Neuroscience Research Department

DIRECTOR: Dr. James L. Kennedy

The Neuroscience Research Department seeks to understand how the brain functions in mental illness and addictions. To do this, we study all levels of the brain, from the molecules through the cells and neurons to the whole brain.

To study these levels, we focus on certain areas of research. In one area, we look at the chemicals that transfer messages from one brain cell to another, the neurotransmitters. These include dopamine, serotonin, noradrenalin and glutamate. We look at the genetic code, the "blueprints," that the body uses to manufacture different brain proteins and chemical messages. If we see a genetic variant in a group of people who have a particular addiction or mental illness, then we can study why that genetic variation might create a change in brain function, which would, in turn, result in or influence the addiction or mental illness.

Another important theme in our research is the action of medications and drugs of abuse. Understanding the details of this action could help us develop novel medications, with better efficacy and reduced side-effects.

Recently, we have made some very interesting developments in our research methods. As you will see in the following pages, we have expanded the range of animal models available to study drug response and behaviour. By administering drugs, using learning paradigms and discovering or creating genetic alterations in the animals, our researchers are revealing mechanisms of action that relate to psychiatric conditions.

For example, building on a fascinating discovery by Dr. Marla Sokolowski at the University of Toronto, investigators in the Neurogenetics Section looked at a gene that controlled fruit fly behaviour. One variant of the gene created a "rover" fly, which wandered around extensively. Another variant of the same gene determined a "sitter" fly, which did not roam around as much. This fruit fly gene, PKRG1, controls a biochemical process that has a direct parallel in humans. We tested for genetic variations in the human PKRG1 gene in people with attention-deficit/hyperactivity disorder. While our initial results have not shown an effect of this fruit fly gene in human hyperactive behaviour, it nevertheless is a fascinating model for future investigation.

We continue to use the nematode *C. elegans*, introduced into the department by Dr. Van Tol, in our studies. This tiny worm, less than a quarter of an inch long, has a very simple brain that consists of a small number of neurons. Despite its brain simplicity, the worm can still learn, for example, to turn right instead of left to find a food source. The simplicity of this tiny animal is a great help in investigating how brain cells function and how they alter behaviour, illuminating processes that are relevant to humans.

Another exciting development is the use of microarray technology. This technology allows us to investigate the activity of up to 10,000 or more genes or proteins at a time in the brain.

Neuroscience Research Department

An example of our use of microarray technology is the work done recently by Dr. Albert Wong and colleagues as a collaborative effort across the Molecular Neurobiology, Neurogenetics and Neuroimaging Sections. Dr. Wong treated a group of rats with the antipsychotic medication haloperidol. He then took portions of the brain tissue from the treated rats and compared these tissues with those from a group of untreated rats. From the comparison, he was able to create a mixture of all the message molecules from the rats' brain tissue affected by the haloperidol. He then poured the mixture over a series of microarrays—small glass wafers with thousands of genetic probes printed on them. This process allowed us to see which genes were increased and which were decreased in the rat brain following treatment with the medication. In one experiment, more than 10,000 components of the brain could be investigated; from this Dr. Wong found about a dozen genes that were significantly changed by the action of the medication.

One of these genes, called 14-3-3, was then tested in a large group of people who had been diagnosed with schizophrenia; we found that certain variants of this gene occurred more often in the group of people who had schizophrenia than in a control group of people who did not have schizophrenia. Our Neuroscience Department researchers are now trying to understand better what this 14-3-3 molecule does in the brain and how it might play a role in schizophrenia.

The use of microarray technology immediately creates an enormous amount of information. A study of 10 drugs, for example, may involve microarrays for 20,000 genes and 30,000 proteins each. Multiplication of all these numbers leads to millions of potential interactions, any one of which might be involved in a mental illness. In this context, computers and information processing are central.

This field of information management, also called *bioinformatics*, is becoming more and more critical to our activities in neuroscience research. Powerful computers and vast amounts of information from microarray methods and the human genome databases can be combined with equally vast amounts of clinical information, including lists of symptoms and

variable response to drugs, to create a massive database. Computers can then probe these enormous databases to try to find the metaphorical "needle in a haystack"—a key part of the puzzle of how the brain functions in a psychiatric disorder.

We are submitting some of our discoveries for patent protection. Patent protection encourages private companies to invest in research toward new treatments or diagnostic tests. We are fortunate to have Dr. Klara Vichnevetski providing support and guidance to our researchers in the processes of patenting selected findings. This increasing collection of patents at CAMH also represents a potential source of significant financial return for our hospital, our researchers and our laboratories.

The Neuroscience Research Department continues to work closely with the Clinical Research Department. Dr. Leslie Atkinson is leading a large team of scientists to investigate the response of young children to stress; this project may help us understand how humans develop both adaptive and maladaptive responses to stress. We are also studying childhood-onset depression, attention-deficit/hyperactivity disorder, aggressive behaviour and autism.

We are beginning to investigate ways to bring neuroscience research results to a wider population; to do this, we are collaborating on education programs and joint research with the Social, Prevention and Health Policy Research Department. For example, we may conduct large-sample surveys that ask questions such as, "If we developed a blood test that could tell you if you were at risk for an addiction, how valuable would this be?" or "If a test could predict your risk for depression, would you ask your doctor for it?"

Meaningful discoveries in the molecules and mechanism of the brain can be used to develop new treatments for mental disorders and addictions. In the Neuroscience Research Department, one of our great strengths is that we are able to interact with clinical researchers. We expect that our discoveries at the laboratory bench will have applications in which predictive tests and new treatments can be evaluated at the bedside and the clinic. These evaluations will then be brought back to neuroscientists to help us refine our experiments, offering progressive improvements to psychiatric care.

Biobehavioural Pharmacology

SECTION HEADS: Drs. A.D. Le & Denise Tomkins

THE RESEARCH GOALS OF THE BIOBEHAVIOURAL Pharmacology Section are: to understand the underlying behavioural and neurobiological mechanisms that initiate and maintain alcohol dependence; and to use this understanding to explore therapeutic agents for treating alcohol dependence. The majority of our research focuses on issues related to alcohol's reinforcing ability and relapse to alcohol drinking behaviour, with an emphasis on the role of stress in relapse. We continue to explore the role of specific central neurochemical systems in regulating these behavioural processes, in addition to examining the possible role of genetic factors involved in problem drinking and concurrent problems with other substances, such as nicotine.

Neurobiological Mechanisms of Stress-Induced Relapse to Substance Use

Our research has focused on the mechanisms underlying relapse to alcohol or other drug use caused by stress. Our working hypothesis has been that exposure to stress produces behavioural disinhibition (loss of control), which leads to relapse to substance use.

We have found that, in rats, the median raphe nucleus plays a critical role in stress-induced relapse to alcohol use. This brain area sends projections containing the neurotransmitter serotonin throughout the limbic system (a brain system that underlies emotion and learning).

While serotonin originating from the median raphe nucleus is important in stress-induced relapse, we found that another neurotransmitter, GABA, also has a critical role in relapse to alcohol. Injection of the drug muscimol, an agonist of $GABA_A$ receptors, into the median raphe caused reinstatement of alcohol-seeking. We also found that injecting bicucculine, a blocker of $GABA_A$ receptors, into the medial septum (a limbic brain structure involved in behavioural inhibition) also modestly reinstated alcohol-seeking. This is further evidence that the GABA system is involved in relapse. Changes in the activity of a direct or indirect GABA-containing projection between the median raphe and the medial septum may underlie these effects.

Our preliminary data show that direct injection of muscimol into the median raphe also activates *c-fos*, a marker of activated neurons, in various brain structures in a way that is similar to that induced by exposure to footshock stress. We believe that the observed effect on relapse to alcohol induced by injection of muscimol into the median raphe is due to its impairment of inhibitory control rather than through effects on incentive mechanisms, as the results of conditioned place preference testing did not indicate that injections of muscimol into the median raphe were rewarding.

Interaction between Alcohol and Nicotine

We have also furthered our research into the co-abuse of alcohol and tobacco. We have previously shown that repeated exposure to nicotine can enhance alcohol self-administration in rats.

Using our animal model of relapse, we have found that treatment with nicotine can also promote relapse to alcohol, by producing alcohol-seeking in animals whose alcohol self-administration has been extinguished. In animals that have previously experienced both alcohol and nicotine, injection of nicotine potently reinstated alcohol-seeking.

The results from these studies have strong implications for the treatment of concurrent alcohol and tobacco addiction.

5-HT Receptor Subtypes and Alcohol Reinforcement Processes

Studies in humans and animals suggest that the central neurotransmitter, 5-HT, is associated with problem alcohol use and dependence. We continue our work to assess how modulating activity at various 5-HT receptor subtypes affects alcohol self-administration behaviour. Through this work, we hope to better understand the neurobiological mechanisms underlying excessive alcohol consumption.

One receptor of particular interest is the $5\text{-}HT_{1B}$ receptor. Human studies suggest that a locus predisposing people to antisocial alcoholism is linked to the $5\text{-}HT_{1B}$ receptor gene.

Previously, we clearly demonstrated that $5\text{-}HT_{1B}$ receptors play an important role in regulating alcohol intake in our animal models. Over the past year, we have extended these findings by exploring the behavioural basis of this phenomenon, as well as the specific brain areas that regulate it. Our data suggest that $5\text{-}HT_{1B}$ receptors are important for regulating both the initial drive to obtain alcohol as well as its consumption, particularly in highly motivated animals. Furthermore, we have now confirmed that two brain areas, the amygdala and the ventral tegmental area, are important in mediating these effects. Interestingly, activation of $5\text{-}HT_{1B}$ receptors within the ventral tegmental area leads to decreased alcohol intake, while in the amygdala, the same manipulation leads to increased alcohol intake. This difference in action shows that the regulatory effect of $5\text{-}HT_{1B}$ receptors within the brain is site-specific.

The collection of data on the amygdala is particularly intriguing, as very few reported pharmacological manipulations have increased alcohol intake in animal models. Our findings may suggest that the amygdala exerts an important modifying influence on alcohol consumption under normal circumstances. Because this brain area has been linked with

mediating the conditioned effects of psychoactive drugs that may elicit craving in humans, it is interesting that 5-HT_{1B} receptors also modify motivation to obtain alcohol in our animal models.

The 5-HT_{2C} receptor is also of interest to us. We previously reported a role for 5-HT_{2C} receptors in tonically regulating alcohol intake.

Recently, we have been trying to find how other members of the 5-HT_2 receptor family may modify the motivation to consume alcohol, by comparing our previous findings with those elicited by 5-HT_{2A} and 5-$HT_{2B/2C}$ manipulations. Our data demonstrate that 5-$HT_{2A/2B}$ receptors do alter alcohol intake; however, this alteration only occurs when the receptor is activated, not inhibited, and is most likely due to non-specific effects. Taken together, these findings suggest that, in this family of receptors, only the 5-HT_{2C} receptor constantly modulates alcohol intake.

We are continuing this line of research in the hope of better understanding the neural circuitry that helps regulate drinking behaviour.

$GABA_A$ Receptor Subunits, Drinking Behaviour and Voluntary Intake

Compelling evidence suggests that central GABAergic systems play an important role in regulating alcohol's effects, particularly those effects mediated via the $GABA_A$ receptor.

We continue to investigate regional differences in the expression of the $GABA_A$ receptor subunits. These differences have been demonstrated in the brains of high-alcohol preferring rats, and humans with drinking problems; therefore, they may represent one of the neurobiological factors underlying problem alcohol use.

The data generated thus far demonstrate that regional differences in $GABA_A$ receptor expression and subunit conformation affect drinking behaviour. We have found that these differences also affect the binding profile of some pharmacological agents that interact with this receptor complex, including muscimol, flunitrazepam and diazepam, but not others, such as zolpidem. There appears to be a complex interaction between inherent alcohol preference, alcohol drinking history and the binding ligand employed.

Currently, we are analysing and interpreting the extensive database generated over the last year. Our data will provide important insights not only into the genetic and non-genetic $GABA_A$ receptor influences on alcohol preference and consumption, but also into potential interactions with, and/or influences over, other clinically used pharmacological agents that interact with this receptor complex, such as the benzodiazepines.

Sex Differences in Susceptibility to Alcohol-Induced Cognitive Deficits

To develop more effective treatment and prevention strategies for problem alcohol use in women, we must conduct basic research on the differences in alcohol's effects on brain function in men and women.

Clinical evidence suggests that women are more vulnerable to some of the negative effects of alcohol than men. While tests of psychomotor performance have consistently reported that men and women are equally impaired by alcohol ingestion, women appear to be more sensitive to the cognitive deficits induced by long-term alcohol exposure, particularly on tasks demanding divided attention or delayed recall.

Over the past year, we have continued to explore the long-term effects of alcohol exposure on cognitive function and behaviour, with specific emphasis on potential differences in susceptibility between males and females.

To support the clinical relevance of our experimental approach, we showed that, similar to humans, chronic alcohol exposure caused equal impairment of female and male rats

on measures of psychomotor performance. As predicted from the human literature, female rats showed greater impairments on measures of delayed recall compared to their male counterparts when chronically exposed and then withdrawn from alcohol. Alcohol-exposed female rats also demonstrated a blunted response for obtaining a sweetened food reward compared to males. One possible interpretation of this finding is that alcohol exposure has elicited a dysphoric state in the females but not the males. We are interested in this difference because, clinically, women are more likely to present with concurrent alcohol problems and depression than are men. Currently we are examining the brain mechanisms that may potentially explain these sex differences.

In our work, we hope to unravel some of the gender differences in susceptibility to alcohol-induced cognitive impairments and uncover the role of $GABA_A$ receptors in these impairments. Ultimately, this research could help identify risk factors, protective factors and treatments for alcoholism that are specific to women.

THE BIOPSYCHOLOGY SECTION STUDIES THE biological foundations of normal and abnormal behaviours relevant to psychiatry. Our work focuses on the role that brain neurotransmitter systems—particularly the serotonin and dopamine systems and the interactions between these systems—play in controlling behaviour. We mainly use pharmacological and/or lesioning procedures to manipulate specific aspects of neurotransmitter function and to observe the resulting changes in behaviour. Our work includes studies of the neurochemical mechanisms involved in addictive behaviour, cognitive behaviour relevant to schizophrenia and impulsive behaviour.

Serotonin Receptors and the Effects of Drugs of Abuse

Dopamine has been the neurotransmitter most closely linked to the behavioural and neurochemical effects of drugs of abuse. However, manipulating serotonin (5-HT) function also leads to changes in the behavioural effects of drugs of abuse.

In recent years, we have shown differing roles of two serotonin receptor subtypes, the $5\text{-}HT_{2A}$ and $5\text{-}HT_{2C}$ receptors, in modulating effects of cocaine. We have found that activating the $5\text{-}HT_{2C}$ receptor reduces the locomotor stimulant and reinforcing effects of cocaine. Blocking this receptor leads to the opposite profile of effects. In contrast, blocking the $5\text{-}HT_{2A}$ receptor reduces the stimulant effects of cocaine. In interpreting these results, we are trying to find whether the effects of $5\text{-}HT_{2C}$ receptor agonists and antagonists alter the effects of other types of reinforcers, or whether they are specific to drug reinforcers.

We now have shown that, while the $5\text{-}HT_{2C}$ agonist also reduces the reinforcing effects of food, the $5\text{-}HT_{2C}$ antagonist does not enhance the reinforcing effects of food. The former effect is consistent with the notion that elevations in serotonin result in a generalized reduction in motivated behaviour. However, the latter finding implies that reducing serotonin

We have found that treatment with nicotine can also promote relapse to alcohol.

transmission via the 5-HT$_{2C}$ receptor may have an effect that is restricted to cocaine.

Amphetamine Sensitization and Schizophrenia

Dr. Fletcher is part of a large, multidisciplinary project, led by Dr. Shitij Kapur (Schizophrenia Research Section), that received funding from the Ontario Mental Health Foundation to investigate amphetamine sensitization as a model for schizophrenia. Part of the rationale for this study is the observation that repeated psychostimulant use can induce psychosis in humans. In the Biopsychology Section, we will first examine whether amphetamine sensitization results in cognitive deficits that are also found in schizophrenia.

We have focused our attention on measuring changes in three behaviours; these behaviours are impaired in some people who have schizophrenia. The first is prepulse inhibition of the acoustic startle reflex, which reflects abnormal sensorimotor gating. The second is latent inhibition, which measures the ability to tune out, or ignore, irrelevant stimuli. The third can loosely be described as "cognitive flexibility," which is measured in humans by the Wisconsin Card Sorting Test.

Our results to date strongly indicate that amphetamine sensitization markedly disrupts these behaviours; this disruption is analogous to the disruptions that are observed in schizophrenia. Our results suggest that amphetamine sensitization appears to be a valid model for cognitive disturbances in schizophrenia.

In our future work, we will examine whether deficits caused by amphetamine sensitization can be reversed by antipsychotic drugs, and we will explore the neurobiological changes that may underlie these deficits.

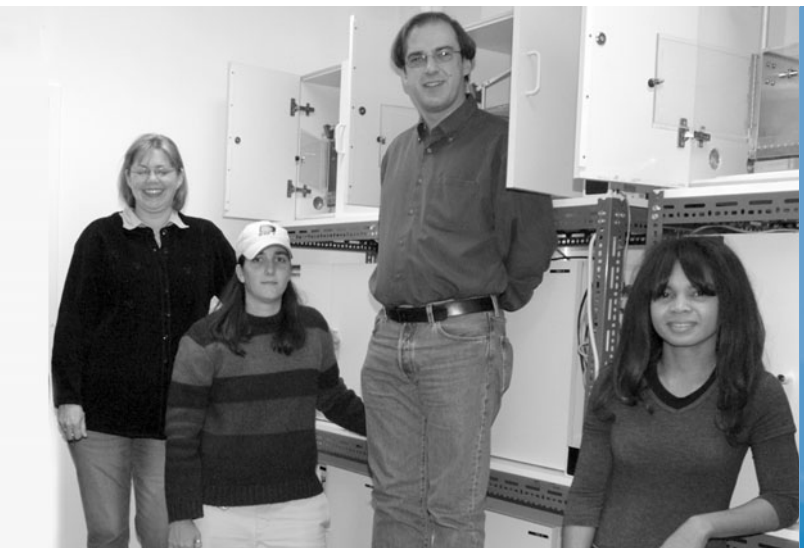
Serotonin and Impulsivity

Impulsive behaviour is associated with reduced serotonin function, both in humans and in animals. However, we know little about which areas of the brain are involved in mediating impulsive behaviour, or which serotonin receptors are involved. We continued to explore both questions in the past year.

We compared the effects of lesioning either the prefrontal cortex or the nucleus accumbens to deplete serotonin innervation of these areas in tasks where subjects receive food reinforcement if they show a degree of inhibitory control. Our results to date suggest that depleting serotonin in the nucleus accumbens, but not the prefrontal cortex, leads to reduced inhibitory control.

In a second project, in collaboration with Dr. Guy Higgins (Schering-Plough Research Institute, New Jersey), using subjects receiving reinforcement for inhibitory control, we have found that 5-HT$_{2A}$ receptor antagonists reduce impulsive behaviour, but 5-HT$_{2C}$ receptor antagonists enhance this behaviour. Overall, our findings suggest that the relationship between reduced 5-HT activity and impulsivity is complex and may depend on which brain areas and which receptor subtypes are affected.

Our observation that 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors have opposing influences on impulsive behaviour is similar to our finding that these receptors exert opposing influences over the expression of cocaine-mediated effects. This work is beginning to reveal the diversity and complexity of 5-HT receptor function at the behavioural level.



Clinical Neuroscience

SECTION HEAD: Dr. Usoa Busto

THE CLINICAL NEUROSCIENCE SECTION CONDUCTS human experimental research to better understand the different factors that influence substance use and dependence as well as other forms of compulsive or addictive behaviour such as gambling. These factors can relate to the drug, the host and the environment.

HOST FACTORS CONTRIBUTING TO SUBSTANCE USE DISORDERS

Dr. Usoa Busto

WE CONTINUE OUR RESEARCH INTO HOST FACTORS contributing to substance use disorders, including multiple drug use, psychiatric comorbidity and genetics.

Depression and Dopaminergic Pathways

Ongoing studies in this area continue to examine the role of the brain reward system in major depressive disorder (with Drs. Claudio Naranjo, Helen Mayberg and Simon Graham). We have shown that the brain reward systems (mesocorticolimbic dopaminergic pathways) are altered in people who are severely depressed and that specific areas of the brain are involved in the response to a dopaminergic probe.

Nicotine and Depression

The role of nicotine in modulating symptoms of depression in depressed smokers and non-smokers is another area under active research (with Drs. Laura Cardenas, Martin Zack, Sylvain Houle, Shitij Kapur and Helen Mayberg). Data from ongoing positron emission tomography (PET) studies show that dopamine release in depressed smokers is significantly lower compared to depressed non-smokers. This difference suggests the possibility of a hypofunctional mesocorticolimbic dopaminergic system (Cardenas et al., 2002).

Dr. Peter Selby and colleagues have also received funding to train professionals to be more effective in treating nicotine dependence. We now have a protocol approved to examine the mechanisms of craving for nicotine in both current and abstinent smokers (with Drs. Selby and Laurie Zawertailo).

Brain Responses to Amphetamine and Hydromorphone

This year, we completed a neuroimaging study showing that changes occur in specific areas of the brain in the response to amphetamine (Tremblay et al., 2003, in preparation).

Other research in humans has shown that oral d-amphetamine administration is associated with a prolonged displacement of [11C] raclopride, which is sustained at six hours post-drug (Cardenas et al., in press), even though the subjective effects of the drug dissipate within three hours. This finding demonstrates longer-term effects of d-amphetamine on dopamine release than has been previously demonstrated.

We also completed a study using hydromorphone as a probe for the dopaminergic and opioid systems. Results suggest that hydromorphone acts differently from amphetamine on dopaminergic/opioid pathways—in people with depression, hydromorphone caused a reported decrease in negative symptoms (e.g., sedation) and little change in positive symptoms.

Hypnotic Medications in Older Adults

We continue to study the effects of hypnotic medications in older adults (with Drs. Beth Sproule and Nathan Herrmann), in the hope of learning more about age as a host factor for substance use problems.

In our comparison of prescription versus non-prescription sleeping medications, we have shown that valerian produced effects that were similar to placebo. We have concluded that valerian, when compared to a benzodiazepine and an antihistamine, is not an effective medication to help older adults with sleep problems (Glass et al., in press). We are currently

conducting a larger trial comparing the benzodiazepine with the antihistamine.

Prescription Drug Dependence

We have received approval for a novel adjunctive treatment for opioid dependence, using the NMDA antagonist dextromethorphan to alleviate withdrawal and tolerance. We also plan to further examine the mechanisms of craving in people who are opioid-dependent using functional magnetic resonance imaging (F-MRI) of brain activity (with Drs. Simon Graham and Laurie Zawertailo).

Abuse Liability of Drugs

The intrinsic pharmacological characteristics of drugs of abuse (such as potency, the ability to produce reinforcing effects and drug kinetics) are essential to drug-taking behaviour. One line of our research looks at the comparative abuse liability of available drugs and new compounds.

We have recently completed a study examining the comparative pharmacology, behavioural effects and abuse potential of heroin and hydromorphone in human subjects (with Drs. Bruna Brands and David Marsh), and we have funding for another crossover study of hydromorphone in people who are opioid-dependent (with Drs. Brands and Marsh). We also plan to expand our research to study aspects of alcohol dependence, such as gender differences in cognitive effects of alcohol (with Drs. Denise Tomkins, Constantine Poulos, Martin Zack and Laurie Zawertailo).

PHARMACOLOGICAL MODULATION OF ADDICTION-RELATED COGNITIVE NETWORKS AND RELATED PROCESSES

Drs. Martin Zack & Constantine X. Poulos

THE MAIN FOCUS OF OUR RESEARCH IS TO LEARN how addiction-related memory structures are activated, and how this activation underlies and interacts with the motivation or craving to engage in addictive behaviour, comorbidity factors (e.g., concurrent addiction and mental health problems), environmental factors (e.g., stress) and, in some cases, measures of addictive behaviour itself.

As we examine the key processes of activation and inhibition, we look at ways in which medications, alcohol, other drugs and addictive reinforcers like gambling alter semantic memory structures in healthy people and in people with addictive disorders. We are trying to determine if addiction-related disturbances contribute to deficits in self-regulation by activating or by inhibiting the cognitive processes that maintain regulation under normal circumstances. Activation of semantic memory networks is important for three reasons. 1) It occurs involuntarily and, in some cases, without conscious awareness. 2) It can bias decisions and overt behaviour toward addictive reinforcers and away from adaptive alternatives. 3) It can be measured simply and accurately, using response time tasks administered by computer. For these reasons, studying the activation of semantic memory networks is a useful way to define the motivation behind addictive behaviour.

We have developed a procedure, called The Lexical Salience Task, to assess the effect of a *pharmacological* prime rather than a verbal prime on substance use or other addictive behaviour. For example, if activation of brain catecholamines contributes to the motivation to gamble, this procedure can show us if the difference in reading speed to gambling words

In our comparison of prescription versus non-prescription sleeping medications, we have shown that valerian produced effects that were similar to placebo.

(e.g., wager) versus neutral words (e.g., window) is greater under a dose of the catecholamine agonist, amphetamine, than under placebo. Similarly, if activation of brain GABA transmission contributes to motivation to drink, the difference in reading speed to alcohol words (e.g., beer) versus neutral words (e.g., board) should be greater under a dose of the GABA agonist, diazepam, than under placebo. We have investigated these two areas in the research projects outlined below.

The Role of Dopamine in Gambling

In a previous study, we found that, in people who have gambling problems, amphetamine: primes gambling cognitions; inhibits neutral cognitions; increases urge to gamble; and decreases confidence to avoid gambling. Amphetamine had no such effects in controls.

Although dopamine is the primary neurochemical activated by amphetamine, amphetamine also activates other neurochemicals, including norepinephrine and serotonin.

In our current study, we hope to isolate the role of dopamine in motivation to gamble, gambling-related cognitions and actual betting behaviour in people who have gambling problems. We will assess the ability of the selective dopamine antagonist, haloperidol, to reduce desire to gamble and gambling-related semantic activation induced by a brief gambling episode in problem gamblers and in age- and gender-matched controls. We will also investigate the effects of dopamine blockade on patterns of betting behaviour during the gambling episode.

We predict that, relative to placebo, haloperidol will reduce post-gambling desire to gamble as well as activation of gambling cognitions on The Lexical Salience Task. If dopamine also influences patterns of gambling behaviour, haloperidol should dampen the overall escalation in bet size and reactivity to wins and losses that characterize problem gambling behaviour.

This project is funded by a grant from The Ontario Problem Gambling Research Centre.

Priming Effects of Benzodiazepines on Alcohol-Related Cognitions and Drinking Behaviour

This project examines the priming effects of two benzodiazepines on automatic alcohol-related cognitions and drinking behaviour in people who have drinking problems. We will compare the effects of diazepam, a drug with high abuse liability, with those of clonazepam, a drug with low abuse liability. We will also examine how drug dose, severity of alcohol problems and the degree of co-existing anxiety affect cognitive and behavioural responses to these drug probes. Our findings will lay the foundation for future research with other pharmacological probes to better characterize the specific neurochemical substrates of motivation to drink in people who have drinking problems and varying degrees of anxiety.

Preliminary data indicate that low-dose diazepam (5 mg) primes alcohol-related cognitions on The Lexical Salience Task. The degree of lexical priming also predicts the volume of beer consumed in a taste-test drinking procedure (cf. Marlatt et al., 1973). In addition, the degree of priming correlates with the severity of alcohol use, as measured by drinks per week. Drinks per week did not mediate the correlation between lexical priming and taste-test drinking.

Consistent with a previous study (Zack et al., 1999), high-dose diazepam (15 mg) significantly reduced the ability of negative-affect words (e.g., tense) to prime alcohol-related cognitions (e.g., beer) in a conventional lexical decision semantic priming task in drinkers with no history of benzodiazepine use. Notably, diazepam had no effect on neutral, categorical priming (e.g., cat-dog), and negative-affect alcohol priming was clearly evident under placebo.

Together, these diazepam data indicate a homeostatic priming and satiety process, moderated by dose, in people who have drinking problems. The findings corroborate the utility of drug priming of addiction-related memory networks as a means of assessing medications that may reduce problem drinking. This procedure may be especially useful for investigating individual differences in the neurochemical basis of alcohol priming to predict which medication(s) will be beneficial to a particular profile of drinking problem.

Effects of Alcohol on Stress-Induced Cognitive Activation in Young Drinkers

This project is being carried out in collaboration with Dr. Colin M. MacLeod of the Department of Psychology at the University of Toronto.

We are building on previous grant-funded research to evaluate the effects of alcohol on automatic alcohol- and anxiety-related cognitions, induced by a stressor, in university undergraduates with high or low anxiety sensitivity. People who have high anxiety sensitivity are more likely to use alcohol to cope with negative mood states; they have higher rates of drinking problems than do people who have low anxiety sensitivity. The current study will determine the possible mediating role of semantic network dampening in the negative reinforcing effects of alcohol in high-anxiety-sensitive drinkers.

Initial data indicate that, relative to placebo, a moderate dose of alcohol (BAC = .06%) primes alcohol-related cognitions in low-anxiety-sensitive but not in high-anxiety-sensitive drinkers. Relative to a soft drink, placebo alcohol

(de-alcoholized beer) reduces activation of anxiety-related and alcohol-related cognitions in both low- and high-anxiety-sensitive subjects.

If these preliminary results persist when the sample is complete, they will provide a basis for examining interventions to modify alcohol-induced and expectancy-related memory activation in young people at risk for alcohol problems (cf. Breslin, Zack & McMain, 2002).

This project is funded by a grant from The Alcoholic Beverage Medical Research Foundation.

Deficient Inhibitory Control and MDMA

This study is being carried out in collaboration with Dr. Paul Fletcher of the Biopsychology Section. Dr. Stephen Kish (Human Neurochemical Pathology Laboratory) and Dr. Constantine X. Poulos (Clinical Neuroscience Department) are consultants.

Chronic use of 3, 4-methylenedioxymethamphetamine (MDMA, ecstasy) has been linked with lasting damage to brain serotonin (5-HT) neurons in rodents and non-human primates. People who use MDMA consistently display deficits in memory, which correlate with deficits in 5-HT function. Although some research has found impulsivity in chronic MDMA users, these previous findings are of limited value, as study participants used a variety of other psychoactive drugs as well as MDMA.

In this study, we are trying to assess fully the cognitive inhibitory processes involved in impulsivity in MDMA users and to determine the impact on impulsivity of prior MDMA versus other drug use.

We have assessed three groups of subjects: (1) people who use MDMA plus low levels of other substances (the normative pattern of MDMA use), (2) people who use marijuana-only and (3) drug-free controls. We are also testing a fourth group (4) people who use MDMA plus marijuana-only (a minority of MDMA users).

All subjects were drug- and alcohol-free at the time of testing. They had been abstinent from all drugs for at least five days, as verified by urinalysis. Assessment of hair samples will verify drug use in the six months preceding testing.

The data in hand suggest that, across a range of tasks, people in the marijuana-only group show as much or more impulsivity as do the people who use MDMA plus other drugs. Both of these groups tended to be more impulsive than controls, although the differences were not consistent. In contrast to these group mean results, correlational analyses indicated a consistent positive correlation between lifetime use of MDMA (tablets) and impairment on the various tasks, whereas level of marijuana use was unrelated to impairment on any task.

These results suggest that, in chronic MDMA users, prior use of marijuana may contribute to some observed deficits in impulse control, but also that heavier MDMA use is associated with poorer impulse control. Because levels of MDMA and marijuana use were not inter-correlated, these substances may exert separate adverse effects on impulse control. However, marijuana is retained in the body long after ingestion, so the results may be affected by residual or hangover effects of recent use (i.e., one week pre-testing). We plan to assess prior users of MDMA and marijuana-only users with at least six months abstinence to clarify the lasting effects of these drugs on impulse control.

This project is funded by the CAMH Grants in Psychiatry program.

We predict that, relative to placebo, haloperidol will reduce post-gambling desire to gamble as well as activation of gambling cognitions.

Human Neurochemical Pathology Laboratory

SECTION HEAD: Dr. Stephen Kish

THE MANDATE OF THE HUMAN NEUROCHEMICAL Pathology Laboratory is to understand the causes of neuropsychiatric disorders by examining the human brain.

We continue to focus on studies of brain monoamine neurotransmitter systems in people who use amphetamine derivatives (including ecstasy) and in people with Parkinson's disease.

Ecstasy

Ecstasy is a widely used amphetamine derivative taken for its mild stimulant property and for its ability to increase the desire for friendliness.

We initiated a neuroimaging investigation, using positron emission tomography (PET) to determine if ecstasy causes brain damage in young users of the drug. All subjects were tested by forensic hair analysis to confirm that the person actually used the drug (rather than another drug or combination of drugs sold as ecstasy).

We found the following:

1. Many people in the Toronto area who assume that they are using only ecstasy are unknowingly using more dangerous drugs (including other amphetamine derivatives), which "contaminate" the "ecstasy" tablets. The general public needs to be educated more about the lack of consistency in the quality of "ecstasy" that is being sold.
2. Our forensic drug hair analyses suggest that most, but not all, ecstasy users respond, to the best of their knowledge, truthfully to questions about past drug use.
3. Although rare, a very small number of people in the Toronto area can be identified as using ecstasy in the absence of other drugs that cause brain damage.
4. We have conducted brain scans of a small number of these "pure-ecstasy users" and have preliminary data about ecstasy's ability to damage brain serotonin neurons. A large replication study is now in progress.

Parkinson's Disease

Parkinson's disease (PD) is a movement disorder commonly associated with clinically significant depression.

Recent studies now confirm the clinical impression that the depression in PD affects the quality of life of the patient more than the motor disability.

Based on the longstanding hypothesis that a brain serotonin deficiency might be responsible for the depression in PD, we conducted a PET investigation to measure the number of serotonin neurons in people with PD who are depressed.

Contrary to the hypothesis, our preliminary data suggest that people with PD who are depressed, who are early in the course of their disorder, do not show a reduced number of brain serotonin neurons. A replication study is in progress.

Laboratory of Cellular and Molecular Pathophysiology

SECTION HEAD: Dr. Jerry Warsh

RESEARCH IN THE LABORATORY OF CELLULAR AND Molecular Pathophysiology Section investigates the cellular and molecular processes that lead to the development of the major psychoses, principally bipolar affective disorder. We also explore the molecular pharmacology of current mood stabilizer and antidepressant medications to understand their mode of action, in hopes of finding more specific cellular targets for drug action against which new drugs can be developed. The research team includes Dr. Jerry Warsh, clinician scientist, Dr. Peter Li, senior basic scientist, and their graduate student and postdoctoral trainees.

Our team is internationally recognized for groundbreaking, innovative research on intracellular signalling abnormalities; these abnormalities are now recognized to play a critical role in the predisposition for, and development of, bipolar I disorder.

The closer we come to understanding the specific chain of cellular disturbances that lead to bipolar disorder, the more effectively we can work to develop new strategies to treat and prevent it. To reach our goals, we have set up new equipment and are developing techniques to measure cellular changes in patients. This measurement infrastructure will help us translate our research findings into clinical tests, which may make it easier to diagnose subtypes of bipolar disorder and guide the choice of mood-stabilizer medications in treatment. Our research also sets the stage for the development of new drugs to treat this disorder and prevent relapses.

The important directions of the research in the section have been recognized in recent research grants from the Canadian Institutes for Health Research, the Ontario Mental Health Foundation and the National Alliance for Research in Schizophrenia and Depression.

cAMP Signalling System and Bipolar Disorder

During the past year, we elaborated more in-depth details of the nature and extent of abnormalities in the cyclic adenosine monophosphate (cAMP) signalling system in brain of people with bipolar disorder. These abnormalities appear to funnel through a key receiving protein, cAMP-dependent protein kinase. This protein "translates" dissonant signals into cellular signalling cascades. The resilience of cells in brain tissue can be affected if the cAMP signalling system is disrupted.

We have found key evidence that cAMP signalling is increased in bipolar disorder in specific brain regions involved in mood regulation. Also, the target protein (cAMP-dependent protein kinase) changes its composition, levels and response in a way that may be maladaptive.

The patterns of changes in cAMP-dependent protein kinase suggest that the processes that regulate its composition and positioning have been changed in a way that affects its breakdown in brain neurons. This is a second, key piece of evidence we have found, suggesting that altered proteomic mechanisms are likely involved in the development of bipolar I disorder.

Calcium Signalling in Bipolar Disorder

We continue our in-depth analysis of parts of the intracellular calcium signalling system, which is also disturbed in bipolar disorder. Calcium signalling also plays critical roles in maintaining the resilience of cells: disruption of calcium signalling can lead to cell death. The abnormalities found in the cAMP signalling cascade in bipolar disorder take on even greater importance in light of their relationship with calcium signalling: there are several bridging points at which cAMP signalling modifies what the calcium signalling systems are doing.

Molecular Pharmacology of Mood Stabilizers

Investigations on the molecular pharmacology of mood stabilizers have led us to identify several novel genes that are regulated by long-term lithium treatment. One of the genes codes for a key enzyme in the metabolism of a type of sugar, inositol; inositol is converted to chemical messengers, the high-energy inositol polyphosphates. The other gene encodes a membrane-spanning protein that may act as a "signal complex," co-ordinating the localized formation of signalling lipids and the positioning of the target signalling protein, protein kinase C, at the inner side of nerve cell membrane.

These observations have uncovered previously unknown targets of lithium. Because these targets are affected in the same range as the blood levels achieved during lithium treatment, they are likely related to lithium's therapeutic actions.

Molecular Neuroscience

SECTION HEAD: Dr. Hubert H.M. Van Tol

THE GOAL OF THE MOLECULAR NEUROSCIENCE Section is to understand the mechanisms by which neural communication takes place. We seek to understand the molecular components involved in communication between neurons, how these components may contribute to mental illness and how they serve as therapeutic targets.

The section has four principal investigators directing their own research groups. Dr. Hubert Van Tol is a University of Toronto Professor in the Departments of Psychiatry, Pharmacology and Institute of Medical Science, and Canadian Research Chair in Neurobiology (tier 1). Drs. Fang Liu and Albert Wong are University of Toronto Assistant Professors in the Department of Psychiatry, and Dr. Xian-Min Yu is an Assistant Professor at the University of Toronto Faculty of Dentistry.

Our investigators use molecular, genetic, biochemical and electrophysiological approaches to study the molecules involved in neuronal signalling. Our scientists mainly use in vitro approaches and model systems, including transgenic mice and the nematode *C. elegans*, for their research. We collaborate with other scientists—usually from the Neurogenetics Section at CAMH—to extend our findings to human disease.

We associate with many neuroscientists in Toronto (http://www.uoftphysiology.com/neurosciencenet/governance.html) and outside Toronto; we are also members of the CIHR group The Synapse (http://www.utoronto.ca/synapse/).

MOLECULAR NEUROBIOLOGY I

Dr. Hubert H.M. Van Tol

THIS GROUP FOCUSES ON THE DOPAMINE SIGNALLING system in the central nervous system. The dopamine signalling system is often considered the origin of, and/or one of the main targets for therapeutic intervention for, the symptoms of several psychiatric and neurological disorders, including schizophrenia, bipolar disorder, Huntington's disease, Parkinson's disease, Tourette's syndrome, addictions and attention-deficit/hyperactivity disorder. We hope to understand the individual components involved in the dopamine signalling system, so we can evaluate how the system contributes to development of disease, improve therapeutic interventions and minimize treatment side-effects.

In our current research, we are trying to unravel the intracellular signalling pathways that mediate the effects of dopamine.

Intracellular signalling cascades are initiated through the interaction of dopamine with a specific receptor on the plasma. In humans, five different dopamine receptors have been identified. These receptors mediate different physiological and biochemical effects, but are all members of the so-called G protein-coupled receptor (GPCR) family.

New research by our group has revealed that these GPCRs, particularly the dopamine receptor subtypes that are targets for antipsychotic medication, can activate growth factor receptors, such as the platelet-derived growth factor receptor beta. Growth factor receptors are critical for the development, survival, differentiation and synaptic plasticity of neurons.

This was a novel observation; however, we did not know its relevance in vivo. In collaboration with Dr. John F. MacDonald (Department of Physiology, University of Toronto), we found that transactivation is also critical for the mechanism by which dopamine receptors can reduce N-methyl-D-aspartate (NMDA) receptor activation in hippocampal and cortical neurons. We continue our studies to understand the mechanism by which dopamine receptors modulate NMDA receptor activity. The NMDA receptor is an ion channel that is activated by the major neurotransmitter glutamate, and it is known to be critically involved in synaptic plasticity, learning and memory and has been strongly implicated in psychosis. Our work identified a novel signalling

cascade by which antipsychotic medication may modulate NMDA receptor signalling.

G protein-activated inwardly rectifying K+ channels (GIRK; a.k.a. Kir3) are known effectors of dopamine receptors. These channels regulate the excitability of the cell and play an important role in the feedback regulation of dopamine release. We still do not know the precise nature of the channel-receptor relationship. We used molecular and biochemical approaches to show that the dopamine receptor and GIRK channel form a stable complex early during their synthesis. The observation that the receptor-channel complex is stable may help us understand how temporal control of synthesis of the individual components regulates GPCR-activation of different signalling pathways. We continue to investigate the molecular determinants of this interaction.

MOLECULAR NEUROBIOLOGY II
Dr. Fang Liu

OUR LAB CONTINUES TO FOCUS ON THE MOLECULAR mechanisms by which G-protein coupled dopamine D1 receptors exert functional cross-talk with NMDA receptors. Previously, we found that dopamine D1 receptors modulate NMDA glutamate receptor-mediated functions through a direct interaction of these two proteins. One interaction is involved in the inhibition of NMDA receptor-gated currents, and the other is implicated in the attenuation of NMDA receptor-mediated excitotoxicity.

The D1 receptor subtype is not a target for classic antipsychotic medication, but has been shown to play a role in working memory. This subtype is often thought to contribute to the "negative" symptoms of schizophrenia, which are not readily treated with classic antipsychotic medication.

The NMDA receptor is one of the ligand-gated ion channels that is activated by the major excitatory neurotransmitter glutamate. Functionally, this ion channel is implicated in synaptic plasticity, learning and memory, but it also plays an important role in excitotoxicity and stroke. Psychotropic drugs like phencyclidine (PCP), that mimic schizophrenic symptoms, are known blockers of NMDA receptors. Furthermore, genetic disruption of the gene encoding this channel in mice results in animals that exhibit behaviour changes related to schizophrenia.

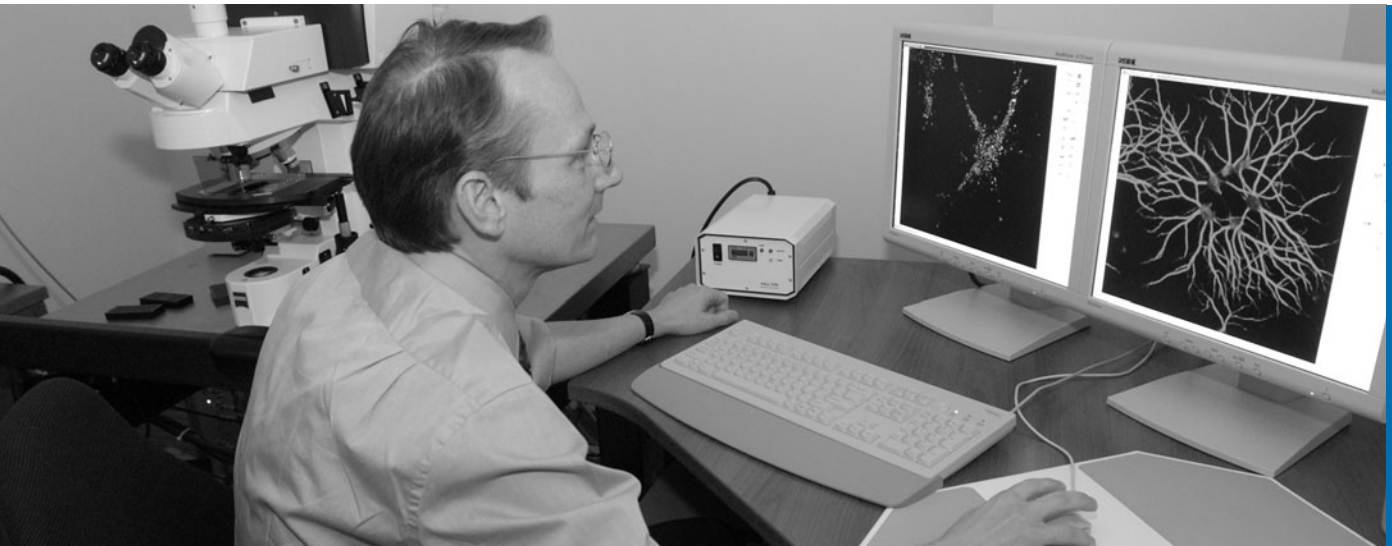
Our ongoing study appears to be the first to provide the possible functional implications of inhibition of the NMDA-mediated cell death without jeopardizing NMDA-mediated excitatory neurotransmission, which is essential for maintaining the normal function of the central nervous system. Thus, the selective modulation of multiple NMDA receptor-mediated functions by direct interactions with D1 receptors may form a new avenue to identify specific targets for drug development to modulate NMDA receptor-governed synaptic plasticity, neuronal development and disease states.

MOLECULAR PHYSIOLOGY
Dr. Xian-Min Yu

OUR RESEARCH FOCUSES ON THE REGULATION AND biophysics of the NMDA receptor, one of the ligand-gated ion channels activated by the major excitatory neurotransmitter glutamate. As indicated above, this ion channel is implicated in synaptic plasticity, learning and memory, excitotoxicity and stroke, and schizophrenia.

Complementary to our investigations on the organization and function of the NMDA receptor signalling complex, we continue to collaborate with Dr. Fang Liu in studies of NMDA channels and their interaction with D1 dopamine receptors (see Dr. Fang Liu).

We continue to study how kinase, kinase activator(s) and kinase substrate(s) may exist in the same complex and



how this structure affects the initiation and maintenance of the constitutive regulation of NMDA receptors by Src family PTKs.

Our earlier research shows that NMDA channel activity is sensitive to intracellular sodium ion concentrations and that this sodium sensitivity of the channel was regulated by Src kinases.

We continue to study how, during NMDA receptor activation, Na+ influx may enhance Ca2+ influx and remove Ca2+ influx induced-inhibition of NMDA receptors by remote NMDA.

MOLECULAR PSYCHIATRY

Dr. Albert H.C. Wong

SCHIZOPHRENIA IS A COMPLEX GENETIC DISORDER best reflected by a multiplicative multilocus model. Its complexity is a huge challenge for genetic studies, a challenge best met by using candidate gene analysis in family-based association studies. Candidate genes for these studies are mainly selected on the basis of their role in development or the functioning of the dopamine system or on the basis of being a target for drugs inducing psychosis. Current molecular technologies, particularly micro-array technologies, allow for the rapid screen of the expression of many genes.

Our research aims to discover factors that contribute to the development of schizophrenia. Our main approach is to use rodent models for schizophrenia and post-mortem brain tissue of schizophrenia patients to identify genes that are altered in their expression and are consequently considered candidate genes underlying the disorder. Once identified, these candidate genes are further analyzed in human genetic studies. Genes with an altered expression in schizophrenia may be labelled as candidate disease genes.

This approach led to the discovery of 14-3-3eta and syntaxin1a. Both these genes demonstrated a genetic association with schizophrenia. These genes affect the release of brain chemicals and are also involved in brain development. Now, our studies are looking into how these genes lead to schizophrenia.

Molecular Pharmacology

SECTION HEADS: Drs. Susan R. George and Brian F. O'Dowd

IN THE MOLECULAR PHARMACOLOGY SECTION, WE continued our research on the biology of neurotransmitter receptors for the G protein-coupled receptors (GPCRs) for dopamine, opioids and apelin. In this work, we focus on the ability of the receptors to interact directly with each other to alter pharmacology and signal transduction. We have also continued investigating D1, D3 and D5 receptor-gene-deleted mice to analyze the role of the individual receptors in discrete behaviours.

Our search for novel human genes continues; in this past year we identified novel orphan receptors and a mutated receptor in the human population. These genes, potential candidate genes in neuropsychiatric disease, will now be included in the search for and development of diagnostic tests or novel drugs.

We have also developed a novel cell-based assay, which has several components. This assay can, for example, perform rapid screening for compounds targeting GPCRs, including the many orphan GPCRs we have identified. The assay can also screen for receptors or proteins that interact with each other.

During the past year, we have submitted 19 papers for publication; 11 of these papers have now been published.

Receptor Biology

Our laboratory previously discovered that receptors for neurotransmitters, such as dopamine, function not as individual molecules, but as highly ordered complexes on the cell surface. This discovery has been shown to be true for many members of the family of GPCRs and is probably universal. We also discovered that individual receptors formed complicated higher-order structures with other receptors, greatly enlarging the complexity of novel functional therapeutic targets in the brain.

We continue to investigate receptor-receptor interactions, and the sites of interaction between two receptors has been precisely identified to involve specific transmembrane regions. Previously, we studied dopamine and opioid receptors, finding that they form homodimeric and heterodimeric (i.e., mixtures of receptors) complexes. We have shown the existence of these receptor complexes in cultured living neurons and human and rat brain by immunocytochemistry and state-of-the-art confocal microscopy. Using confocal microscopy, we are studying further the colocalization of the receptors, not only within single neurons, but also within cellular microdomains of the neurons. Our work has revealed that hetero-oligomerization of receptors may generate novel pharmacological and functional properties and that hetero-oligomerization is a specific process, with rules governing which receptors participate in the hetero-oligomeric complex.

Novel GPCR Assay

We have developed a novel method incorporating a strategy suitable for the identification of chemicals interacting with or modifying the activity of both known and orphan GPCRs, transporters and other plasma membrane receptors. The method will also allow us to evaluate the ability of GPCRs and transporters to selectively oligomerize with other GPCRs, transporters or other proteins to generate novel heteromeric drug targets. We will use this assay method to screen for novel compounds and to identify the dimerization partners of various receptor proteins.

Novel Receptor Genes

We continue to discover novel GPCR genes and to identify novel orphan receptors.

As orphan receptors we cloned are being identified, such as GPR 7 and 8, it is apparent that these are completely novel ligand-receptor systems. Identifying the receptor and its endogenous ligand will now allow us to elucidate its physiological effects and functions.

One of the first receptors we identified was the apelin receptor, which is highly expressed in brain. We have recently completed studies showing localization of the apelin receptor in human brain regions, with a unique nuclear localization within the neuron, highly novel for GPCRs. This suggests unique functions for this receptor, as the vast majority of other GPCRs are located on the cell surface; this receptor will be the focus of detailed study in future.

We are searching through genomic databases and DNA of people with neuropsychiatric diseases for mutations and polymorphisms in the receptor genes that may predispose humans to disease. Recently we discovered a mutated GPCR that was present in a highly significant percentage of the population, including those with and without a neuropsychiatric disease. The prevalence of this mutation is the highest among documented receptor mutations; we plan to study the functional significance of this mutation and its role in mental disorders.

More excitingly, as we uncover the functions of additional orphan receptors, we will be able to elucidate distinct novel CNS functions, the role of the receptors in CNS disorders may become apparent, and the receptors may be targets for the development of new therapeutics.

Role of Receptors in Behaviour

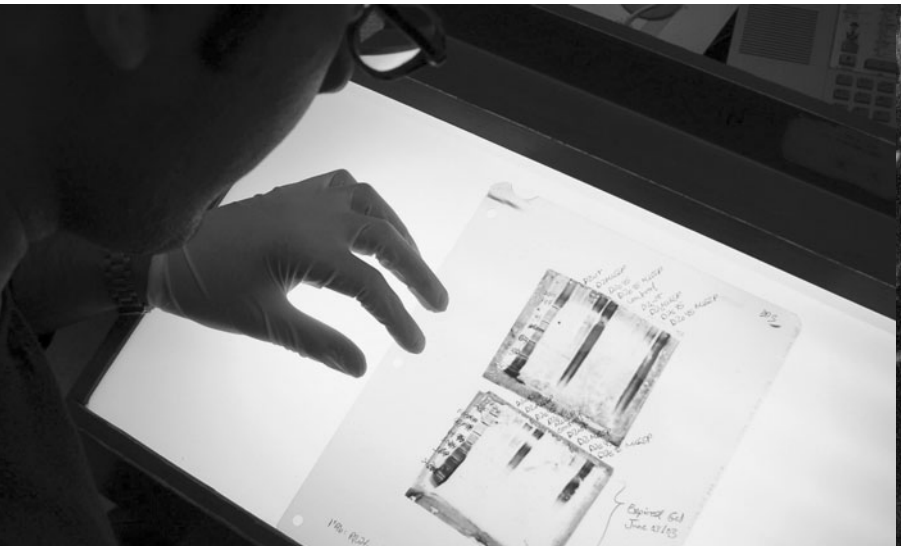
Our analysis of the effects of receptor gene deletions on specific brain functions has helped us understand the role of the receptors in important higher level functioning, with specific corollaries to several human CNS disorders. To explore the functional role of specific dopamine receptors, we studied various receptor gene knockout models for the functional consequence of deletions of the D1, D3, D5 and D1+D3 receptor genes.

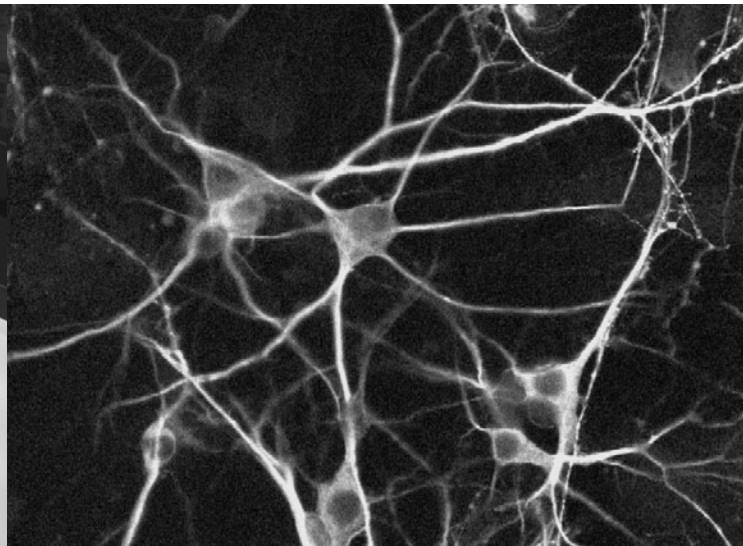
The localization of the D1 dopamine receptor in the hippocampus prompted us to analyse learning and memory processes in the D1-deleted mice.

Our results showed that their fear conditioning responses were intact, although the extinction of the fear memory was abnormally prolonged compared to their wild-type littermates. This finding suggested a role for the D1 receptor in promoting the extinction of fearful memories, with implications for human disorders such as abnormal fear/anxiety states and post-traumatic stress disorder. These mice also show a markedly reduced motivation to work for rewarding stimuli.

To our knowledge, this was the first demonstration of a single gene disruption that has resulted in specific attenuation of drug-seeking and pleasure-seeking behaviour and enhancement of fear memory.

Because the D3 dopamine receptor is colocalized with the D1 receptor in the nucleus accumbens, we studied D1 receptor mediated functions in D3-/- mice, and generated double-gene-deleted animals, deficient in both D1 and D3 receptors. We continue to study the interaction of these receptors to regulate exploratory behaviour and gene expression in specific brain regions.





Neuroimaging

SECTION HEAD: Dr. José N. Nobrega

RESEARCH IN THE NEUROIMAGING SECTION IS AIMED at mapping changes in specific brain areas, neuroanatomical pathways and chemical mechanisms in neuropsychiatric disorders, primarily through the use of appropriate animal models. In 2002, we focused on three broad areas.

MODELS OF DEPRESSION AND STRESS REACTIONS

Learned Helplessness Model of Depression

We continue to test genes identified by cDNA microarray analyses in brains of animals showing propensity to develop depressive-type symptoms in response to stress, using the *learned helplessness model of depression*. This behavioural model also allows us to identify subjects that are resistant to stress-induced reactions.

Our current data indicate that different sets of genes may be associated with propensity versus resistance to stress-induced behavioural deficits. While a number of candidate genes have proved to be false positives in this model, our PhD student, Beatrice Setnik, using in situ hybridization, has identified the first clear changes in gene expression in the frontal cortex of susceptible animals.

Because human females are more prone to depressive episodes than males, we have extended the behavioural model to include gender comparisons. Our initial behavioural evaluations of female animals at different points in the estrous cycle did not reveal significant differences.

We have, however, uncovered a significant overall difference between males and females in effects of stress on plasma levels of homocysteine (Hcy), an amino acid that has been associated with symptoms of depression, stress effects and cardiovascular risk. We found that, while males have higher basal Hcy levels than females, females appear to be more vulnerable than males to stress-induced elevations in Hcy.

Chronic Mild Stress Model of Depression

Using the *chronic mild stress model of depression*, we began to examine possible changes in the GABA-benzodiazepine receptor complex, seeking to isolate anxiety-related components of this model. This work is being conducted in collaboration with Dr. Nylson Silveira-Filho's group at the Federal University of São Paulo, Brazil. An extensive autoradiographic mapping analysis, using [3H]Ro-154513 to label diazepam-insensitive benzodiazepines binding sites, suggested that behavioural changes in this model are not likely to be mediated by alterations in this binding site.

Sleep Deprivation and Depression

We continue to study sleep deprivation, seeking to identify mechanisms involved in its beneficial (antidepressant) effects as well as in its potentially harmful effects. In collaboration with Dr. Sergio Tufik's group at the Federal University of São Paulo, we found that sleep deprivation was followed by localized changes in the expression of b1 thyroid hormone receptors in brain, suggesting a potential involvement of these receptors in antidepressant effects.

We also conducted the first detailed examination of the serotonin transporter in the brain after sleep deprivation, using [11C] DASB. In this collaboration with Dr. Alan Wilson from the PET Centre at CAMH, we developed and validated experimental protocols for the use of short-lived PET tracers in in vitro autoradiographic analyses.

This overall approach is now being extended to other areas, such as the preclinical imaging of potential PET probes for markers of pathology in Alzheimer's disease. This extension is part of a larger effort led by Dr. Paul Verhoeff of the Baycrest Geriatric Centre.

In 2002, we published evidence that sleep deprivation, unlike stressful procedures in general, has unexpected beneficial effects on blood levels of homocysteine. High blood levels of

homocysteine (Hcy) are a risk factor for cardiovascular disease. Stress increases Hcy levels, but sleep deprivation decreases Hcy levels in blood.

On the other hand, we confirmed that sleep deprivation negatively affects the acquisition of a simple avoidance learning task, an effect that was blocked by muscarinic agonists. A detailed autoradiographic analysis indicated that this behavioural effect was not mediated by changes in the muscarinic M1 receptor binding in the brain.

We also completed an extensive examination of two receptors for orexin (hypocretin), a neuropeptide that has been identified a key element in narcolepsy. In situ hybridization analyses revealed that both orexin1 and orexin2 receptors are significantly altered after sleep deprivation. Changes in the expression of the two receptor subtypes were different in kind from each other and were noticed in different parts of the brain. These receptor expression changes were not seen immediately after deprivation, but were seen after the animals were allowed to recover lost sleep for one day.

BRAIN DOPAMINE AND MOVEMENT DISORDERS

Paroxysmal Dystonia

We continue to collaborate with investigators from Germany to build a comprehensive map of brain alterations in *the dt^{sz} mutant hamster model of paroxysmal dystonia*. This year we have identified significant changes in the hamsters' expression of the mRNA encoding two important neuropeptides in the basal ganglia circuits controlling movement, namely enkephalin and dynorphin.

Tardive Dyskinetic Syndromes

Our study continues of the *VCM model of tardive dyskinetic syndromes* induced by long-term antipsychotic treatment. Work published by Peter Turrone, a PhD candidate, in collaboration with Drs. Gary Remington and Shitij Kapur from the Schizophrenia Section, shows that, after long-term haloperidol treatment, variables such as dose and of continual drug availability affect the likelihood that dyskinetic symptoms will emerge. Long-term treatment by single daily injections produces fewer motor side-effects than are seen when the same daily doses are given by continuous release (e.g., via osmotic minipumps).

We believe these effects may relate to sustained versus discrete occupation of D2 receptors by antipsychotic medications, and we are currently testing this hypothesis on other antipsychotic drugs.

BRAIN MECHANISMS OF COMPULSIVE DRUG-TAKING

Behavioural Sensitization to Alcohol

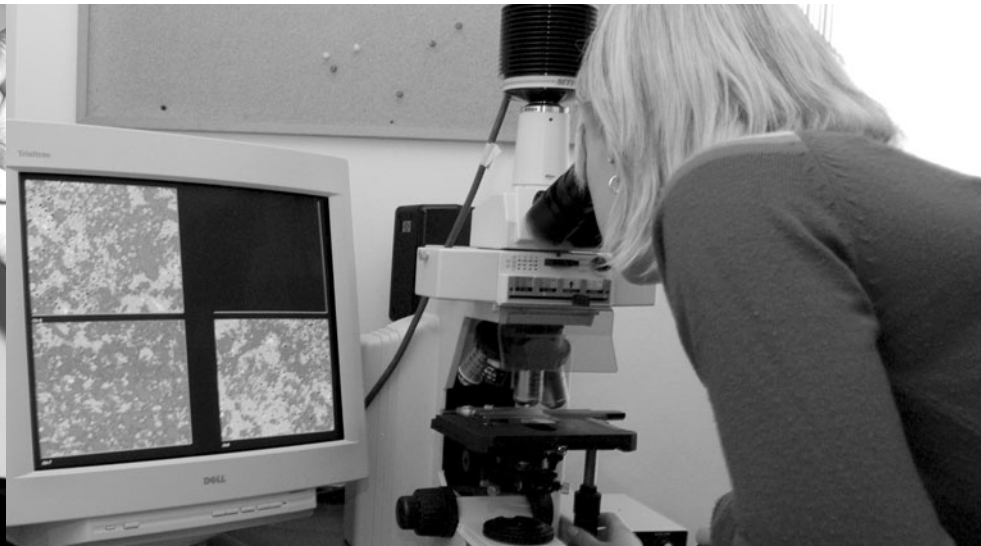
We continue to study the brain mechanisms underlying differential susceptibility to behavioural sensitization to alcohol.

This year, we found that alcohol-sensitized mice have higher [^{3}H] flunitrazepam binding levels in the ventral tegmental area—an important region of the mesocorticolimbic pathway—than do non-sensitized animals. These binding differences may have functional correlates, because our sensitized mice also showed an enhanced locomotor response to a benzodiazepine challenge.

In a separate study, we examined the relationship between ethanol sensitization and learning variables. The results show that the development of ethanol sensitization seems to be positively associated to contextual learning. This confirms that the expression of sensitization depends heavily on contextual cues.

GABA Receptor Changes and Alcohol

In collaboration with Drs. Denise Tomkins (Biobehavioural Pharmacology) and Rachel Tyndale (Pharmacogenetics), we began analyses of GABA receptor changes in brains of two types of rats: one that shows innate high preference for alcohol and one that shows innate low preference for alcohol.



Pharmacogenetics

SECTION HEAD: Dr. Rachel F. Tyndale

PEOPLE HAVE GENETIC DIFFERENCES IN THE AMOUNT and type of drug-metabolizing enzymes they produce. Genetic variation can cause people to metabolize drugs slowly or quickly, resulting in wide ranges in levels of drugs and drug metabolites (products of drug metabolism) among different people. Such variations can result in therapeutic failure and unanticipated toxicity.

Researchers in the Pharmacogenetics Section are interested in genetic variations in enzymes and the effect these variations can have on the metabolism of drugs of abuse. Specifically, we are investigating how genetic variations in drug metabolism affect the pharmacology of specific drugs, the risk for specific drug dependencies and the amount of a drug used by people who are dependent on it (pharmacogenetics). We investigate this using studies involving abuse liability, and epidemiological, genetic, biochemical and therapeutic intervention studies.

At the same time, we are examining how exposure to drugs of abuse may alter or regulate the levels of metabolizing enzymes. These studies use humans and animal models combined with behavioural, biochemical, immunological and molecular biological techniques. We hope that our studies will help us to develop novel approaches to identify and treat people who have a high risk for substance dependence.

Our data illustrate how genetic variation in drug metabolism, such as the inactivation of nicotine or alcohol, can alter the risk for becoming dependent on nicotine or alcohol. We can now initiate studies where we manipulate the activity of an enzyme (e.g., with inducers or inhibitors) to imitate the protection from drug dependence found in the genetic studies.

This year, we also published a number of reviews on pharmacogenetics and drug dependence, as well as a review on the potential roles of CYP enzymes within the brain.

Variation in Nicotine Metabolism

Much of our recent work has focused on how genetic variation in the inactivation of nicotine affects aspects of smoking.

Nicotine is the psychoactive substance (drug) responsible for tobacco dependence; smokers adjust their cigarette consumption to maintain nicotine levels in the brain. In humans, 80 per cent of nicotine is metabolized to the inactive metabolite cotinine.

In our earlier work, we identified and characterized the liver enzyme responsible for this metabolism as the genetically variable CYP2A6. Genetic variation in this enzyme results in slower nicotine removal, prolonged higher brain levels of nicotine and, consequently, decreased smoking. People with defective CYP2A6 were protected from becoming tobacco-dependent and may be at lower risk for cancer due to both decreased smoke exposure and decreased activation of tobacco smoke procarcinogens.

We are studying the role of genetic variation in adults who have already become, or have not become, smokers. In addition, through collaborations in Montreal and California, we are investigating how variable metabolism of nicotine alters the development of nicotine dependence and smoking behaviour. In one study, we have been following adolescents as they learn to smoke; in another, we are studying college students and their smoking behaviours.

Recently, we have been identifying novel defective alleles in this gene. In 2002, we published the characterization of three new gene variants that also alter nicotine metabolism. Many more uncharacterized variants are likely to exist and remain to be investigated. We investigate these novel alleles in a number of ethnic populations as the frequency of a specific form of the enzyme can be very different among different groups.

We can mimic the effect of defective CYP2A6 by administering inhibitors of the enzyme. We have shown that use of an inhibitor can decrease the amount smoked and also decrease the amount of procarcinogen activation.

We have just completed a study of CYP2A6 in collaboration with a group in Kansas City, demonstrating that people who are slower nicotine metabolizers are able to quit smoking more effectively than those with more rapid metabolism. This increases our evidence that inhibiting the enzyme, thereby decreasing nicotine metabolism, may be useful for helping people quit smoking.

Variations in Alcohol Metabolism

In addition to the CYP2A6 gene, we continue to study the genetics of other CYP enzymes, including CYP2E1. CYP2E1 is able to metabolize alcohol and is thought to play a role in metabolizing different compounds in the brain.

We have found that people with a specific genetic form of this enzyme, when exposed to ethanol or other inducers, make much more of this enzyme. Our genetic studies have also shown that these people are more likely to become dependent on alcohol and also on nicotine. Again, like CYP2A6, the frequency of genetic variants for CYP2E1 varies substantially among ethnic groups.

Variations in MDMA Metabolism

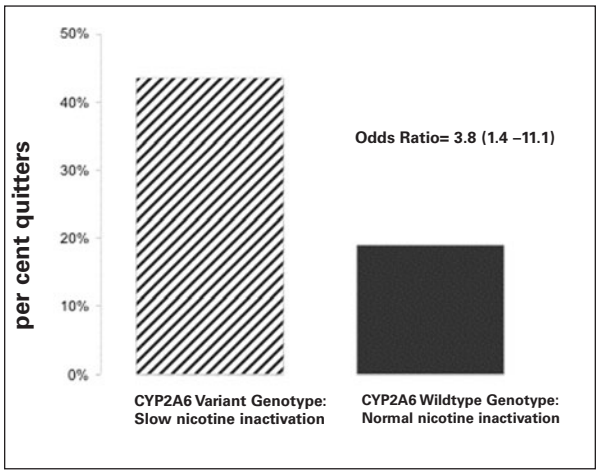
This year, we also discovered that a number of "designer drugs" and clinically used drugs are potent inhibitors of specific drug-metabolizing enzymes. A commonly used recreational drug, MDMA (ecstasy), is metabolized very differently among people with different genetically variable enzymes.

Enzyme Variations in the Brain

In addition to our genetic studies, we have studied the presence and regulation of drug-metabolizing enzymes in the liver and brain. In the brain, we have used animal studies and human autopsy tissues to show that both nicotine and alcohol can profoundly alter the levels of these enzymes and that the effects of these drugs are very different in different parts of the body.

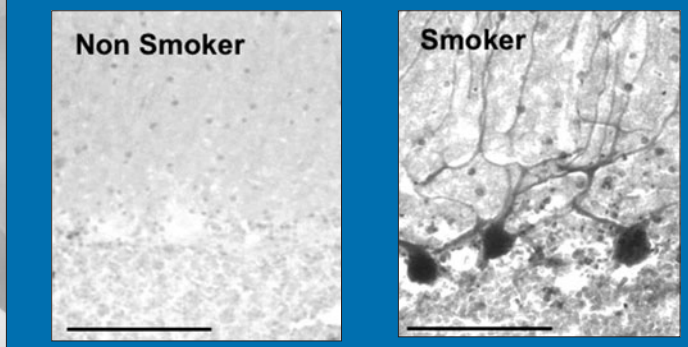
We have characterized the distribution in rat and human brain of three important enzymes, CYP2B6, CYP2E1 and CYP2D6. We found that the enzymes can be increased or decreased in rat brain by exposure to nicotine or alcohol and that the enzymes are higher or lower in brains from humans who were smokers or alcohol-dependent.

Besides alcohol and nicotine, these enzymes can activate or inactivate many drugs that act in the brain (e.g., antidepressants, neurotoxins); having more or less enzyme in the brain may alter the amount of active drug or neurotoxin in this organ. People who smoke or are alcohol-dependent may, therefore, have altered responses to drugs that act in the brain and may be more or less susceptible to neurotoxins. These enzymes may play a role in some of the psychiatric diseases where certain pathways in the brain are damaged (e.g., alcoholism, Parkinson's disease, Alzheimer's disease).



▲ People with genetically slow nicotine inactivation are almost four times more likely to successfully quit smoking than are people with normal rates of nicotine inactivation.

▼ CYP2B6, which metabolizes many drugs of abuse, clinically used drugs and neurotoxins, is found at higher levels in cerebellar Purkinje cells in smokers compared to non-smokers. Bar: 100mm.



Psychiatric Neurogenetics

SECTION HEAD: Dr. James L. Kennedy

Schizophrenia

The neurodevelopmental hypothesis of schizophrenia suggests that structural abnormalities of the brain, acquired during the development of the central nervous system, are responsible for the susceptibility to develop schizophrenia later in life. These structural abnormalities lead to the dysfunctional connectivity ("wiring") of several areas of the brain affected in schizophrenia.

The brain-derived neurotrophic factor (BDNF) is a protein molecule that plays an important role in the development and survival of dopaminergic and serotonergic neurons. Animal models of schizophrenia show altered expression of the BDNF gene: mice that were deprived of BDNF exhibit abnormalities of dopaminergic and serotonergic systems. Antipsychotic and antidepressant medications appear to affect the activity of the BDNF gene. All these pieces of evidence have led us to study BDNF as a genetic risk factor in schizophrenia.

Our first analysis of a study on families, consisting of a schizophrenia patient and both parents, collected from Italy and the Toronto area, shows an association between a DNA variant in the BDNF gene and schizophrenia. We then extended this study to additional variations of the BDNF gene and to a larger collection of these triad families, and also to families that consist of subjects with a mood disorder and their parents.

We are excited that our new analyses on new samples of patients and families continue to show association between two BDNF gene variations (and their combination, i.e. haplotype) and both schizophrenia and bipolar disorder. These findings suggested that BDNF could be a common genetic risk factor both for schizophrenia and for mood disorders.

The investigation of BDNF in schizophrenia has led to a publication in *Molecular Psychiatry* (Muglia et al., 2002) and our strong results for BDNF in bipolar disorder were published in *The American Journal of Human Genetics* (Neves-Pereira et al., 2002).

We are now investigating subtypes of schizophrenia and bipolar disorder to understand whether the BDNF gene is a risk factor for specific clinical symptoms common to these disorders.

Suicide

In the past year, we submitted funding proposals for genetic studies of suicide ideas and attempts in people who participated as research subjects in our schizophrenia and mood disorder investigations. We will investigate to see if genetic variants can predict risk for suicide in these disorders.

Dr. Vincenzo De Luca received a three-year award from the American Foundation for Suicide Prevention for his genetic investigations of suicidal behaviour in schizophrenia and bipolar disorder. Dr. John Strauss also received a similar award from the same foundation to examine genetics of suicide ideas and attempts in child-onset depression.

Also, our staff (Dr. Xingqun Ni and colleagues) are working on a proposal to study suicidal behaviour in the context of borderline personality disorder.

Pharmacogenetics and Pharmacogenomics

Strong evidence suggests that genetic variation plays an important role in inter-individual differences in medication response and toxicity. The rapidly evolving disciplines of pharmacogenetics and pharmacogenomics seek to uncover this genetic variation in order to predict treatment outcomes. The goal of these disciplines is to be able to tailor the therapy to the individual, by using the person's genetic make-up to select the drugs with the greatest likelihood of benefit and the least likelihood of harm.

In our laboratory, we investigated the dopamine D1 receptor gene's ability to predict response to clozapine treatment. So far, we have found that the D1 gene predicts improvement in memory and attention during clozapine treatment (Masellis et al., 2002, New York Pharmacogenetics Meeting presentation).

In addition, we studied candidate genes to try to determine the cause of clozapine-induced weight gain (Basile, Masellis, Potkin, & Kennedy, *Human Molecular Genetics,* 2002; Basile et al., *Lancet*, 2002). Using large, combined datasets, we have further examined antipsychotic-induced tardive dyskinesia and its association with the dopamine D3 receptor, first reported by our group in 1996.

We have also found that the pharmacogenetic principles developed in schizophrenia can be used to study many other psychiatric and addiction disorders (Masellis et al., 2002).

Obsessive-Compulsive Disorder

In collaboration with Drs. Peggy Richter and Emanuela Mundo, over the last year we made an important step forward in understanding the genetic basis of obsessive-compulsive disorder (OCD). Our group detected and, most importantly, replicated the finding that a serotonin system gene encoding the 5HT1D beta receptor (a.k.a. 5HT1B) can be a predisposing factor to OCD (Mundo et al., 2002).

The clue to this gene came from the unusual finding by Zohar et al. that the migraine drug sumatriptan, which binds to 5HT1B, increases OCD symptoms in the short term. Our

genetic finding, also reported in the lay press (*National Post*, Sept. 4, 2002), could have important implications for the molecular diagnostics and design of new therapeutic strategies in OCD.

Another interesting investigation, the first ever of OCD risk genes in children, is being led by Dr. Paul Arnold, who has reported a significant role for the NMDA receptor gene in OCD. He is now examining this gene in a sample of children from Wayne State University who have OCD and have undergone functional MRI studies testing glutamate activity in the living brain.

Attention-Deficit/Hyperactivity Disorder

In collaboration with Dr. Cathy Barr's lab at the Toronto Western Hospital, we investigated the role of catecholamine system genes in children with attention-deficit/hyperactivity disorder (ADHD) (Wigg et al., *American Journal of Psychiatry,* 2002; Barr et al., 2002). Our adult ADHD studies in collaboration with Dr. Umesh Jain (Muglia et al., 2002) continue to show a role for the dopamine D4, but not D3, receptor genes.

Problem Gambling

In collaboration with Drs. Umesh Jain, Nigel Turner, Michael Bagby and others, we obtained a grant from the Ontario Problem Gambling Research Centre to investigate genetic risk factors for gambling behaviour in people who have symptoms of ADHD. Early results suggest that dopamine system genes may be involved in predicting risk for gambling behaviour, but more work is needed to establish these findings.

Seasonal Affective Disorder

Dr. Robert Levitan is leading the effort to study the role of serotonin system genes in people who eat larger amounts of food in the autumn, become depressed and slowed-down in the winter, then return to normal mood and activity in the spring and summer.

Dr. Levitan showed that the serotonin 2A receptor gene was associated with a history of childhood attention-deficit/hyperactivity disorder in adult women who have seasonal affective disorder (Levitan et al., 2002). The next steps in this project include examining the interaction between the serotonin 2A and the dopamine D4 receptor genes.

Childhood Onset Depression

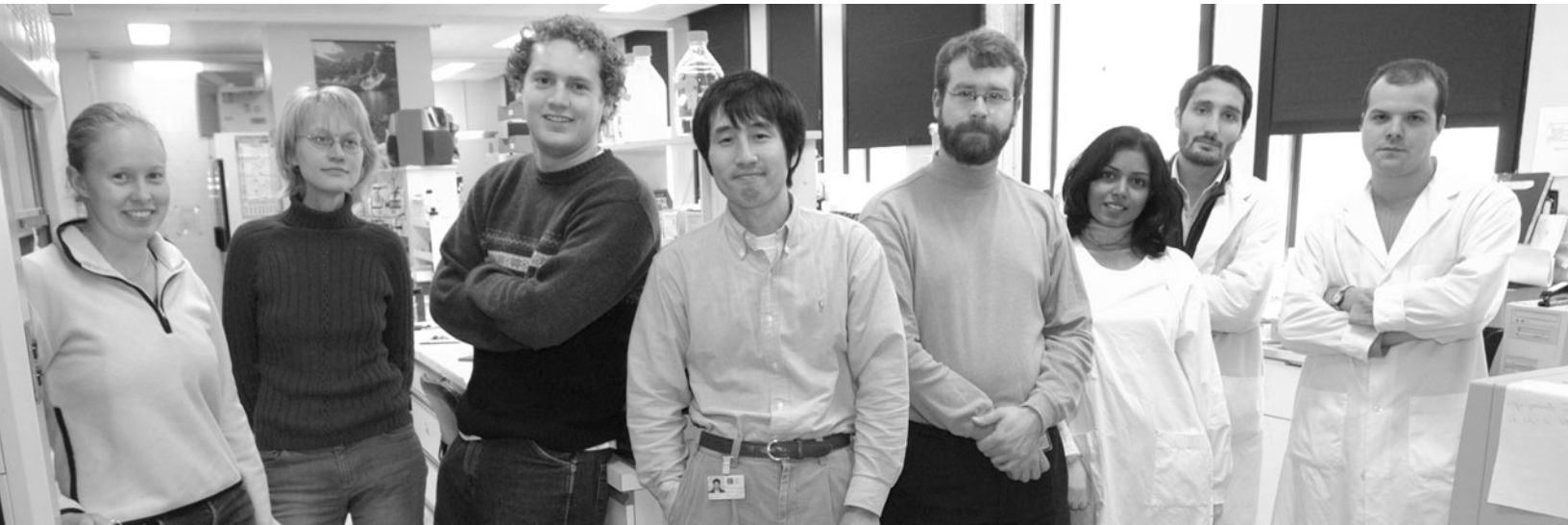
Dr. John Strauss, in collaboration with investigators at the University of Pittsburgh, reported a strong effect of a variant of the BDNF gene in predicting severe mood disorder in children. This finding has led us to study the role of this gene in suicidal behaviour and the tendency of depressed children to develop bipolar disorder in adulthood.

Bipolar Disorder

We have dedicated significant effort over the last year to genetic studies of bipolar disorder, which, together with schizophrenia, represents the group of major psychoses.

We investigated 300 small families (consisting of the person with bipolar disorder and his or her parents), first examining the dopamine D1 receptor gene. We found that a particular combination of genetic variants of D1 create increased risk for bipolar disorder (Ni et al., 2002). Dr. Ni also showed that a suspect gene, for the serotonin 2A receptor, was not involved in bipolar disorder.

In these 300 small families, we also examined how specific variants of the dopamine D4 receptor gene in the parents were transmitted to the affected child. Interestingly, we found that having the D4 gene transmitted from the mother increased risk for bipolar disorder, as opposed to having the same genetic variant coming from the father (Muglia et al., 2002). This finding suggests that regulation of this gene is complicated and likely to be subjected to genomic



imprinting, which links the traditional DNA sequence-based studies to epigenetic developments.

Epigenetics

The epigenetic theory of major psychosis puts the emphasis on looking at possible dysregulation of gene activity, rather than changes in DNA sequence. Psychiatric epigenetics is a relatively new field in psychiatric research. The great advantage of the epigenetic theory is that epigenetic mechanisms, unlike the traditional genetic ones, can explain unclear issues in major psychosis, such as age of disease onset, fluctuating course (remissions and relapses), common major differences in identical twins. To our knowledge, we represent the only group in the world fully dedicated to this development.

Dr. Art Petronis, head of the Epigenetics Laboratory, received a prestigious OMHF Special Project award to study epigenetic mechanisms in schizophrenia and bipolar disorder.

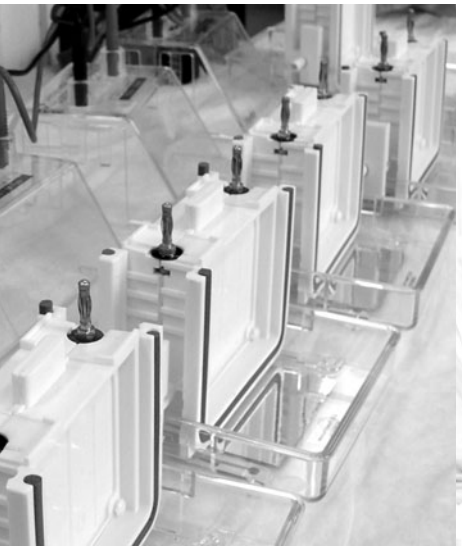
Over the last year, the Epigenetics group performed a series of laboratory experiments looking for molecular differences in identical twins, and compared epigenetic regulation of non-coding regions of the genomes of people who have schizophrenia or bipolar disorder with controls. Two manuscripts that describe the differences in DNA methylation patterns between ill and healthy control individuals are currently submitted to peer-reviewed journals.

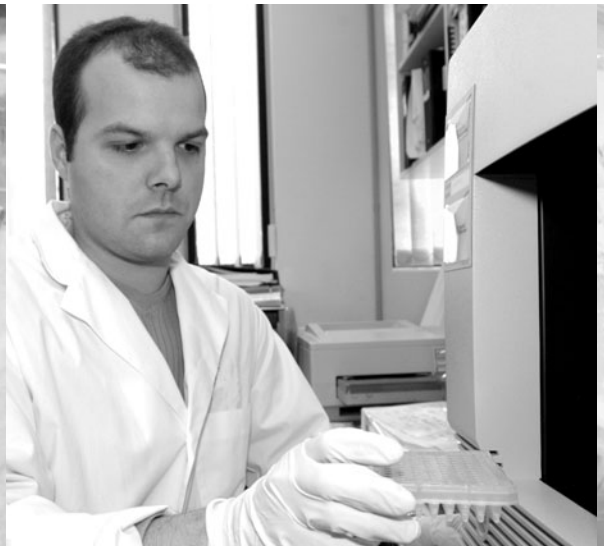
Developmental Neuropsychiatry and Autism

Dr. John Vincent, head of the Laboratory for Molecular Studies in Developmental Neuropsychiatry, has reported the cloning and characterization of a gene, called RAY1, that spans an area of breakage on the long arm of chromosome 7 in a person who has autism (Vincent et al., 2000). This gene has recently, and controversially, been reported to be a tumour-suppressor gene, although several reports have been unable to support this finding.

Dr. Vincent has continued to further characterize this gene and its surrounding DNA and has established the surprising situation that there are at least five other genes within RAY1 (Vincent et al., 2002). Mutations in RAY1 were identified in two families that have several members affected with autism. This work was recently published in a leading journal in the field, *Genomics*.

Dr. Vincent's work has also established the location of breakage regions within the suspected autism region on chromosome 7 for several other people who have autism. In one of these cases, the breakpoint maps to the same point identified in an unrelated person with autism. Both translocations are likely to disrupt a new gene that we have identified. This gene thus represents a very strong candidate for autism, and further characterization is under way.





Smoking and Nicotine Dependence

SECTION HEAD: Dr. Paul Fletcher (acting)

THE MAIN FOCUS OF OUR RESEARCH IN THE SMOKING and Nicotine Dependence Research Section is to better understand the neurochemical basis or mechanisms underlying nicotine dependence.

A limiting factor in finding therapies to prevent smoking is that underlying mechanisms involved in nicotine addiction, such as the positive reinforcing effects of nicotine, still remain unclear. Our research gives us valuable information about the brain mechanisms involved in nicotine addiction; this work should help us identify drug targets and thus help the search for more effective therapeutic agents for smoking cessation.

Previous studies in our laboratory have shown that nicotine is the primary rewarding compound in tobacco smoke. The study of nicotine dependence, like that of other drug dependence, profits from animal models, which allow us to examine the biochemical and behavioural consequences of acute and chronic drug treatment at a depth not possible with human studies.

Under the leadership of Dr. Shafiq Rahman (Research Scientist, CAMH), we continue to explore the mechanisms that mediate the positive reinforcing effects of nicotine in the brain. We use both conventional (qualitative) and quantitative (no-net-flux) in vivo microdialysis techniques in behaving animals to measure dopamine (brain chemical for reward) release in rat brain reward circuits. We have used both microdialysis methods in the nucleus accumbens (NAc) or ventral tegmental area (VTA) after acute and subchronic (pretreatment or pre-exposure regimen) and chronic nicotine treatment.

Nicotine Receptors in the Nucleus Accumbens

Nicotine is thought to exert its rewarding effects by activating dopamine neurotransmission in the mesocorticolimbic dopamine system, which is an integral part of the brain reward system. We have shown that acute and repeated nicotine exposure stimulates the release of dopamine in the NAc, a feature shared by other drugs of abuse, such as amphetamine and cocaine. Nicotine exerts these effects by attaching to and activating specific sites called receptor proteins and, more specifically, a type of receptor called neuronal nicotinic acetylcholine receptor.

Neuronal nicotinic acetylcholine receptors have several subtypes. We have determined that stimulatory effects of nicotine in the brain reward system are mediated by the high- and low-affinity nicotinic acetylcholine receptors. We have also found that low-affinity nicotinic receptors have a special role in modulating brain dopamine function in the NAc after repeated exposure to nicotine.

Nicotine and Release-Controlling Dopamine Autoreceptors

We are also investigating the role of dopamine autoreceptors in the NAc and VTA that control the release of dopamine.

In the NAc, we have found that dopamine autoreceptor subsensitivity may not contribute directly to the sensitization of dopamine releasibility. Additional studies on the role of dopamine autoreceptors in the VTA suggest that dopamine autoreceptor subsensitivity in this area is essential in nicotine-induced sensitization of dopamine release. The regulation of release-controlling dopamine autoreceptors is associated with sensitization and/or adaptive changes that are important for the development of nicotine addiction.

Chronic Nicotine Self-Administration

We have also started to use no-net-flux (quantitative) microdialysis to examine changes in dopamine transmission in the NAc after chronic nicotine self-administration. Recent advances in microdialysis methods show that quantitative methods are useful not only in determining the true extracellular concentration of dopamine but also in testing for

Transgenic Facility

SECTION HEAD: Dr. Hubert H.M. Van Tol

potential changes in dopamine transmission. Moreover, changes in tissue dopamine transmitter content are not necessarily paralleled by corresponding changes at the synaptic level of the reward centre.

We hope to determine whether chronic voluntary nicotine self-administration alters or influences the functional integrity of the dopamine system in the NAc (i.e., dopamine release, uptake, synthesis and metabolism). We are measuring extracellular dopamine levels and in vivo extraction fraction.

Typically, extracellular dopamine levels represent dopamine release in the NAc, and dopamine extraction fractions reflect the uptake process or clearance of a known dopamine concentration. For example, a known dopamine concentration is given through microdialysis probe into the NAc, which is measured after a time interval (dopamine in–dopamine out is equal to extraction fraction).

Our results indicate that chronic nicotine self-administration significantly reduces basal dopamine levels, which is likely due to chronic intermittent exposure to the pharmacological effects of nicotine. More importantly, chronic nicotine self-administration creates a neuroadaptive change in the brain that alters the dopamine transporter and increases uptake in the NAc.

ADVANCES IN MOLECULAR AND GENETIC RESEARCH have increased our need to analyse the function of genes in physiological contexts. New technologies can easily modify genetic material in the germ line of mice or introduce new genetic material in selected tissues or organs through viral-mediated gene transfer. These technologies allow us to study the function of genes in whole animals, extending the molecular and genetic revolution to the realm of behavioural research.

The Transgenic Facility breeds and maintains transgenic mice strains for CAMH researchers. The facility can also help scientists create their own transgenic mice strains or employ viral-mediated gene transfer experiments. This year, the facility continued to lend its services to researchers in the Molecular Neuroscience, Biopsychology and Neuroimaging sections.