciding which of the two cookies they would like to taste and eat. It was hypothesized that the temptation to eat the high calorie cookie would create an internal conflict to the extent that dieters would have compensatory thoughts that "allow" them to eat the high calorie cookie, even though doing so would interfere with their weight loss goals. An example of such a thought is, "It's okay to eat this cookie now because I will not eat dinner later."

Participants

Participants in this pilot study were female students from McGill University (N=10) from 18 to 22 years of age, who were currently dieting to lose five pounds or more, and who were recruited individually by the researchers through acquaintances. All of the people approached for the study were able to participate after the screening process. Recruitment was under the guise of a "Food palatability study for dieters: A study examining the effects of weight loss dieting on food palatability."

Procedure

The aim of this research was to investigate compensatory beliefs in a non-clinical population. As such, researchers screened participants, via phone, for any possible eating disorders using the Eating Disorder Examination Self-Report Questionnaire (EDE-Q; Fairburn & Beglin, 1994). Participants were asked to refrain from eating 2.5 hours prior to the experiment to ensure that they were sufficiently hungry to be tempted by a cookie. After arriving at the assigned room, individual participants were asked to sign a consent form, which detailed that participants would be asked to choose one of two cookies and fully consume it during the course of the study. In truth, this section of the study was not performed, and none of the participants were asked to eat either cookie (i.e. deception was used). Individuals were then told that they were participating in a "Food Evaluation Study", to assess how weight loss dieting affects food palatability. Participants were randomly assigned to either a temptation condition or a control condition prior to testing (n=5 for each of the two conditions). Once the experiment began, participants in both conditions were presented with two identical cookies. Participants were told that the researchers wanted to know what goes through an individual's mind while deciding between two cookies, which would shortly be brought to market. They were then asked to report their thoughts while deciding on which of the two cookies to taste and eat.

Participants in the temptation condition were presented with what were labeled as one high calorie, high sugar cookie (Cookie A) and one low calorie, low sugar cookie (Cookie B). The exact calorie and sugar contents of the respective cookies were not written on tags in front of the respective cookies as participants might interpret these numbers differently based on their personal weight-loss goals. For example, a 150 calorie cookie might appeal more to someone on a 2500 calories per day diet than to someone on a 1200 calories per day diet. The experimenter verbally described one cookie as vanilla flavoured and the other as almond flavoured. These flavours were chosen because of their equally perceived sweetness, as determined in pretests. To control for the possibility that one flavor was favored over another, the tags labeling the cookies were switched, so the almond cookie was high calorie for half of the participants and low calorie for the rest of the participants. The experimenter explained that in previous sessions, participants had mostly found that the low calorie, low sugar cookie was not very good, and tasted quite flat, leaving a bitter after-taste compared to the high calorie, high sugar cookie, which everyone had found to be rich, chewy and "very yummy". This discrepancy between the cookies was emphasized to increase the temptation to eat the high calorie cookie and thereby increase the chances that participants would generate compensatory beliefs.

Participants in the control group were presented with the same cookies, except that both were now labeled as low calorie, low sugar cookies, differing only in the two flavours. Again, the flavour descriptions were only mentioned verbally by the experimenter.

Participants in both groups were then asked to evaluate the two cookies, knowing that they would have to eventually consume the chosen cookie. While considering their choices, participants were asked to describe any thoughts they were having about eating one of the cookies. This portion of the experiment was done in an open response format, i.e. neither priming nor examples were provided, and participants were free to write down any thoughts they had on a lined paper section. Participants were asked to alert the researcher upon finishing the free-write portion.

Participants were then asked to complete a questionnaire about the thoughts they had while deciding which cookie to eat, i.e. to report compensatory beliefs in a closed response format. After completing the questionnaire, participants were asked to once again alert the researcher, who presented an open-ended comments card on which individuals could comment on the experimental procedure and any aspects that they might have found confusing. Finally, a debriefing sheet was distributed to participants with a complete outline of the purpose and experimental manipulations of the study.

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Measures

Eating Disorder Examination Self-Report Questionnaire (EDE-Q). The EDE-Q (Fairburn & Beglin, 1994)) is a 41-item measure adapted from the Eating Disorder Examination (EDE) by Fairburn and Beglin (1994). The EDE is a structured clinical interview assessing the key behavioural features and associated psychopathology of eating disorders (Cooper & Fairburn, 1993). The EDE-Q has been adapted for self-reportability. The EDE-Q was ideal for our screening purposes, as it was condensed and required no clinical training. Diagnoses of probable eating disorders were based on a 28-day time period and the participants' responses to the main diagnostic questions. Participants were identified as probable for eating disorders if they appeared to have the necessary criteria for bulimia nervosa, anorexia nervosa, or eating disorder not otherwise specified, as described in the DSM-IV (American Psychological Association, 1994). None of the ten women screened were diagnosed with an eating disorder, and therefore all participated in the pilot study.

Open response format questionnaire.
On a blank sheet of paper, participants were asked to indicate their thoughts from when they were deciding which cookie to eat. Specifically, participants were asked: "While you are thinking about which cookie you want to eat, we would like you to tell us any thoughts you are having about eating one of the cookies." Each of the participants' answers was later coded for frequency of compensatory beliefs.

Food Palatability Questionnaire.
Participants completed the Food Palatability Questionnaire, a self-developed 20-item questionnaire that included eight compensatory belief items ("I'll eat it but I'll only have salad for dinner") embedded within 12 filler items. Participants were asked to indicate to what extent such a thought was currently on their mind while they were deciding which cookie to eat, which was reported on Likert-type rating scales, ranging from 1 (a little bit on my mind) to 4 (very much on my mind). The mean endorsement of compensatory beliefs across the eight compensatory beliefs was calculated for each participant.

Results

Sample description.
Participants were on average 19.8 years old (Mdn = 19.5, SD = 1.47). Regarding weight loss goals, participants on average intended to lose 7 pounds (Mdn = 5 pounds, SD = 4.22 pounds). The average amount of time since the last meal eaten was 2.85 hours before participating in the experiment (Mdn = 2.5, SD = 0.78).

Compensatory beliefs in the open response format questionnaire. Responses to the open response format questionnaire were reviewed for the occurrence of compensatory beliefs. As expected, more participants in the experimental condition reported a compensatory belief than in the control condition. Specifically, two of the five participants in the experimental condition wrote down a compensatory belief and none of the participants in the control condition wrote down a compensatory belief. These two respondents wrote: "… I can always abstain from eating cookies for the time following the eating of said cookie" and "I would rather eat a high calorie cookie, and overall less food, than eat a low calorie cookie". In the control group, no participant mentioned any compensatory beliefs, with all comments pertaining only to flavour preferences between the two choices presented. Common responses to the open-ended question in the control group indicated the existence of a conflict between choosing among two equally appetizing flavours, similar to the following: "I like vanilla and almond flavors equally, but sometimes find almond too intense… I am leaning towards eating vanilla". The difference in the frequency with which compensatory beliefs were generated between groups (two vs. none) was not statistically significant (chi square = 2.50, df = 1, p = .11), likely due to the small sample size in this pilot study.

Compensatory beliefs in the Food Palatability Questionnaire.
Participants endorsed more compensatory beliefs on the Food Palatability Questionnaire in the experimental condition (M = 2.7; SD = 0.69) than in the control condition (M = 1.6; SD = 0.61). Indeed, a significant difference in means between groups was found, $t(8) = 2.67$, $p < .029$, with a higher average endorsement of compensatory beliefs in the experimental condition than in the control condition. These findings lend initial support to our hypothesis that compensatory beliefs are indeed elicited as a reaction to temptations. Both participants who had reported compensatory beliefs in the open response format also endorsed compensatory beliefs in the Food Palatability Questionnaire (i.e. gave a rating of 2, 3, or 4). All five of the experimental condition participants endorsed a minimum of 4 to a maximum of all 8 compensatory beliefs on the Food Palatability Questionnaire, with a rating of 2, 3 or 4. In the control condition, only three of the participants endorsed a minimum of 5 compensatory beliefs and a maximum of 7 compensatory beliefs, with, at most, a rating of 3. None of the participants in the control condition endorsed compensatory beliefs with a rating of 4 ("very much on my mind").

Discussion

In an attempt to provide initial support for the hypothesis that compensatory beliefs are elicited in tempting situations, we created a setting in which compensatory beliefs could naturally occur as a form of justification for making a goal-inconsistent choice. We assessed compensatory beliefs in an open and a closed response format, and found evidence with both assessment strategies that compensatory beliefs were mentioned and endorsed more often when dieters were tempted to consume high calorie food. That more participants in the experimental condition than in the control condition spontaneously listed compensatory beliefs in an unprompted open response format is encouraging because it suggests that compensatory belief endorsements in the closed response format are not just a result of having "planted" beliefs in the minds of participants who otherwise may not have spontaneously generated such thoughts. This is further supported by the fact that both participants who indicated compensatory beliefs in the open response format also endorsed compensatory beliefs on the Food Palatability Questionnaire.

Limitations
In order to avoid any bias caused by differences in the appearance of the food, identical looking cookies were used for both the experimental and control group throughout the entire study. This attempt to avoid bias towards one cookie over another might, however, have influenced results. Indeed, some participants stated that they believed the

cookies might taste the same, even after it was explained that they were quite different. The similarities between the two cookie choices were noted on multiple occasions during both the experimental condition and the control condition. One comment made during the experimental condition was "Both cookies look the same, so I question whether it is worth it to take the higher calorie cookie", while control condition yielded comments such as "They look the same so, other than flavor, I have no reason to pick one over the other". Thus our attempt to create a temptation situation in the experimental condition might not have been successful in all cases. Therefore, the number and degree of compensatory belief endorsements in the experimental condition might have been higher if the cookies actually appeared to be different (i.e. if the high calorie cookie would have looked more appealing or better tasting than the lower calorie cookie).

The purpose of a pilot study is exploratory. One clear limitation of the pilot study sample is that it was homogeneous in gender and age; moreover, all participants were McGill undergraduate students.

Implications and Future Research Directions

A chronic pattern of unsuccessful dieting may partly be due to holding compensatory beliefs and not acting upon them, or acting upon ineffective compensatory beliefs. This pilot study is a stepping stone towards better understanding these beliefs. It will be followed up with an experiment in a large, heterogeneous sample of dieters from the community to investigate the generation of compensatory beliefs in dieters systematically.

Compensatory beliefs research may provide directions for helping individuals achieve a healthier body weight by showing that weight-loss recommendations should target erroneous compensatory beliefs and make individuals aware that they use them to deal with temptations, as well as help individuals implement effective compensatory behaviours by creating specific plans that state when, where, and how they will implement them (Gollwitzer & Brandstätter, 1997). Insight gained from this research may be used in the future to plan interventions aimed at preventing individuals from using compensatory beliefs, or to help them to implement effective compensatory behaviours.

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Investigating auditory fear memory erasure in the basolateral amygdala

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Abstract

New memories are initially fragile and need protein synthesis in order to be stabilized for long-term storage, a mechanism called cellular consolidation. When recalled, memories are re-activated and become unstable again. They therefore need to be re-stored through a process called reconsolidated. Behavioural studies in rats using auditory fear conditioning have demonstrated that propranolol, a β-adrenergic receptor antagonist, administered following memory reactivation can reduce fear expression (freezing), which has been interpreted as amnesia for the fear memory. It was recently discovered that GluR1-containing AMPA receptors are recruited into the post-synaptic membrane of the basolateral amygdala during auditory fear conditioning, suggesting that synaptic GluR1 increase may be a molecular correlate of long-term memory. The present study aims to investigate what molecular mechanisms accounts for the observed amnesia following a reconsolidation blockade by propranolol. Rats were trained in an auditory fear conditioning task, and fear memory reactivation was followed by systemic propranolol administration. Rats were then euthanized and GluR1 protein levels in baso-lateral amygdala synaptoneurosomes were quantified. We report preliminary evidence to suggest that a reconsolidation blockade by propranolol reduces fear expression with a concomitant reduction in GluR1. Such evidence suggests that a reconsolidation blockade might, at a molecular level, erase a component of the fear memory, providing support for the clinical utility of this treatment for post-traumatic stress disorder.

Memory, the ability to acquire, store and recall learned information, is a fundamental feature of human experience. Observations that new memories are initially sensitive to disruption but strengthen over time laid the foundations for the consolidation hypothesis (Muller & Pilzecker, 1900, as cited in Dudai, 2004). This theory holds that memories pass through two qualitatively different states (Ebbinghaus, 1885 as cited in Squire & Kandel, 2000). At the time of learning, a fragile memory trace is formed in short-term memory (STM) and said to be labile as it is sensitive to disruption. By way of the consolidation process, a short term memory is converted into a lasting and stable memory trace which exists in long-term memory (LTM).

The most striking evidence supporting consolidation theory comes from animal studies; amnesic agents such as protein synthesis inhibitors (Flexner, Flexner, De La Haba, & Roberts, 1965) or electro-convulsive shock (Duncan, 1949) administered to animals shortly after learning resulted in amnesia, while the same treatment after a delay caused no memory impairment. These results suggest that new memories must undergo a time-dependent process to persist in LTM stores. Consolidation is thus defined as a stabilization process that renders a newly acquired memory stable and lasting.

At the cellular level, consolidation occurs as a result of synaptic changes following acquisition of newly learned information. The synaptic plasticity hypothesis (Hebb, 1949) holds that the encoding of new memories results in structural modifications of synaptic connections, causing persistent changes in synaptic strength. Long-term potentiation (LTP) is currently the leading model for this plasticity. LTP was first demonstrated in vivo in rabbits, where it was shown that successive test pulses in the neural pathways leading to the hippocampus were shown to increase the strength of active synapses (Bliss & Lomo, 1973). Numerous subsequent studies (Rogan, Ursula, Staubli, & LeDoux, 1997) have shown that learning and LTP involve similar cellular mechanisms, suggesting that LTP may be the mechanism by which new memories stabilize over time.

LTP is a process triggered by the activation of the excitatory glutaminergic N-methyl d-aspartate receptors (NMDAr). Activation of the NMDAr results in an influx of Ca2+, which initiates a cascade of protein synthesis dependent intracellular reactions that are thought to lead to the growth of new synapses and to the insertion of receptors into the post-synaptic membrane (for review see Milner, Squire, & Kandel, 1998). Specifically, the α-amino-3-hydroxy-5-methylsoxazole-4-propionic acid receptor (AMPAr), another type of glutaminergic receptor, is inserted into the post-synaptic membrane and increases the neuron's sensitivity to glutamate, increasing the likelihood of synaptic transmission (for review see Malenka, 2003).

AMPAr are composed of four subunits, known as GluR1 to GluR4, that have different relative levels of insertion into post-synaptic membranes during LTP (Passafaro, Piech & Sheng, 2001; Rumpel, LeDoux, Zador, & Malinow, 2005; Yeh, Mao, Lin, & Gean, 2005). Both in vitro and in vivo rodent studies have demonstrated increased insertion of only the GluR1-containing AMPAr in the basolateral amygdala (BLA), the brain structure thought to be responsible for fear learning, following auditory fear conditioning (Yeh et al, 2005; Rumpel et al, 2005). Moreover, it was shown that blocking the synaptic incorporation of GluR1 (Rumpel, 2005) or knocking out the gene that codes for the GluR1 subunit (Humeau, Reisel, Johnson, Borchardt, Jensen, Gebhardt et al, 2007) impedes associative fear conditioning. These findings indicate that GluR1 may provide an essential contribution to the molecular mechanism of memory formation and maintenance, and thus may be a molecular correlate of the memory trace.

Previously, scientists believed that once the synaptic modifications necessary for consolidation were made, the memory was permanently hardwired into the brain. Misanin, Miller & Lewis (1968) challenged this hypothesis when they found that 24 hours after the acquisition of a passive-avoidance task, a cueing-procedure followed by electroconvulsive shock (ECS) resulted in memory loss. This study was the first to suggest that a consolidated memory could be susceptible to amnesic treatments. Recently, Nader, Schafe & LeDoux (2000) confirmed these results using auditory fear conditioning. This robust learning paradigm involves the pairing of a tone, the conditioned stimulus (CS), with a footshock, an aversive unconditioned stimulus (US). As such, rats learn that the CS predicts the footshock, and eventually fears the CS when it is presented alone. Nader et. al. (2000) demonstrated that post-training

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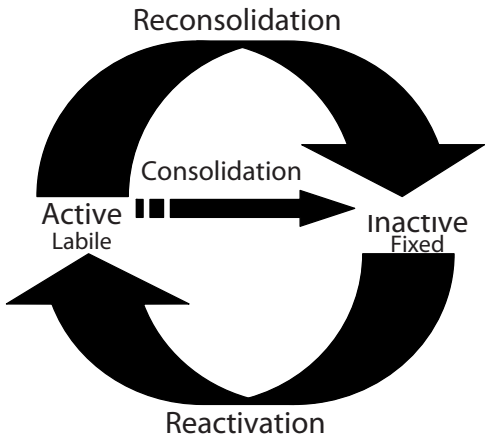

Figure 1: The dynamic nature of memory

infusion of the protein synthesis inhibitor anisomycin into the BLA immediately following memory reactivation produced amnesia. Interestingly, anisomycin infusion in the absence of reactivation did not produce amnesia, suggesting the observed amnesia hinges on memory reactivation. The authors concluded that if a protein synthesis inhibitor infused after reactivation causes amnesia, then reactivation must destabilize a well-consolidated memory and instigate a second stabilization process requiring de novo protein synthesis in order for the memory to persist in LTM. This process, termed reconsolidation, suggests that persistence of memory involves not only the storage of memories following their acquisition, but also the re-storage of these memories following recall.

Reconsolidation has been demonstrated across species ranging from C. elegans to humans, and for different types of learning, from aversive to appetitive conditioning (Nader, 2007). The memory process is therefore believed to be dynamic in nature, where a memory cycles between being in an active and an inactive state. As such, reconsolidation studies suggest that it is not the time delay following encoding that determines the durability of a memory, but its qualitative state; memories are in an active state when they are acquired and recalled, following which they require a time-dependent stabilization process to persist in LTM (see Figure 1).

The discovery of reconsolidation has important theoretical implications, as it challenges the long-standing notion that memories are fixed when consolidated, but also introduces a novel clinical treatment for disorders associated with debilitating traumatic memories, such as post-traumatic stress disorder (PTSD). Anisomycin, the amnesic agent used by Nader et. al. (2000), cannot be administered to humans, and as a result drugs with a similar amnesic effect that can be administered to humans have been investigated to determine the validity of such a treatment.

Propranolol, a β-receptor antagonist, has been examined because of the involvement of endogenous stress hormones in the memory system and because propranolol can safely be administered to humans. When a rat experiences a footshock, or when a human undergoes a traumatic experience, stress hormones are released (McGaugh, 2000). Also, β–adrenergic agonists infused into the amygdala in rats (Cahill & McGaugh, 1996) and injected in humans (Chamberlain, Muller, Blackwell, Robbins & Sahakian, 2006) enhance memory storage. Put together, these findings suggest that the release of stress hormones at the time of a traumatic event may contribute to an 'over-consolidation' process that renders fear memories highly resistant to extinction (Orr, Metzger, Lasko, Macklin, Peri, & Pitman, 2000). Following from this, being a b-receptor antagonist, propranolol was used to block the memory-enhancing effects of stress hormones during consolidation in both rats (McGaugh, 2000) and humans (Chamberlain et al., 2006). Pitman, Sanders, Zusman, Healy, Cheema, Lasko et al (2002) administered propranolol to humans immediately following an acute traumatic event, and found that this administration reduced subsequent PTSD symptoms.

Since propranolol blocks consolidation in both rats and humans it was believed that perhaps propranolol might block memory reconsolidation. Indeed, Debiec & Ledoux (2004, 2006) used auditory fear conditioning in rats and found that administration of propranolol following reactivation blocked the expression of fear memories, suggesting that propranolol blocked reconsolidation. Their study provides an animal model for the treatment of traumatic memories by way of reconsolidation blockade by propranolol, and suggests that propranolol may be an effective treatment for PTSD.

Our study aims to investigate the molecular processes that might account for the observed amnesia, as the mechanism by which reconsolidation blockade reduces fear expression in rats is currently not clear. Although it has been proposed that blocking reconsolidation actually removes a part of the memory (Nader, 2007), this has not yet been validated at a molecular level. Furthermore, since GluR1 is thought to represent a component of the memory trace,it is apt to examine whether a blockade of reconsolidation by propranolol can decrease this molecular memory tag. This would validate the hypothesis that the reduction of freezing observed by reconsolidation blockade can be directly attributed to memory erasure.

Using an auditory fear conditioning paradigm, three hypotheses were tested. Firstly, when propranolol is injected following memory reactivation, can it reduce freezing behaviour? Secondly, does fear conditioning cause a measurable increase in synaptic GluR1 three days after training? Lastly, does a blockade of reconsolidation by propranolol reverse the conditioning-induced increase in GluR1? In order to test these hypotheses, 12 rats were randomly assigned to four experimental groups (n=3) (Table 1). The rats in the propranolol group (CS+P) were habituated, trained, and were given a propranolol injection immediately following reactivation. The rats in the vehicle group (CS+V) were habituated, trained, and given a saline injection immediately following reactivation. The rats in the non-reactivated propranolol group (No CS+P) were habituated, trained, and given a propranolol injection without memory reactivation. This group is a necessary control to determine that propranolol, in the absence of reactivation, does not affect memory processing. The rats in the naïve group were habituated and administered saline. This last group provides a baseline measure of GluR1.

Methods

Subjects

Twelve adult male Sprague-Dawley rats from Charles River Laboratories, weighing 275-300g on arrival, were individually housed and maintained on a 12/12-hour light/dark cycle, with lights on at 7:00 a.m. All testing was performed during the light period. Rats were handled once a day for three consecutive days before the testing began.

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Groups	Training	Reactivation	Drug	Freezing	GluR1
Naïve	None	None	Saline	None	Baseline
CS+P	CS-US	CS	Propranolol	Low	⬇
CS+V	CS-US	CS	Saline	High	⬆
No CS+P	CS-US	No CS	Propranolol	High	⬆

Table 1: Drive efficiency of the SNOM probe as indicated by the ratio between the drive amplitude and the amplitude of the cantilever tunes

Behavioural Apparatus

Two distinct test chambers were used for this study (Med-Associates). The first chamber, (context A) was used for training. It had a metal grid floor (1.5 cm bar spacing), stainless steel sidewalls, a transparent Plexiglas front wall, and was enclosed in a custom-built sound-attenuating isolation cubicle. During training, all lights and ventilation fans in each cubicle were on.

The second chamber (context B) was used for habituation and tone testing. These chambers were located in a second room of the laboratory. Each chamber was 30 x 25 x 30 cm; two sidewalls were stainless steel and two were Plexiglas with an opaque sheet of alternating 2 cm-wide vertical black and white stripes. The floor was a Plexiglas opaque white surface, scented with peppermint before each rat was inserted. Each conditioning chamber was enclosed in a sound-attenuating isolation cubicle. The house lights were dimmed while the ventilation fans remained off. The amount of time each rat spent freezing during the 30 s interval preceding the CS presentation for each test, as well as the time spent freezing during the CS, was measured.

Molecular Apparatus

A Cryostat (Microm Instrumentation, Germany) was used to collect amygdala slices. A Teflon homogenizer was used to homogenize tissue samples in a buffer to fractionate cells. Millipore filters were used to filter the cell fractions from other cellular components. A microcentrifuge was used to separate the heavier fractions, enriched with synaptoneurosomes, from lighter ones (less than 5 µm). An electrophoresis module (Bio-rad) was used in the present study to further separate synaptic GluR1 from other cellular proteins. A transfer module (Bio-rad) allowed a current to run through the polyacrylamide gel apposed to a PVDF (Millipore) membrane, and was used to transfer proteins from the gel to the membrane. The Storm Laser scanner (Storm 860, Amersham Biosciences) was used to quantify GluR1 proteins in the PVDF membrane via Image Quant software (Blot Imaging System).

Behavioral Procedures (Figure 2)

Habituation. All rats were habituated for 15 minutes on three consecutive days in Context B. This was intended to acclimate the rats to the lab and the behavioural chambers, to minimize generalization between the two contexts, and to eliminate any contextual conditioning, ensuring that the CS is the primary predictor of US exposure.

Training. On the fourth day, only rats in the CS+P, CS+V and No CS+P were transported to a brightly lit waiting area and remained there for five minutes before training began in Context A. Rats were then individually placed in a chamber, and after a two minute acclimatizing period, they were given three forward-presented pairings of the CS-US. The CS was a 5 KHz, 65 dB, 30 s tone that co-terminated with the US, a 1.5 mA , 1 s footshock delivered through the metal grid floor. The inter-trial interval (ITI) between each tone-shock presentation was 60 s. Rats were then returned to their home cages.

Reactivation. On day five, twenty-four hours after training, when the fear memory is thought to have completed cellular consolidation (Nader et al., 2000), rats in the CS+P, CS+V and no CS+P groups were transported to a dimly lit room and remained there for five minutes. Rats in the CS+P and CS+V groups were then placed in Context B. After 120 s of acclimatization, a single tone (5 KHz, 65 dB, 30 s) was played, but no shock was given. Immediately following the memory reactivation, the animals in the CS+P group received an intraperitoneal injection of propranolol (Sigma Aldrich, Ontario), while animals in the CS+V group received a saline injection. Rats in the no CS+P group received the propranolol injection at this time, without undergoing the memory reactivation session. Propranolol was dissolved in a saline solution at a dose of 10 mg/ml and administered at a dose of 20 mg/kg, as used in Debiec & Ledoux (2004, 2006). Freezing behaviour, operationally defined as the cessation of all movement except respiratory-related movements (LeDoux, 2000), is a species-typical fear response, and is used as a measure of fear. Freezing behaviour was scored with Freeze-View software (Actimetrics) by an experimenter blind to the experimental condition.

Post-reactivation short-term memory test (PR-STM). On day five, four hours following reactivation, rats in the CS+P, CS+V and no CS+P group underwent the PR-STM test. These rats were transported to a dimly lit room and remained there for five minutes. Rats were then placed into Context A, and after a 120 s acclimatization period three tones with the same parameters as used in the reactivation trials were delivered, and the freezing behaviour scored.

Typically, PR-STM tests are performed as an internal control in order to rule out any nonspecific effects of the drug, in this case the effects of propranolol on the memory abilities of the rats (Nader et al, 2000). The rats in the CS+P, CS+V, and no CS+P groups should show no significant difference in freezing on this test, indicating that propranolol is affecting long-term memory storage without interfering with normal memory processing.

Post-reactivation long-term memory test (PR-LTM). On day six, twenty hours after the PR-STM test, rats in the propranolol, vehicle and non-reactivated propranolol group were

Figure 2: Experimental design

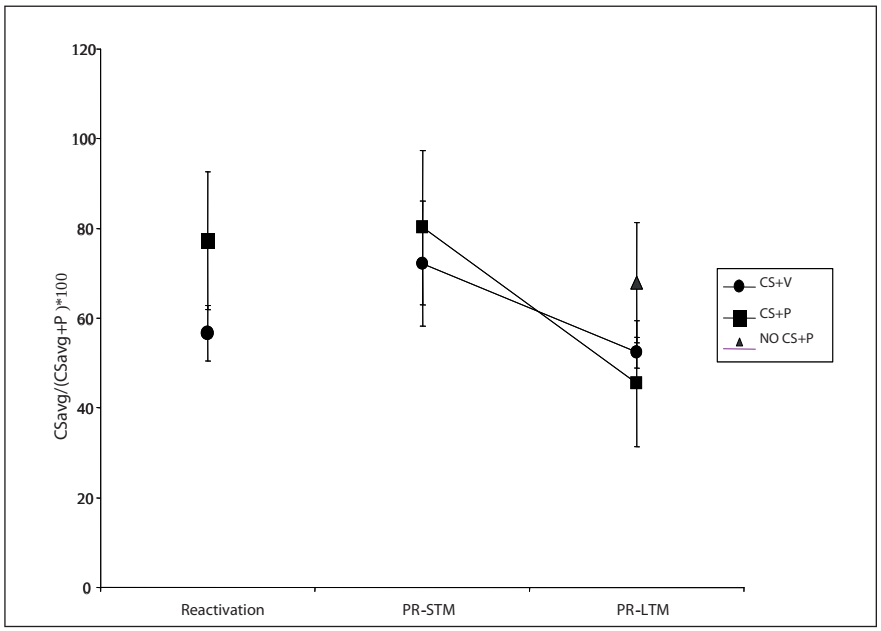

Figure 3: Averaged freezing data for the three experimental groups (CS+V, CS+P, No CS+P) in each experimental condition (Reactivation, PR-STM and PR-LTM).

transported to a dimly lit room and remained there for five minutes. Rats were placed into Context A, and after a 120 s acclimatization period, three tones with the same parameters as used in the reactivation and PR-STM trials were delivered and freezing behaviour scored.

Euthanasia. On day seven, twenty-four hours after the PR-LTM test, the animals were deeply anesthetized with urethane (1 ml/kg) and decapitated. Brains were dissected, immediately frozen on dry ice, and stored at -80 ºC for later molecular analysis.

Molecular Analysis

Brains were sliced at -20 ºC on a cryostat until the BLA was reached. BLA tissues were collected with a hollow needle and homogenized at 4 ºC using a Teflon tissue grinder in lysis buffer consisting of 10 mM Hepes/1.0 mM EDTA/2 mM EGTA/0.5 (Roche, Mississauga, ON).

Synaptoneurosome preparation. Homogenates were passed through two 100-µm-pore nylon mesh filters, then through a 5-µm-pore filter. Filtered homogenates were centrifuged at 3600 g for 10 min at 4°C. Resultant pellets were resuspended in 20 µL boiling 1% SDS for 10 min and stored at -80°C.

Western Blot Analysis. Equal amounts of proteins (30 µg) from each sample were boiled for 10 min in SDS electrophoresis sample Laemmi buffer containing beta-mercaptoethanol (Bio-Rad) and were run on a 8% SDS-polyacrylamide gel, along with a molecular weight marker (BioRad), and transferred to PVDF membranes (Bio-rad, Hercules, CA). The membranes were incubated overnight at 4°C in blocking solution (0.1 % Tween 20 and 2% bovine serum albumin in TBS), and incubated for 2 hrs at room temperature with the anti-GluR1 antibody (Chemicon). After being washed in TBS, membranes were incubated with the secondary fluorescent antibody (molecular probes).

GluR1 quantification. The membranes were scanned with a Storm Laser scanner and the signals quantified.

Results

Statistical Analysis

The behavioural data was analyzed using independent-samples t-tests or a two-way mixed analysis of variance (ANOVA), with testing interval as the within-subjects factor and group as the between-subjects factor. The molecular data was analyzed using a one-way analysis of variance (ANOVA). Significant interactions for both behavioural and molecular data were further analyzed using Tukey's post-hoc test. Type one error rate was set at 0.05. Statistica version 6.0 statistical software was used to perform all analyses.

Behavioural Results

For both the PR-STM and PR-LTM tests, the average freezing score (time spent freezing) across the three CSs was calculated (CSavg). In order to distinguish and isolate the freezing induced by the CS from the freezing induced by the context, we took the CSavg for each rat and divided it by the sum of that rat's pre-CS and CSavg freezing. This allowed us to isolate each rat's average freezing to the tone for each test, and to factor out any contextual freezing (Figure 3). All statistics were done on this modified data.

To determine that there were no significant differences between the propranolol and vehicle groups during reactivation, an independent-samples t-test was conducted. The two groups demonstrated comparable freezing during reactivation, as an independent-groups t-test, $t(4) = -1.589$, $p > 0.05$, revealed freezing of animals in the vehicle group ($M = 87.34$, $SEM = 5.67$) was not significantly different than animals in the propranolol group ($M = 73.40$, $SEM = 19.64$). A two-way mixed design ANOVA, with group (CS+P, CS+V) as between-subjects factor and test (PR-STM, PR-LTM) as within-subjects factor revealed non-significant results ($F(1, 4) = 0.15$, $p > 0.05$). However, it was observed; on the PR-LTM test, the propranolol rats froze less ($M = 45.48$, $SEM = 14.03$) than the vehicle rats ($M = 52.37$, $SEM = 5.67$).

To further quantify the amnesia observed in the propranolol group, we calculated the Amnesia Index for each group (Figure 4) (Debiec, LeDoux & Nader, 2002). This was calculated by dividing each rat's freezing score during PR-LTM by its freezing score during reactivation. A t-test, $t(4) = 0.66$, $p > 0.05$, was conducted on the averaged values and indicated no significant difference between the mean amnesia index of the vehicle group ($M = 93.13$, $SEM = 9.33$) compared to that of the propranolol group ($M = 68.98$, $SEM = 35.39$). However, the propranolol group did freeze 24.15% less than the vehicle group, indicating that propranolol treatment following reactivation produced amnesia.

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Molecular Results

We divided the BLA synaptoneurosome GluR1 levels of each rat by the average naïve value and ran the statistics on this normalized data (Figure 5). A one-way ANOVA, $F(3, 8) = 4.52$, $p < 0.05$, revealed a main effect of group. A Tukey post-hoc test revealed a significant difference between the GluR1 levels of the naïve group ($M = 100$, $SEM = 13.64$) and the vehicle group ($M = 160.72$, $SEM = 8.55$; $p < 0.05$). The Tukey post-hoc test also revealed lower levels of GluR1 in the propranolol group ($M = 125.29$, $SEM = 8.20$) as compared to the vehicle group ($M = 160.72$, $SEM = 8.55$), although these results were not statistically significant ($p > 0.05$). The non-reactivated propranolol group showed higher levels of GluR1 ($M = 136.09$, $SEM = 15.30$) than the propranolol group ($M = 125.29$, $SEM = 8.20$), and lower levels than the vehicle group ($= 160.72$, $SEM = 8.55$), but not as low as the naïve group, all of which were non-significant ($p>0.05$).

Discussion

Although not statistically significant, the results obtained demonstrate trends in the direction of our hypotheses. With regards to the first hypothesis, we demonstrated that the propranolol group demonstrated a non-significant decrease in freezing on the PR-LTM test, thereby partially replicating Debiec and LeDoux's (2004) finding. The second hypothesis was supported; trained rats that received a saline injection showed a significant increase in synaptic GluR1 levels in the BLA compared to naïve rats, which replicates the study by Yeh et al (2005). Interestingly, the results of the present study are, to our knowledge, the first demonstration that an increase in GluR1 can be detected three days after training, providing further evidence that GluR1 insertion in post-synaptic membranes can be viewed as a molecular component of the memory trace. The third hypothesis was partially supported; the propranolol group demonstrated decreased freezing with a concomitant reduction in GluR1 compared to trained rats, but these results were non-significant and thus only trends can be reported. Nonetheless, since propranolol does not affect LTM in the absence of reactivation, it appears that the effect of propranolol on the fear memory is not confounded by any effects it may have on memory systems altogether. As such. these results suggest that a blockade of reconsolidation by propranolol may reduce fear responses while reducing a molecular correlate of the memory.

This molecular information helps clarify what molecular events account for the amnesia observed when reconsolidation is blocked. Since behaviour is used as a measure of memory retention, there is some question as to the nature of the observed amnesia. Nader (2007) suggests reactivation destabilizes a memory, and blocking its reconsolidation thus interferes with its re-storage. Others suggest that the drug administration following reactivation causes amnesia by damaging the BLA. Specifically, Rudy, Biedenkapp, Moineau & Bolding (2006) cite studies showing that anisomycin can cause apoptosis, a form of programmed cell death. According to this view, the observed amnesia is not the result of deletion of a fear memory, but rather from the destruction of the tissue that would normally store it. Typically, a PR-STM test is taken four hours after reactivation and drug infusion in order to control for any nonspecific effects of the drug on the memory system altogether. This would seem to be a valid test for any lesion effects of a drug, and indeed with anisomycin, there are no such observed deficits in freezing (Nader, 2000). However, Rudy et al. (2006) argue that the lesion effect produced by anisomycin could be delayed for several hours, so that a rat would be impaired at 24 hours, but not four hours, following anisomycin infusion.

Another challenge to the observed amnesia resulting from a reconsolidation blockade was advanced by Lattal & Abel (2004), who suggest that the rats experience a transient impairment in the ability to retrieve the memory. Supporting this view, they found that rats, when tested a day after receiving anisomycin treatment following reactivation, were amnesic. However, when these animals were tested 21 days after this manipulation, the anisomycin-infused rats froze similarly to control rats, indicating that the fear memory spontaneously recovered over time. From these findings, they suggested the fear memory remains intact following reconsolidation blockade, and the amnesia observed after one day reflects a transient inability to retrieve the memory (Lattal & Abel, 2004).

The debate as to the nature of the observed amnesia results from the use of a behavioural measure of memory retention; when a rat does not exhibit fear responses following some manipulation, it is inferred that the rat is amnesic for this memory. Since there is no valid molecular measurement for the integrity of a memory, behaviour is the only way to assess memory retention. The present study, however, provides preliminary evidence to suggest that the observed amnesia is directly attributable to a reduction of GluR1, a molecular correlate of the memory. This therefore suggests that the amnesia following a reconsolidation blockade by propranolol may represent "true amnesia", in that it actually erases a component of the memory.

In light of the preliminary findings of the current study, questions still remain as to how much of the memory is erased. A recent study conducted by Rose & Rankin (2006) investigated reconsolidation in the nematode C. elegans for a nonassociative learning task called habituation. The C. elegans were repeatedly presented with a habituation stimulus (a tap). Initially, the C. elegans swam in the opposite direction of the tap, but after repeated presentations, they showed a decreased response to this stimulus. Twenty-four hours after training, the habituation memory was reactivated by the presentation of several taps followed by the delivery of a heat shock, which works like anisomycin to disrupt protein synthesis. When tested 24 hours after reactivation for the memory of the tap, these animals behaved like controls, suggesting the heat shock successfully blocked reconsolidation. Interestingly, this study also investigated GluR1 levels in the control and the heat-shock group, and found that when reconsolidation was blocked, the C. elegans not only behaved like controls, but their GluR1 levels were equivalent to that of controls. This suggested that a reconsolidation blockade actually re-set the GluR1 levels of the trained rats to that of the untrained controls.

The present study, although examining rodents using a fear conditioning paradigm, supports the results obtained in the Rose and Rankin (2006) study. Interestingly, Rose and Rankin (2006) additionally suggest that the memory trace is not only reduced, but actually erased, to the point where the C. elegans have no molecular trace for the learning of this task. This stands in contrast to the data obtained in the present study, as we demonstrate trends suggesting that propranolol reduced synaptic GluR1 to the level of the trained saline group but not to the level of the naïve rats. Importantly, Rose and Rankin (2006) provides additional evidence that the amnesia induced by a reconsolidation blockade actually decreases a molecular component of the memory, producing a "true amnesia".

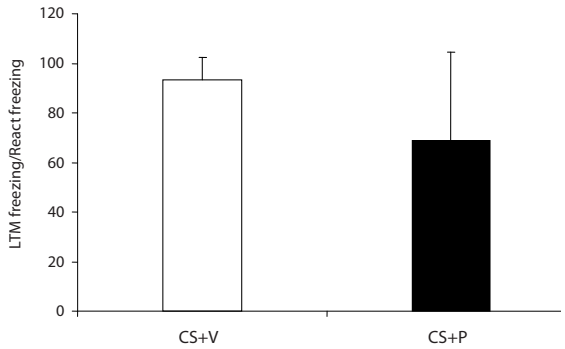

Figure 4 : Quantification of the amnesia induced by Propranolol as compared to Vehicle rats.

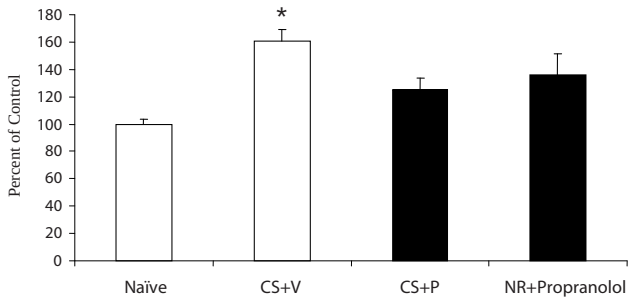

Figure 5 : GluR1 synaptoneurosome quantification in the BLA for each experimental group (Naïve, CS+P, CS+V, no CS+P) normalized to the Naïve.

Beyond these theoretical implications, our study also supports the use of propranolol in clinical treatment for PTSD. This is particularly significant because the most common treatment for PTSD is exposure-based psychotherapy, a form of extinction involving a patient's repeated exposure to the feared object or situation in the absence of any overt danger. Although believed to attenuate the associated emotional response, clinical experiments show it has a poor long-term outcome (Davis, Myers, Chhatwal, Ressler, 2006). It was initially believed that extinction represented "unlearning" at the synaptic level, in that it simply reversed the plasticity associated with acquisition. Such a theory does not reflect the literature, as extinction in both rats and humans is not long-lasting (Myers & Davis, 2002). As a result, it is currently believed that extinction is a new and distinct form of learning, resulting in the formation of an inhibitory association between the CS and US that competes with the original memory trace. This theory is more consistent with the literature, as the conditioned fear response often returns when the animals are tested in a different context, re-exposure to the US prior to testing reinstates the fear memory, and the fear responses to the CS spontaneously recover over time (Myers & Davis, 2002).

A recent study conducted by Mao, Hsaio, Ya-Hsin, Gean, & Po-Wu (2006) using a light-shock conditioning paradigm, found that extinction applied 24 hours after training reduced fear-potentiated freezing without influencing surface GluR1 levels. From this, it was proposed that although extinction reduced fear-potentiated freezing at a behavioural level, it may not affect the original memory trace at a molecular level, and this could explain why extinction training is often short-lasting. In other words, this study suggests that GluR1 might be responsible for the persistence of the memory after extinction. Interestingly, when DCS, a partial agonist for the glycine site on NMDAr was used, the rats' fear-potentiated freezing was reduced, as was the conditioning-induced increase in GluR1. From this, the authors suggested that extinction training with DCS may transform the effect of light-alone trials from inhibitory learning (extinction) to erasure (reconsolidation blockade). The preliminary results from our experiment further suggest that reconsolidation blockade may be an effective treatment for PTSD, as they indicate that a reconsolidation blockade may actually decrease a portion of the emotional component of the memory.

One limitation of the present study is that propranolol was administered systemically and not infused into brain regions of interest, as done in the Nader et. Al (2000) study with anisomycin. This raises the possibility that propranolol may exert nonspecific effects on the memory system. Although we included a control group that received propranolol without memory reactivation, propranolol may cause some long-term permanent changes in fear expression by peripheral sites of action , producing amnesia-like behaviour. Murchison, Zhang, Zhan, Lee, and Thomas (2004) addressed such a problem; prior to fear conditioning, rats were given other β-adrenergic receptor antagonists such as nadolol and sotalol, which do not readily cross the blood-brain barrier, and found no effects on freezing. This suggests that the effects of propranolol are CNS-dependent, and the observed amnesia was not due to interference with fear memory expression, but from its direct effects on the BLA. In addition, although in this study propranolol was administered systemically, we detected a specific effect on synaptic GluR1 levels in the BLA, indicating the behavioral effect of the drug was mediated at least partially via the BLA.

Another limitation of the present study concerns the increase in GluR1. Although this study is the first to our knowledge that demonstrates that levels of GluR1 remain elevated three days following the initial learning, this finding may be confounded by the test trials (reactivation, PR-STM, PT-LTM). It is possible that each test trial may reinstate GluR1, helping to maintain elevated levels of GluR1 so that it can be detected three days after the initial learning. In order to address this confound, a separate control group is required where rats are conditioned and sacrificed three days later without any test trials. This would ensure that GluR1 levels remain high even without the reminder trials, further confirming that GluR1 is a molecular correlate of the memory trace.

In conclusion, the preliminary results obtained in this experiment need to be replicated with a larger sample size in order to statistically validate the observed trends. Also, the use of central infusions of both propranolol and anisomycin into the BLA would provide further evidence that a blockade of reconsolidation actually reduces synaptic GluR1 in the BLA. This would directly correlate the observed behaviour with BLA synaptic GluR1, providing firmer conclusions. Nonetheless, the present study does demonstrate that propranolol injection following reactivation, at a behavioural level, reduces fear expression and, at a molecular level, reduces a correlate of long-term memory. These preliminary results suggest that a blockade of reconsolidation actually erases a component of the memory.

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Above all, mSURJ seeks to publish well-written articles which demonstrate scientific acumen while remaining accessible to a broad readership outside the specific discipline. mSURJ accepts articles based on the following criteria:

- clarity, organization, and style
- presentation and interpretation of result
- motivation of the study based on literature
- contribution to advancing the field of study

Peer-review
mSURJ considers the peer-review system an essential tool in manuscript selection. Its expedience is twofold:

i. It allows the board to reach informed decisions based on a fair, consistent assessment of the article
ii. It strengthens the quality of submitted work by identifying specific areas requiring improvements.

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Authors are required to identify three (3) potential reviewers at McGill University who are knowledgeable in the particular field. Including graduate students as suggested reviewers is recommended, as this yields mutually-beneficial academic experience for the authors and the reviewers. At least one of the reviewers should be a professor or recognized expert in the field. Reviewers may not be article co-authors or work under the same faculty supervisor as the authors. Note: it is imperative that reviewers do not contact reviewers prior to or during the review process, even in order to confirm their availability.

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The short-listed articles undergo the peer-review process, as a second evaluation stage. Copies of the manuscript are sent to the selected reviewers. Upon receipt of the reviews, the comments are combined with those of the editorial board to reach a decision over the article's suitability for publication.

Regardless of the outcome, reports of reviewers are forwarded verbatim to authors, along with a point-form evaluation of the article evaluation of the article justifying the decision. Three decisions are possible:

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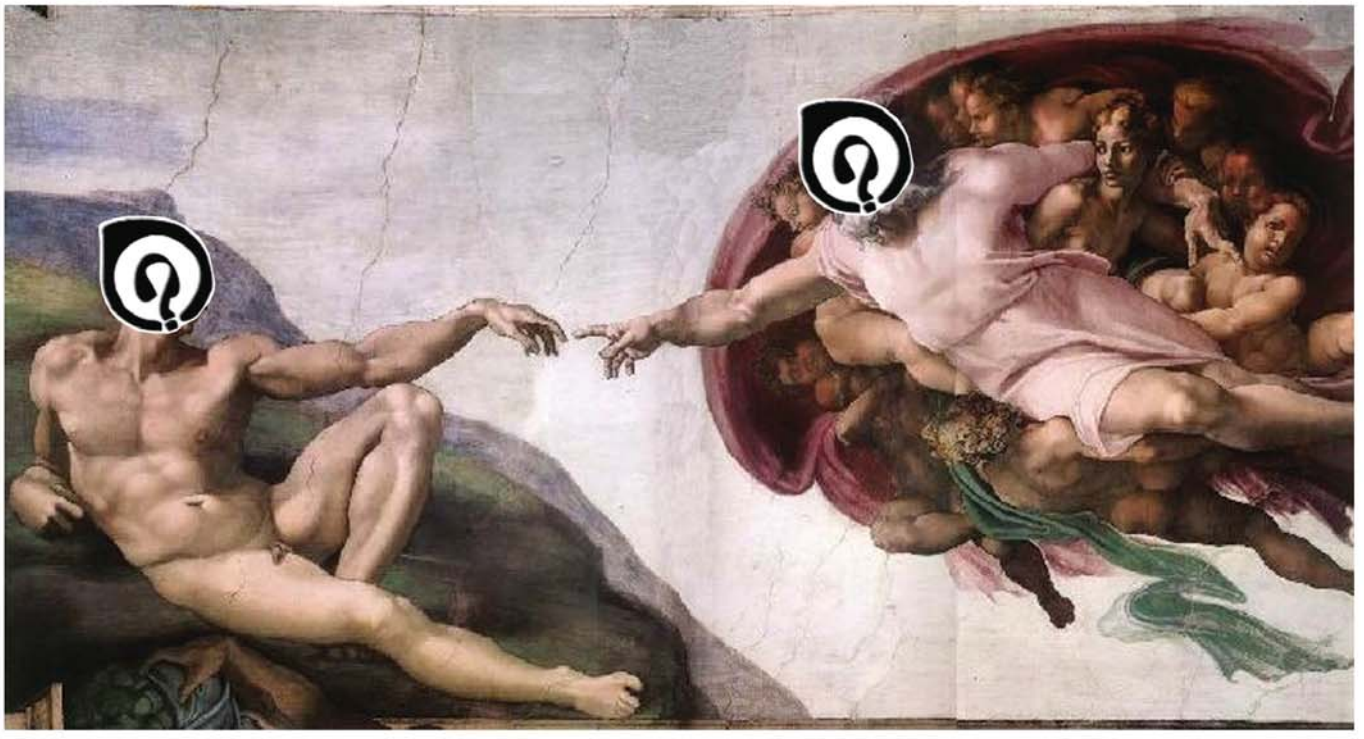

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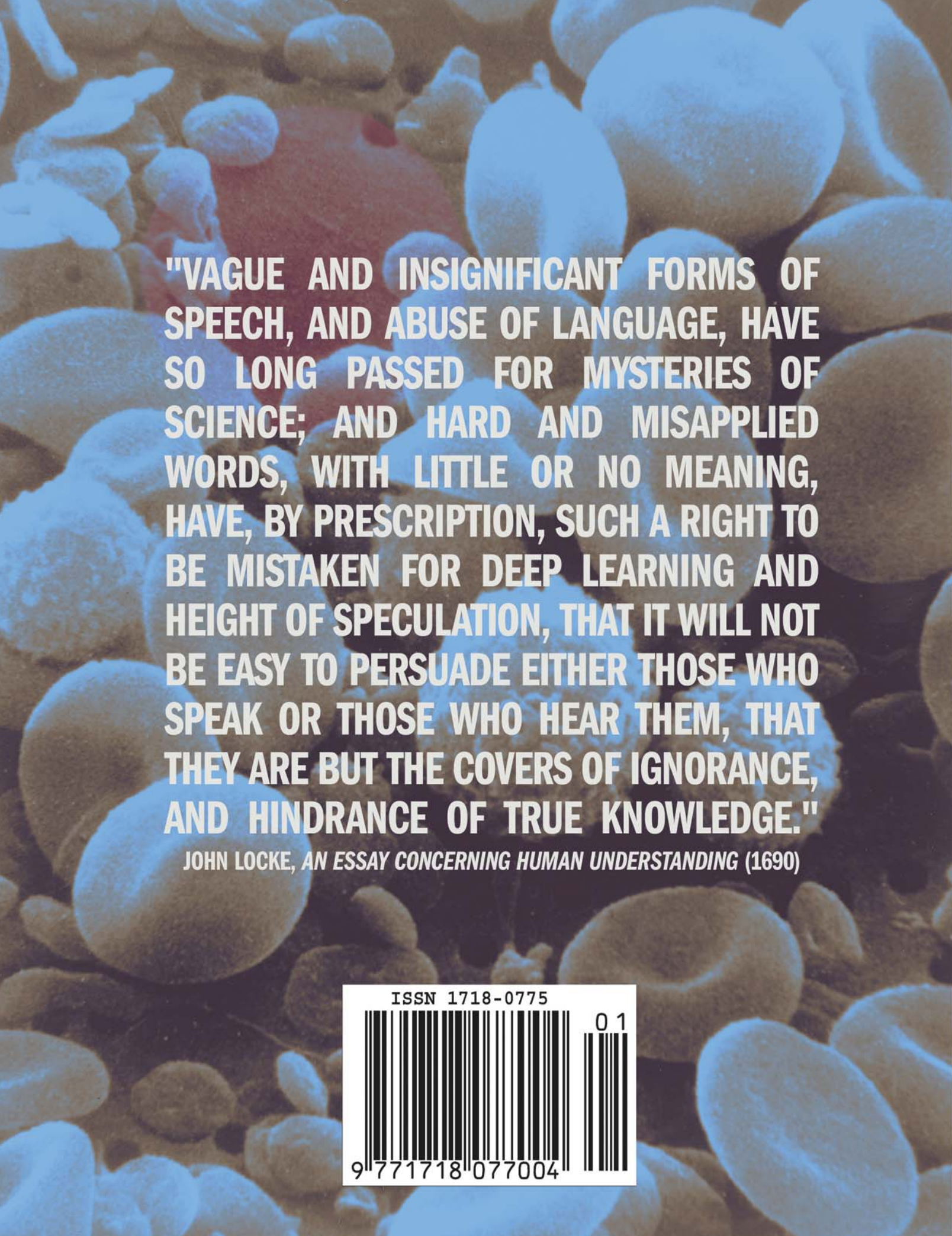

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JOHN LOCKE, *AN ESSAY CONCERNING HUMAN UNDERSTANDING* (1690)

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