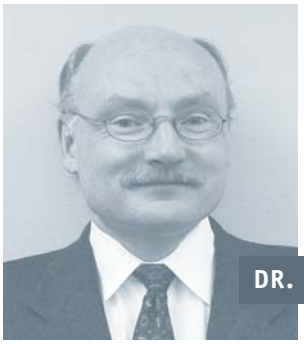


Neuroscience
RESEARCH DEPARTMENT

DR. JAMES KENNEDY, DIRECTOR

Mental illness and addiction will continue to affect people's lives the same way until research reveals the mechanisms involved in these disorders. Understanding how alcohol, other drugs and psychiatric problems affect the human body and brain is crucial to the development of more effective approaches to treatment and prevention.

The Neuroscience Research Department focuses on neurobiological mechanisms underlying mental illnesses, addiction and their respective treatments. The Neuroscience Research Department benefits from state-of-the-art, in-house research facilities. These facilities include the Positron Emission Tomography (PET) Centre, which allows researchers to scan the brains of live human subjects, and the Transgenic Research Centre, which can alter the genetic makeup of laboratory mice to mimic human diseases such as schizophrenia, bipolar disorder or addiction.

Each neuroscience research section has its own health theme and neurobiological emphasis. We are investigating how variations in the action of dopamine — a "neuro-transmitter," or chemical that allows signals to pass

between cells in the brain — have been linked to a range of problems, from schizophrenia, bipolar disorder and Parkinson's disease to dependence on alcohol, cocaine, nicotine, and amphetamines. Disturbances in signal transduction and molecular and genetic mechanisms within cells are also of great interest, and are now believed to be critical determinants of mental illness and addiction. Examining the genes for neurotransmitters, and other systems known to be involved in drug response, may lead us to predict the type and amount of medication best for each individual patient. Pharmacogenetics, the study of how genes relate to drug response, may also help to predict those people who are at higher risk for addictions.

By exploring these research strategies, researchers within the Neuroscience Research Department broaden our understanding of mental illness and addiction and lay the basis for potential new treatments of the future.

The following pages show how the work of our neuroscientists is recognized throughout the world, and how we are making enormous strides toward unravelling the complexities of mind and brain. Our diverse research is well integrated, and a rich cross-fostering of ideas is evident in innovative combinations of methods across the department. One example of this integration is our recent initiative that combined molecular genetics, epigenetics, and PET scans to simultaneously assess the human brain's blueprint and biologic activity in depression and in Parkinson's disease. Following forward from our initial pilot studies linking PET images to genetic variants, we now have the world's largest collection (more than 250) of DNA samples from patients who have undergone PET scans.

In the past few years, we have focused on consolidating and building on the strengths of our neuroscience research group, and establishing future priorities. In the months and years to come, neuroscience will gain increasing importance in the education, clinical and research activities of the Centre. The growth and activities of our department have been incorporated into the Functional Plan for the new Queen Street site development, which promises more space and resources to further enhance our world-class efforts in neuroscience research and training.

Biobehavioural Pharmacology

NEUROSCIENCE RESEARCH DEPARTMENT

DRS. A.D. LE & DENISE TOMKINS, HEADS

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.

During the past year, we have increased our knowledge of the role of specific neurochemical systems within the brain that regulate alcohol consumption in general, as well as identified potential systems that may predispose an individual to problem drinking. We have begun to explore sex differences in susceptibility to alcohol's effects, with a particular emphasis on cognitive function, which is an increasing public health issue. We hope our research will, in time, help develop therapeutic agents, identify potential risk factors, and provide research-based information on alcohol's effects on the brain and its ability to function.

Stress and Relapse to Alcohol

Relapse is a major challenge in treating alcohol dependence. Exposure to stressful situations has been identified as a key factor in relapse to alcohol use. A major research effort in the section attempts to elucidate neurochemical mechanisms underlying stress-induced relapse to alcohol.

Over the past year, we have advanced our understanding of the mechanisms of stress-induced relapse. Using our animal model, we found that the brain neuropeptide involved in the co-ordination of stress responses, corticotrophin releasing factor (CRF), plays a critical role in stress-induced relapse to alcohol. Administration of a CRF receptor antagonist can block stress-induced relapse to alcohol; these CRF receptor antagonists may potentially be used to treat alcohol dependence.

CRF might control relapse to alcohol by interacting with a specific serotonergic pathway in the brain. Such a serotonergic pathway has been shown to play a significant role in inhibitory control of behaviour. A number of CRF receptor antagonists are currently being developed for treating anxiety and depression.

Co-Abuse of Alcohol and Nicotine

Another finding is the mechanism underlying the co-abuse of alcohol and tobacco. We have shown previously that nicotine can enhance alcohol self-administration in experimental animals, and treatment with a nicotinic receptor antagonist can reduce alcohol consumption. Genetics have been shown to play a critical role in problem alcohol use in humans. We have found that there might be a common genetic determination for alcohol and nicotine abuse. Animals selectively bred for high alcohol consumption also self-administer more nicotine than those bred for low alcohol consumption.

Of most interest to our researchers is our finding on the effect of exposure to nicotine on alcohol consumption. Animals exposed to nicotine for a short period during their adolescence have a much higher preference for alcohol when tested during adulthood. This effect of nicotine is age-dependent, as similar treatment with nicotine in adult animals did not affect alcohol consumption when tested three months later.

This finding is consistent with the notion that nicotine might act as a "gateway" drug.

5-HT Receptor Subtypes and Alcohol Reinforcement Processes

Multiple neurotransmitter systems help to modulate the impact that alcohol has on the behaviours linked to problem alcohol use and alcohol's dependence liability. We have been selectively manipulating central neurotransmitter function in animal models of alcohol drinking behaviour. We hope to further our understanding of the neurobiological mechanisms underlying excessive alcohol consumption.

Studies in humans and animals suggest an association between the central neurotransmitter, 5-HT, and 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.

One receptor of particular interest is the 5-HT_{1B} receptor. Human studies have suggested that a locus that predisposes people to antisocial alcoholism is linked to the 5-HT_{1B} receptor gene. Over the past year, we have clearly demonstrated that 5-HT_{1B} receptors play an important role in regulating alcohol intake in our animal models. So far, this effect of the 5-HT_{1B} receptors appears limited to substances with pharmacological activity, such as alcohol and cocaine, because consumption of other fluids and other general behaviours are less sensitive to effects of 5-HT_{1B} receptor manipulations.

We continue to study two brain areas, the amygdala and the ventral tegmental area, that may be important in mediating 5-HT_{1B} receptor effects on alcohol intake. Results to date suggest that activation of 5-HT_{1B} receptors within the ventral tegmental area lead to a decrease in alcohol intake, while in the amygdala, the same manipulation leads to an enhancement of alcohol intake.

These differential findings in discrete brain regions demonstrate that the regulatory effect of 5-HT_{1B} receptors within the brain is site-specific. Furthermore, the 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, that can be reversed by activation of 5-HT_{1B} receptors within this area.

We are continuing this line of research in the hopes of better understanding the neural circuitry important in regulating 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. These differences might represent one of the neurobiological factors underlying problem alcohol use.

Previously, we found significant differences in the $GABA_a$ receptor between animals with a propensity to self-administer alcohol and those without. We found support in reports of altered brain, cerebral spinal fluid and plasma GABA levels associated with alcohol dependence and withdrawal and in reports of altered $GABA_a$ receptor binding, and region- and subunit-specific changes in $GABA_a$ receptors, in the brains of people who are alcohol-dependent. A tentative link between various $GABA_a$ receptor subunit genes and a risk for alcoholism may be related to differences in the expression of alcohol's behavioural effects.

This ongoing project combines behavioural and biological approaches to investigate if higher levels of $GABA_a$ receptor subunits within discrete brain loci are a predictor and/or a consequence of high-alcohol drinking behaviour. Much of the research on neurochemical effects of alcohol has used force administration. We consider potentially crucial differences between "self-administered" versus "experimenter-administered" alcohol, as different neurochemical systems may be involved in voluntary drug-seeking behaviour and in forced intake, the former being more analogous to drug-taking by humans.

The data generated thus far demonstrate that regional differences in $GABA_a$ receptor expression and subunit conformation 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. These data will provide important insights not only into the genetic and non-genetic $GABA_a$ receptor influences on alcohol preference and consumption, but also on 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

We are studying the differences in alcohol's effects on brain function in men and women. In our work, we hope to unravel some of the gender differences in susceptibility to alcohol-induced cognitive impairments and provide new knowledge about the role of $GABA_a$ receptors in these impairments. Ultimately, this research could help identify risk and/or protective factors for alcoholism specific to women and help develop effective treatment and prevention strategies. Over the past year, we began to explore the long-term effects of alcohol exposure on cognitive function and behaviour, with specific emphasis on examining potential differences in susceptibility between males and females.

Clinical evidence suggests that women are more vulnerable to the negative effects of alcohol than men are, even when doses are adjusted for body composition. In addition to being more susceptible to alcohol-related diseases (e.g., liver damage), women appear to be more sensitive to the cognitive deficits induced by long-term alcohol exposure. For example, women are more impaired during tasks demanding divided attention or delayed recall, even when blood alcohol levels are comparable to those of men. Interestingly, in tests of psychomotor performance, males and females have been consistently reported to be equally impaired following alcohol ingestion.

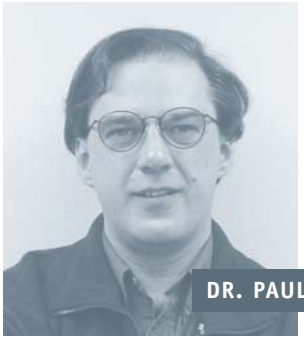
Despite the urgency of attending to such critical sex differences, alcohol research has customarily employed males (humans and animals) to avoid the hormonal confounds introduced by the female estrous cycle. However, research to date has found no consistent evidence that alcohol-induced impairments differ over the female menstrual cycle.

Furthermore, in animal studies where sex differences have been studied, investigators have not considered sex differences in the pharmacokinetic profile of alcohol. Females have generally been exposed to equivalent, or even higher, doses compared to their male counterparts during these studies. Functionally equivalent doses of alcohol were not administered in these studies, as females (both animal and human) generally achieve higher blood alcohol levels due to sex differences in body composition. This limits the interpretability of these data.

Our recent studies were designed to examine sex differences in susceptibility to ethanol-induced impairments of cognition. In these studies, the doses are titrated to ensure that equivalent blood ethanol levels are achieved in both sexes over an extended period of time. We are currently testing the following hypotheses:

1. Female rats will require lower doses of ethanol to be administered to achieve similar blood ethanol levels to that observed in their male counterparts.
2. On measures of psychomotor performance, female and male rats will be equally impaired following acute and chronic exposure to equivalent functional levels of ethanol.
3. Females will show greater impairments on measures of spatial learning and delayed recall compared to their male counterparts when exposed to equivalent levels of ethanol over an extended period of time. These sex differences will not necessarily be observable during acute ethanol exposure, but will be observable both in the ethanol-free state following chronic ethanol exposure, and following a subsequent ethanol challenge test.
4. Females and males will not show impairments under experimental conditions that assess sensorimotor ability and motivation during the spatial learning and delayed recall tasks, demonstrating that differences in performances between the sexes are due to cognitive deficits, and not other factors such as their inability to complete the task due to motor deficits, visual deficits and overall motivation to perform the task.
5. Chronic exposure to functionally equivalent levels of ethanol in male and female rats will result in differential alterations in $GABA_a$ receptor subunit expression in a sex-, brain region- and subunit-specific manner. Furthermore, these differences in brain regions implicated in cognition are correlated with the deficits observed on measures of spatial learning and delayed recall.

Our data thus far support our hypothesis that sex differences in some, but not all, behaviours occur when equivalent blood alcohol levels are maintained over an extended period of time. This first step in the project confirms the validity of our test regimen. Over the coming year, we will expand on this pilot data and specifically explore sex-related differences in alcohol's long-term effects on cognitive function.



Biopsychology

NEUROSCIENCE RESEARCH DEPARTMENT

DR. PAUL J. FLETCHER, HEAD

The Biopsychology Section focuses on the role that brain neurotransmitter systems play in controlling behaviour. We are particularly interested in the serotonin and dopamine systems, and the interactions between these systems. Our general strategy is to use pharmacological and/or lesioning procedures to manipulate specific aspects of neurotransmitter function, and to observe the resulting changes in behaviour. Our current studies explore neurochemical mechanisms involved in addictive behaviours, cognitive behaviours relevant to schizophrenia and the mode of action of antipsychotic drugs.

Serotonin and Drug Use

A long-standing project in the Biopsychology Section explores the consequences of altered serotonin function on reward-related behaviour, with special emphasis on drug-seeking behaviour. Over the past two years we have been especially interested in the role of a specific serotonin receptor subtype, the 5-HT_{2C} receptor, in modulating the behavioural effects of drugs of abuse.

5-HT_{2C} Receptors and Cocaine

We previously found that stimulation of 5-HT_{2C} receptors, with the 5-HT_{2C} receptor agonist Ro60-0175, attenuated a variety of behavioural effects elicited by cocaine, including cocaine self-administration.

We have now determined that blocking the activity of 5-HT_{2C} receptors enhances the effects of cocaine. Specifically, the 5-HT_{2C} receptor antagonist SB242,084 increases the locomotor stimulant effect of cocaine, increases intravenous self-administration of cocaine and potentiates cocaine's ability to induce relapse to cocaine-seeking in subjects whose self-administration behaviour has been extinguished. Our complementary findings with 5-HT_{2C} agonists and antagonists demonstrate that 5-HT_{2C} receptors exert a bi-directional influence over the expression of the effects of cocaine.

The 5-HT_{2C} receptor is expressed in a part of the brain, the ventral tegmental area (VTA), that gives rise to the mesolimbic dopamine pathway, which is critically involved in mediating the effects of many drugs of abuse. Our most recent findings indicate that injecting the 5-HT_{2C} receptor agonist Ro60-0175 into the VTA reduces cocaine-induced locomotor activity, and cocaine self-administration. Serotonin, acting via 5-HT_{2C} receptors, modulates the effects of cocaine specifically in the VTA, perhaps by indirectly altering the function of the mesolimbic dopamine pathway.

5-HT_{2C} Receptors and Other Drugs

A parallel series of studies examined the effects of 5-HT_{2C} receptor blockade on the locomotor stimulant effect of several other drugs of abuse, including amphetamine, phencyclidine, morphine, nicotine, and methylenedioxymethamphetamine (MDMA). The receptor blockade SB242,084 significantly increased the activation induced by all of these drugs. The effect was most pronounced in the case of MDMA. MDMA releases both serotonin and dopamine. The fact that 5-HT_{2C} receptor blockade greatly enhances the stimulant effect of MDMA could indicate that, under normal circumstances, serotonin acting via the 5-HT_{2C} receptor subtype might inhibit the activating effects of MDMA. Removal of this inhibition then leads to greater activation.

5-HT_{2C} Receptors and Addiction

The 5-HT_{2C} receptor has several different polymorphisms and isoforms. We have observed that 5-HT_{2C} receptor blockade leads to exaggerated responses to drugs of abuse. This indicates that individual differences in 5-HT_{2C} receptor function could be one neurobiological mechanism underlying vulnerability to addiction. We recently found, in collaboration with Dr. Peter Clifton (University of Sussex, England), that mice lacking 5-HT_{2C} receptors show an increased behavioural response to MDMA. Thus, we have evidence that a genetic alteration in 5-HT_{2C} receptor function has an identical effect to pharmacological blockade of 5-HT_{2C} receptors.

Models of Schizophrenia: Amphetamine Sensitization

In a different line of research, we have been collaborating with Dr. Shitij Kapur and Dr. Catherine Tenn (Schizophrenia Research Division, CAMH) to explore the usefulness of amphetamine sensitization as a model for schizophrenia. Repeated amphetamine use can induce psychosis in humans, while in animals the behavioural responses to amphetamine are augmented, or sensitized, with repeated use. Some people with schizophrenia show augmented DA release, as inferred by a greater shift in the binding of [3H]raclopride following a challenge with amphetamine. As well, some people with schizophrenia exhibit a disrupted prepulse inhibition of the acoustic startle response, which is thought to reflect altered information processing.

We have shown that these behavioural and neurochemical abnormalities are also present in rats exposed to a sensitizing regimen of amphetamine. Thus, amphetamine sensitization could be a useful model for understanding pathophysiological mechanisms in schizophrenia, as well as mechanisms of action of antipsychotic drugs.

Models of Schizophrenia: Damage in the Prefrontal Cortex
Dysfunctional dopamine activity is linked to schizophrenia, and schizophrenia is a neurodevelopmental illness. We know that the mesolimbic dopamine system is modulated by the prefrontal cortex, including dopamine elements within the prefrontal cortex. We have begun to explore the effects of early damage to dopamine in the prefrontal cortex on adult behaviour. To date, we have found that even modest damage to dopamine projections to the prefrontal cortex has repercussions for the expression of adult behaviours. In particular, early-life damage to prefrontal cortex dopamine appears to greatly facilitate the development and expression of amphetamine sensitization.

DR. USOA BUSTO, HEAD

Clinical Neuroscience

NEUROSCIENCE RESEARCH DEPARTMENT

Many factors influence compulsive drug-taking behaviours. These factors vary according to the drug, the host and the environment. The Clinical Neuroscience Section studies the behavioural and pharmacological effects of drugs in humans and the different factors that may contribute to drug-taking behaviour.

CLINICAL NEUROSCIENCE I

DR. USOA BUSTO

Host Factors Contributing to Substance Use Disorders

A major line of our research explores host factors contributing to substance use disorders, including multiple drug use, psychiatric comorbidity and genetics.

We continue to examine the role of the brain reward system in major depressive disorder (with Drs. Claudio Naranjo, Helen Mayberg and Simon Graham). Our findings suggest that dopamine and the brain reward system are dysfunctional in severely depressed patients and that specific areas of the brain are involved in the response to a dopaminergic probe. In a neuro-imaging study, which is the logical continuation of the brain reward system clinical study, we are currently looking at the specific areas of the brain where changes in the response to amphetamine actually occur.

Another area of our research explores the role of nicotine in modulating symptoms of depression in depressed smokers and non-smokers (with Drs. Laura Cardenas, Martin Zack, Sylvain Houle, Shitij Kapur and Helen Mayberg). Preliminary data from positron emission tomography (PET) studies suggest that dopamine release in depressed smokers was significantly lower in comparison to depressed non-smokers. This suggests a hypofunctional brain reward system in people with depression. Age is another host factor contributing to substance use disorders. We are studying the effects of hypnotic medications in older adults (with Drs. Beth Sproule and Nathan Herrmann). We hope to document the advantages and disadvantages of prescription versus non-prescription sleeping medications in an older adult population.

Prescription Drug Dependence

Pain and depression may influence dependence on opioid medications, particularly prescription opioids. This year, we started a collaborative study with the Clinical Research Department, examining the characteristics of, and comorbid disorders in, patients dependent on prescription opioids. Our findings, which have been recently presented, show that there are four patterns of opioid abuse: Heroin only, prescription drugs only, prescription drugs and heroin (current) and prescription drugs with a past history of heroin abuse/use. The prescription-only patients are older and have more substantial pain and psychiatric comorbidity than all the other groups. This study will lay the foundation for future research in people who are dependent on prescription drugs and who have comorbid problems, such as chronic pain and depression (with Drs. Beth Sproule and Bruna Brands).

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. We continue to research the comparative abuse liability of currently available drugs as well as new compounds. Our ongoing work looks into the comparative pharmacology, behavioural effects and abuse potential of heroin and hydromorphone in human subjects (with Drs. Bruna Brands and David Marsh).

PHARMACOLOGICAL MODULATION OF ADDICTION-RELATED COGNITIVE NETWORKS AND RELATED PROCESSES

DRS. MARTIN ZACK AND CONSTANTINE X. POULOS

Our research explores cognitive processes that mediate self-regulation of addictive behaviour. Using a cognitive neuroscience approach, we study ways that alcohol and other drugs affect the way people — with and without addictive disorders — process information.

Psychoactive drugs can activate or inhibit the cognitive processes that regulate behaviour under normal circumstances. Our methodology consists of cognitive science tasks (e.g., reaction time, vigilance, attention, psychomotor inhibition), often administered by computers, and pharmacological probes (e.g., drugs of abuse, drugs that tap specific neurotransmitter systems).

To further our understanding of the neurochemistry underlying addiction, our general strategy is to assess how drugs modulate addiction-related cognitive networks in memory. Cognitive networks are sets of concepts linked in memory around a common theme (e.g., alcohol, smoking, gambling, panic, phobic concerns). Such networks distinguish clinical populations, including addicted people, from healthy controls. Pharmacological or environmental cues can activate these networks. Cognitive activation can then bias decisions or overt behaviour toward substance use or gambling, either directly or by engendering subjective states (e.g., negative affect, anxiety, craving) that motivate these behaviours. Because the biasing effects of cognitive activation can occur automatically and involuntarily, they may contribute to the compulsive aspects of addictive behaviour.

This is particularly important in people who have concurrent disorders, such as people who are anxious and have drinking problems, because their addiction networks may be intertwined with networks related to their anxiety disorder. It also permits us to use drug probes to evaluate gambling-related cognitions in people who have gambling problems. This approach is especially important because there are no animal models to provide information about the neurochemical basis of gambling.

Priming of Gambling-Related Cognitions by Amphetamine
In this project, funded by a grant from the National Center on Responsible Gaming, we examined how amphetamine, a psychostimulant, activates motivation to gamble and automatic gambling-related cognitions in people who have gambling problems, people who have drinking problems, and controls.

The results indicate 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 amphetamine increased desire for alcohol in people who have drinking problems, it did not reliably alter confidence to avoid drinking, nor did it affect alcohol-related cognitions.

These findings support the possibility that gambling addiction is mediated by the same neurochemical circuits activated by psychostimulant drugs.

Priming of Alcohol-Related Cognitions by Benzodiazepines
In this project, we examine the effects of two benzodiazepines on motivation to drink and automatic alcohol-related cognitions in people who have drinking problems. We are comparing the effects of diazepam, a drug with high abuse liability, with those of clonazepam, a drug with low abuse liability. The project also examines the moderating effects of drug dose, severity of alcohol problems, and degree of co-existing anxiety on cognitive and behavioural responses to these drugs. Our findings will lay the foundation for future research, using 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.

Effects of Alcohol on Stress-Induced Cognitive Activation in Young Drinkers with High- and Low-Anxiety Sensitivity
This project, funded by a grant from the Alcoholic Beverage Medical Research Foundation, evaluates the effects of a moderate dose of alcohol on automatic anxiety-related cognitions induced by a stressor in university students with a high or low sensitivity to anxiety-provoking stimuli.

Anxiety sensitivity is a trait variable that also predicts the sub sequent development of anxiety disorders. People with a high sensitivity to anxiety use alcohol to cope with negative mood states more often, and display higher rates of alcohol use problems, than do people with a low sensitivity to anxiety. We hope to determine a possible mediating role of cognitive activation in the negative reinforcing effects of alcohol in young people with a high sensitivity to anxiety. This may help us develop strategies to prevent or reduce the transition to full-blown comorbid anxiety and alcohol use disorders later in life.

Effects of Chlorpromazine on Smoking-Related Cognitions
In this study (a collaboration with Dr. Bill Corrigall, CAMH), we gave a low dose of chlorpromazine, a typical antipsychotic, to male smokers with no psychiatric disorder. Our intent was to evaluate the effects of chlorpromazine on the subjects' automatic smoking-related cognitions and motivation to smoke.

Our results indicate that chlorpromazine augments activation of smoking cognitions induced by overnight abstinence. This effect correlates with self-reported craving for cigarettes. Smoking a single cigarette also had a greater dampening effect on activation in subjects who received chlorpromazine than in subjects who received a placebo.

We observed a parallel pattern on a cognitive task that involved no specific smoking-related stimuli. These results indicate that information processing may contribute to extremely high smoking rates in people receiving typical antipsychotic medications.

Deficient Inhibitory Control and MDMA (Ecstasy)
In this study, funded by the Grants in Psychiatry program, CAMH, we identified a consistent linear increase in impairment of inhibition with chronic use of MDMA in people who used no other drugs. Using controls of healthy non-MDMA users, we identified empirical cut-offs for the levels of MDMA use that coincide with a significant increase in "disinhibition." This finding may provide a rough index of the point where the neurotoxic effects of MDMA begin to translate into demonstrable impairment in self-control.

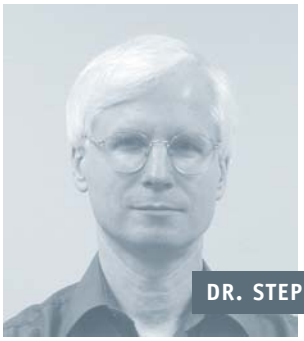
Current work extends this evidence to investigate how other potentially neurotoxic drugs and marijuana may affect the impairment of people who use MDMA and these substances. (Drs. Paul Fletcher and Stephen Kish, CAMH, are collaborators on this project)

Alcohol-Gambling Associations

This study investigated the cognitive mechanisms involved in concurrent gambling and alcohol problems. Some people who gamble report a tendency to drink more when they win. These people displayed greater activation of alcohol cognitions by win-related verbal stimuli (e.g., a jackpot) than did gamblers with no specific drinking bias, or gamblers who said they were less likely to drink when they won.

Our results support the possibility that cognitive activation contributes to the motivation to drink in certain people who have gambling problems. Involuntary-gambling/alcohol associations specify a process that may explain how gambling and alcohol become linked in comorbidity and provide a target for interventions designed to treat such individuals. (Drs. Sherry Stewart and Ray Klein of Dalhousie University are collaborators on this project.)

Project funded by a grant from the Ontario Problem Gambling Research Centre (held jointly with Dalhousie University).



Human Neurochemical Pathology Laboratory

NEUROSCIENCE RESEARCH DEPARTMENT

DR. STEPHEN KISH, HEAD

The mandate of the Human Neurochemical Pathology Laboratory is to understand the causes of neuropsychiatric disorders through direct examination of the human brain by either brain scan procedures in living subjects or by neurochemical investigations in autopsied human brain. During the year 2001, we published eight articles in peer-reviewed neuroscientific journals. The laboratory continues to divide its time between studies of drug use (ecstasy) and psychiatric problems in patients with movement disorders (Parkinson's disease).

DRUG USE

Ecstasy

Ecstasy (MDMA), a derivative of amphetamine, is widely used by people of all age groups worldwide. Among known risks of the drug (e.g., death in a very small number of users), the most serious concern is that ecstasy might cause permanent damage to brain neurons that use serotonin as a neurotransmitter, as suggested by animal data.

In collaboration with the PET brain scan unit at CAMH (Drs. Sylvain Houle, Alan Wilson, Natalie Ginovart), we have begun to measure the number of serotonin neurons in brain of chronic users of ecstasy as compared with that in a control group. The results of this study will help define the risks of taking ecstasy and may also help us to understand the role of serotonin in different psychiatric conditions, such as depression and panic anxiety, sometimes observed in people who use ecstasy.

MOVEMENT DISORDERS

Depression in Parkinson's Disease

Recent data suggest that depression has a greater impact on the quality of life of the patient with Parkinson's disease than does the movement disorder (rigidity, tremor, slow movement) itself. Work of Dr. Oleh Hornykiewicz suggests that damage to the brain serotonin system might explain the depression in Parkinson's disease. In collaboration with Dr. Mark Guttman (Human Neurochemical Pathology Lab), responsible for the largest Parkinson's disease practice in Canada, Dr. Jerry Warsh, a CAMH psychiatrist specializing in mood disorders, and the PET unit, we are comparing the number of serotonin neurons in brain of depressed patients with Parkinson's disease, non-depressed patients with Parkinson's disease, and control subjects. The results of this study, supported by the Michael J. Fox Foundation, will help us understand the nature, cause, and treatment of the disabling depression in Parkinson's disease.

Laboratory of Cellular and Molecular Pathophysiology

NEUROSCIENCE RESEARCH DEPARTMENT

DR. JERRY WARSH, HEAD

Research in the Laboratory of Cellular and Molecular Pathophysiology Section investigates the cellular and molecular pathophysiology of the major psychoses, principally bipolar affective disorder, and the molecular pharmacology of antibipolar and antidepressant medications. The research team includes Dr. Jerry Warsh, clinician scientist, Dr. Peter Li, senior basic scientist, and their graduate student and postdoctoral trainees.

Our groundbreaking, innovative research has led us to discover abnormalities in signalling processes inside nerve cells. These abnormalities play a critical role in the development of bipolar I disorder.

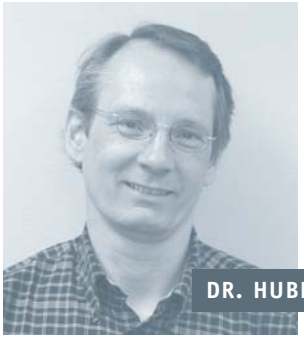
A year ago, we identified patterns of changes, in several genes and their protein products, that affect intracellular calcium signalling in a subtype of bipolar I disorder. This year, we identified two novel target genes whose expression is regulated by lithium treatment; these genes may represent therapeutically relevant targets of this medication.

Inositol monophosphatase type 2 is an enzyme found to be altered in cells from people who have bipolar disorder. Lithium, a mainstay in the treatment of bipolar disorder, blocks the activity of this family of enzymes. Using special gene-hunting techniques, our research team continues to identify genes that may be affected by lithium and other mood stabilizers. This year, we identified a gene that encodes diphosphoinositol polyphosphate phosphohydrolase, an enzyme that helps metabolize inositol polyphosphates. We identified another gene that encodes a transmembrane-4-superfamily protein, CD151. This is a scaffolding protein that interacts with several signalling molecules in the inositol lipid signalling pathway.

These observations clearly support lithium's therapeutic potential to regulate the expression of a distinct set of genes involved in the signalling pathway(s) known to be dysregulated in bipolar disorder.

Our findings of the past year continue the current goal of our research — to translate our findings into clinical tests to more easily diagnose subtypes of bipolar disorder; and to predict which patients will respond to lithium or other mood-stabilizing medications.

Our research grants, awarded by the Canadian Institute for Health Research and the Ontario Mental Health Foundation, now total $1,295,000. These grants allow our lab to conduct the studies necessary to produce clinical tests for use at the bedside and in the community. Our findings also set the stage to develop new drugs for treating and preventing relapses in bipolar disorder. The closer we come to understanding the specific chain of cellular disturbances that lead to this disorder, the more effectively we can work to develop new strategies to treat and prevent it.



Molecular Neuroscience
NEUROSCIENCE RESEARCH DEPARTMENT

DR. HUBERT H.M. VAN TOL , HEAD

The goal of the Molecular Neuroscience Section is to understand the mechanisms by which neural communication takes place. By taking a deterministic approach to fundamental problems in neurotransmission, we seek to understand the molecular components involved in communication between neurons and how these components may contribute to mental illness and how they serve as therapeutic target.

The section has three principal investigators directing their own research group. Their research involves molecular, biochemical and electrophysiological approaches to study the molecules involved in neuronal signalling. Our scientists principally apply *in vitro* approaches and use model systems, including transgenic mice and the nematode *C. elegans*, for their research. They often extend their findings to human disease through collaboration with other scientists, most notably with the Neurogenetics Section at CAMH. The research section is widely associated with many neuroscientists inside Toronto (http://www.uoftphysiology.com/neurosciencenet/governance.html), and outside Toronto, and is associated with 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. This system is often presumed the origin, 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 humans, the neurotransmitter dopamine is synthesized in the brain in neurons located in the midbrain area, most notably the substantia nigra and the ventral tegmental area. These neurons project to their target areas where dopamine is released in a regulated manner. The importance of proper function of these neurons is seen in examples such as the loss of dopamine neurons of the substantia nigra, which is the cause for Parkinson's disease. Evidence is emerging that excessive dopamine release plays a role in schizophrenia, and in the study of addiction, several drugs of abuse have been found to stimulate dopaminergic transmission.

Dopamine released from these neurons binds to specific targets known as dopamine receptors. Five different dopamine receptors have been identified in humans; these receptors are all members of the G-protein-coupled receptor (GPCR) family. Besides the target (postsynaptic) neurons, dopamine receptors are also present presynaptically on the dopamine neurons themselves. Thus dopamine neurons can serve as a component of the feedback mechanism for controlling their own release.

Activation of the dopamine receptors by the neurotransmitter will activate a cascade of intracellular signalling molecules. This cascade will ultimately mediate a change in the activity of various ion channels or modulate the status or expression of the molecules involved in neurotransmission, thus modulating the excitability of the cell and the transmission of a signal. Many areas are still poorly understood, such as the factors controlling dopamine neuron development, regulation of neurotransmitter release, and mechanisms of dopamine receptor-mediated changes in intracellular signalling.

Novel Dopamine Signalling Pathways

Dopamine receptors belong to the superfamily of receptors that mediate their signal through heterotrimeric G-proteins. Evidence is emerging to show that this family of receptors may also directly interact with other cellular components that will either regulate the receptor or serve as effector. We identified that dopamine receptors can bind Src homology 3 (SH3) domains. SH3 domains can be found in a variety of proteins involved in intracellular signalling, and these domains serve a role in bringing proteins together in the cell. Using yeast two-hybrid and phage displays screening protocols, and more directed protocols, we have been identifying SH3-domain-containing proteins that can directly interact with dopamine receptors.

We and others have observed that this type of interaction may modulate receptor internalization and the activation of mitogen-activated protein kinases (MAPK). Our observation has directed our research on the functional significance of this interaction into these two areas. In past study, we found that dopamine D2 and D4 receptors activate the MAPK pathway through transactivation, a process by which platelet-derived growth factor receptors are activated. 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) activation in hippocampal neurons. The mechanism of transactivation is not well understood and is the subject of our ongoing studies. The observation that dopamine receptors can transactivate growth factor receptors, and thus a large variety of intracellular signalling pathways, may give us new insight in how dopamine receptors control neuronal development and survival, differentiation, and synaptic plasticity.

GIRK Channel Complex

G-protein-activated inwardly rectifying K+ channels (GIRK; a.k.a. Kir3) are the effector of various GPCRs, including the dopamine D2, D3 and D4 receptors. Four different Kir channel subunits, Kir3.1, 3.2, 3.3 and 3.4, form a tetrameric complex to make a functional channel. These channels regulate the excitability of the cell by maintaining the membrane potential to the resting potential. The presence of these channels in the presynaptic dopamine neurons, particularly Kir3.2, may play an important role in the feedback regulation of dopamine release through its activation via presynaptic dopamine D2 receptors.

We know that these channels are activated in a membrane-delimited manner, arguing that the channel and receptor have to be close to each other to mediate functional activation. However, we 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 stability of the receptor-channel complex does not depend on receptor activation or G-proteins, but its initial formation depends on G-beta-gamma G-protein subunits.

In collaboration with Dr. Terrence Hebert (Montreal Heart Institute, University of Montreal) we used bioluminescence resonance energy transfer in live cells to extend these findings to other receptors, including the beta 2-adrenergic receptor. 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. Our ongoing work investigates the molecular determinants of this interaction.

Model Systems: *C. elegans* and Dopamine Signalling

The nematode *C. elegans* is a model system that can be analysed with powerful genetic tools. Its genetics, anatomy, development, behaviour and nervous system have been well studied. By mammalian standards, *C. elegans* has a very simple nervous system. However, it encodes most of the known molecular components of mammalian brains.

We confirmed that *C. elegans* produces dopamine and several of its metabolic products. Others identified several key components of the dopamine system, including tyrosine hydroxylase and the dopamine transporter.

The dopamine receptor of *C. elegans* remains elusive. Using bioinformatic approaches we identified up to 15 candidate dopamine receptors. One of these receptors encodes on functional and pharmacological grounds for a dopaminergic receptor. In collaboration with Dr. Joseph Culotti (Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto), we identified the neurons in *C. elegans* that express this receptor and mutant strains in which the receptor is disabled.

With Dr. William Schafer (University of California, San Diego), we observed that mutant strains with this receptor disabled displayed an altered habitutation response to non-localized mechanical stimuli ("tap response"). This is consistent with the observation that this receptor is expressed in the mechanosensory neurons ALM, PLM and PHC. We are analysing these mutant strains, using genetic suppressor screens to identify genes linked to the dopamine receptor functioning. This way, we hope to identify new components involved in the functioning of the dopamine signalling system. Based on the observed genetic similarities between humans and *C. elegans*, these genes may fulfill a similar role in the functioning of the mammalian dopamine system.

Model Systems: Candidate Genes for Schizophrenia Using a Rodent Model System

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. Genes with an altered expression in schizophrenia may be labelled as candidate disease genes.

We collaborated with Dr. Barbara Lipska et al. (Clinical Brain Disorders Branch, NIMH) to pursue a non-human model system for schizophrenia (Lipska et al., 1993) to screen for candidate genes. This model contains not only the appropriate behavioural abnormalities, but also the delayed development component and differences in genetic susceptibility for the disorder. We screened up to 30,000 genes for six different parameters of the model. This allowed us to identify several genes that may be involved in schizophrenia. Several of the identified genes are being analysed in ongoing genetic family-based association studies in collaboration with Drs. James L. Kennedy and Fabio Macciardi (Neurogenetics Section, CAMH).

To date, we have found that one of the 14-3-3 genes, a multifunctional protein involved in intracellular signal transduction in neurons, shows an association with schizophrenia.

MOLECULAR NEUROBIOLOGY II

DR. FANG LIU

This year, our lab focused on characterizing the molecular mechanisms by which G-protein-coupled dopamine D1 receptors exert functional cross-talk with ligand-gated ion channel NMDA receptors. Previously, we found that dopamine D1 receptors modulate NMDA glutamate receptor-mediated functions through direct protein-protein interactions. Two regions in the D1 receptor carboxyl tail can directly and selectively couple to NMDA glutamate receptor subunits NR1-1a and NR2A. More significantly, we found that 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.

D1 Receptors Modulate Excitotoxicity

We have found a constitutive coupling between the dopamine D1 receptor carboxyl tail and the NMDA receptor NR1-1a subunit. Interestingly, the D1: NR1-1a binding competes with the physical coupling of the calcium binding protein CaM to the NR1-1aCT in a concentrated and Ca2+-dependent manner. Agonist stimulation of D1 receptors, which protect cells from NMDA-mediated excitotoxicity, leads to the uncoupling of the D1: NR1-1a complex, thereby leaving NR1-1a subunits more accessible to promote the formation of the NR1-1a: CaM: PI-3 kinase complex. The NR1-1a: CaM: PI-3 kinase complex can promote PI-3 kinase activity and attenuate NMDA receptor-mediated excitotoxicity.

Our study appears to be the first to define and demonstrate the possible functional implications of the interactions between the NMDA receptor and PI-3 kinase. This finding provides complementary evidence that our previous observation — that the dopamine D1 receptor attenuates NMDA receptor-induced toxicity — does not depend on the reduction of Ca2+ influx but depends upon the PI-3 kinase and the subsequent activation of downstream anti-apoptotic signalling pathways, such as Akt.

D1 Receptors Modulate NMDA Receptor Currents

Agonist-induced sequestration of plasma membrane G-linked receptors is the primary mechanism by which these receptors are desensitized. Research shows that agonist stimulation leads to D1 receptor internalization. Collaborating with Dr. Yu-Tian Wang (professor and HHMI International Scholar, University of British Columbia), we found that the direct protein-protein coupling between dopamine D1 and NMDA receptors enables activation of D1 receptors to induce a rapid internalization of NMDA receptors from the plasma membrane surface, thereby reducing NMDA receptor-mediated currents. Moreover, D1-stimulated NMDA receptor translocation can be antagonized by co-expressing the mini-gene that is able to interrupt the D1: NR2A interaction.

These data suggest that NMDA receptor activity may be modulated via rapid trafficking away from the cell surface plasma membrane. This is consistent with the notion that NMDA receptor-mediated responses may be a product of the number of synaptic receptors.

MOLECULAR PHYSIOLOGY

DR. XIAN-MIN YU

The pathophysiological process underlying the development of schizophrenia remains a mystery for modern medicine. Data obtained from clinical and basic research studies have convincingly indicated that abnormal NMDA receptor activity is an important factor involved in the development of this disorder.

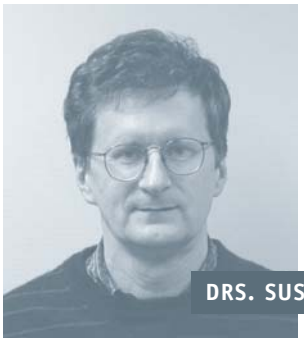
Through our research, we hope be able to characterize mechanisms underlying the activity-dependent neuroplasticity associated with physiological and pathological processes in the central nervous system (CNS). To try to discover how neuronal activity regulates synaptic responses in the CNS, we have tried to identify the novel intracellular mediator(s) that couple the neuronal activity to the modulation of neurotransmitter receptor functions.

In the CNS, Src-family protein tyrosine kinase regulation of NMDA subtype glutamate receptors has been found to play an important role in learning and memory, ethanol sensitivity, and epilepsy. However, we do not yet understand the mechanisms underlying this regulation. We found that Src protein tyrosine kinase, its activator, protein tyrosine phosphatase alpha (PTPa) and substrate, NMDA receptors, are linked by the same scaffold protein, post-synaptic density 95 (PSD95). Through the distal phosphatase domain (D2), PTPa binds to the PDZ2 domain of PSD95. Removing PTPa does not affect the association of Src with NMDA receptors, but it may knock out the constitutive regulation of NMDA receptors by Src-family protein tyrosine kinases. Furthermore, our work demonstrates that application of PTPa functional domains (D1+D2) into neurons enhances NMDA receptor-mediated synaptic responses. Conversely, blocking endogenous PTPa inhibits NMDA receptor activity and the induction of long-term potentiation (LTP) in hippocampal neurons.

The regulation of ligand-gated ion channels, such as NMDA receptors, by protein kinases and phosphatases has been extensively studied. According to previous studies, protein kinases and phosphatases appear to act in opposition when regulating ligand-gated ion channels (i.e., kinases up-regulate, while phosphatases down-regulate, the channel activity). However, our present study gives the first direct evidence indicating that a phosphatase may also up-regulate ligand-gated ion channel functions and excitatory synaptic transmission in the CNS. PTPa is the first phosphatase found to be actively involved in LTP induction. To understand activity-dependent neuroplasticity in the CNS, it is essential to further characterize mechanisms underlying the function of PTPa in LTP induction (*The EMBO Journal*, 2002).

Another ongoing project in the Molecular Physiology group is the collaborative research with Dr. Fang Liu's Molecular Neurobiology II group. We are investigating the regulation of NMDA channels by a novel protein-protein binding interaction between dopamine D1 receptors and ligand-gated ion channels, exploring the theory that different types of neurotransmitter receptors modulate each other via their direct binding interaction (see page 22).

Our findings have created new avenues to advance our understanding of the regulation of NMDA receptor functions in the CNS. We believe that further studies will help us understand mechanisms underlying normal in vivo NMDA receptor activity in the CNS. Such understanding is essential for developing novel therapeutic approaches to treat a wide range of clinical problems such as mental illness.

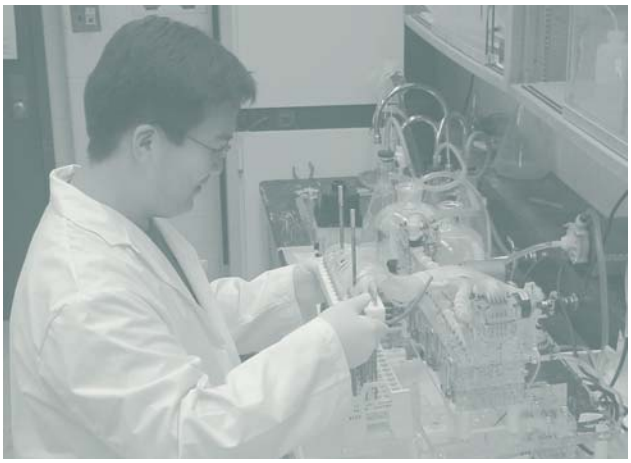


Molecular Pharmacology

NEUROSCIENCE RESEARCH DEPARTMENT

DRS. SUSAN R. GEORGE AND BRIAN F. O'DOWD, HEADS

During the past year, the Molecular Pharmacology Section continued work on the biology of neurotransmitter receptors for dopamine, opioid peptides, apelin and others that we identified, including the ability of the receptors to interact directly with other receptors to alter signal transduction. We have continued investigating receptor-gene-deleted mice models and models over-expressing neurotransmitter/neuropeptide to study the role of the individual receptors in brain function. Our search for novel human genes resulted in the identification of several novel receptors. Many of these are highly expressed in brain. They are potential targets as candidate genes in neuropsychiatric disease and may lead to the development of novel drugs. During the past year, we have published 13 peer-reviewed papers on our research in this section.



Receptor Biology

Almost 10 years ago, our laboratory 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 substantiated and shown to be true for all members of the family of G-protein-coupled receptors.

We also discovered that individual receptors can form complicated higher-order structures with other receptors, greatly enlarging the complexity of novel functional therapeutic targets in the brain.

We are now investigating receptor-receptor interactions, using functional assays we developed, to establish the physiological roles of this process in receptor and cell function.

We have determined the sites of interaction between two interacting receptors, and have narrowed this to a particular region of the receptor. We are intensively studying the dopamine (5 distinct receptors, D1 to D5) and opioid (3 distinct receptors) receptors and a novel receptor that we cloned, the apelin receptor. The dopamine receptors form homomeric and heteromeric (i.e., mixtures of receptors) complexes. The apelin receptors have a highly novel expression in the cell — we are further investigating this. After activation by the specific neurotransmitter, the receptors on a cell or neuron undergo a process by which they become insensitive to further activation, termed desensitization. We have recently identified the specific amino acids within the D1 receptor responsible for this effect, in distinction from other amino acids that mediate the internalization of the receptor from the cell surface into the interior.

Novel Receptor Genes

Our successful work on the discovery of novel receptor genes has resulted in the identification of ~50 additional ones to date, many of which are expressed highly in human and rat brain and will form novel drug targets. Our laboratory and many others worldwide are conducting the physiological characterization of these receptors. 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.

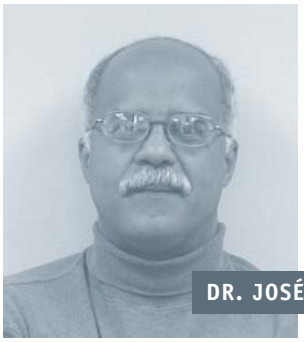
Role of Receptors in Behaviour

Our development of several mouse models lacking individual or multiple receptor genes has proven to be an extremely valuable strategy to determine the complete repertoire of functions mediated in the brain.

Mice lacking the D1 dopamine receptor lose their preference for alcohol drinking and for sugar pellets. They appear unable to perceive rewarding stimuli and will not press a lever to obtain these substances. They also have a spatial learning deficit and an inability to forget fearful events.

We have developed a colony of D3 dopamine-receptor-lacking mice. These mice show less anxiety on some behavioural tests.

The loss of both D1 and D3 receptors prevents the manifestation of reduced anxiety, implicating an interaction between these two receptor systems in mediating the level of anxiety. We have engineered a mouse model overexpressing apelin. At present, we are characterizing the regions of brain where this is occurring and will investigate the behavioural consequences.



Neuroimaging

NEUROSCIENCE RESEARCH DEPARTMENT

DR. JOSÉ N. NOBREGA, HEAD

In the Neuroimaging Section, we aim to identify specific brain areas, neuroanatomical pathways and chemical mechanisms involved in neuropsychiatric disorders. This is done through detailed post-mortem analyses of anatomically preserved brains from animal models or human subjects. Research continues in three major areas.

Models of Depression

We are analysing brain alterations in four different models of depression, in particular the role of thyroid hormones and their receptors in brain. We continue to study models involving reactivity to stress as well as genetic models provided by collaborators from McMaster University, the University of Maryland and the Federal University of São Paulo. These analyses are complemented by similar investigations of the effects of various types of antidepressant interventions, including sleep deprivation, on the same brain systems and pathways.

In the chronic mild stress anhedonia model, we have found significant and widespread decreases in the expression of alpha1 thyroid hormone receptors in brain. When animals were treated with the antidepressant imipramine, both the behavioural deficits and brain receptor changes reverted to normal levels. These brain changes were not seen in the helplessness model of depression, suggesting that they may be associated with specific types of depressive symptoms.

Brain analyses in these models were expanded with the introduction of cDNA microarray techniques for large-scale gene screening.

We have reported upregulation and downregulation in a number of genes in the frontal cortex of animals showing vulnerability to depressive symptoms after stress. Unexpectedly, we found that a different set of genes was affected in animals showing resistance to depressive symptoms following stress.

In the sleep deprivation model, we reported the negative results of a fairly comprehensive assessment of neuropathology, using gene markers of apoptosis and autoradiographic indices of necrosis in brain. This agrees with other evidence that sleep deprivation does not induce neuronal loss.

The neuropeptide orexin has been identified as a key element in human narcolepsy. We have started to examine orexin, and have reported increases in the expression of the precursor prepro-orexin after sleep deprivation and after sleep rebound. We are currently completing *in situ* hybridization analyses of expression of orexin1 and orexin2 receptors after sleep deprivation.

Brain Dopamine and Movement Disorders

In collaboration with investigators from Hanover, Germany, a long-term project continues to build a comprehensive map of brain alterations in a genetic model of paroxysmal dystonia. This year we reported significant changes in glutamatergic AMPA receptors in basal ganglia as well as changes in NK-3, but not NK-1, Substance P receptors in dystonic hamster brains.

We have made significant progress in our ongoing work with a model of tardive dyskinetic syndromes induced by long-term antipsychotic treatment. In collaboration with clinical researchers from the Centre's Schizophrenia Division, we have identified and reported important differences related to role of antipsychotic dose and route of administration (continuous vs. intermittent availability) in defining the risk of late-onset dyskinetic symptoms. We have also reported early gene activation data on a modified clozapine molecule. This finding is in line with the hypothesis that dopamine D2 receptor occupancy is a key factor in defining atypicality for antipsychotic drugs. However, our current work with mice lacking dopamine D1 or D2 receptors suggests that long-term dyskinetic effects must involve other factors in addition to D2 receptor occupation.

Brain Mechanisms of Compulsive Drug-Taking

For the last few years, in collaboration with a group from São Paulo, Brazil, we have been systematically investigating brain mechanisms underlying differential susceptibility to alcohol sensitization.

We have reported that animals showing differential propensity to alcohol sensitization have increased levels of D2 binding in specific areas of the limbic forebrain. We reported separately that similar changes were not seen in D1 receptors or in the dopamine transporter. We have also found and reported increased levels of NMDA binding in mice showing resistance to ethanol sensitization.

In collaboration with investigators from the Biobehavioural Pharmacology and the Pharmacogenetics laboratories at CAMH, we reported localized brain changes in 3H-flunitrazepam, and 3H-muscimol binding in animals showing a differential propensity to consume alcohol. We have not observed changes in other components of the $GABA_a$ receptor system, including 3H-zolpidem and 3H-RO-154523 binding sites, and alpha1 or alpha6 receptor subunits examined by *in situ* hybridization.

Pharmacogenetics

NEUROSCIENCE RESEARCH DEPARTMENT

DR. RACHEL F. TYNDALE, HEAD

Genetic variations in people's ability to metabolize drugs can result in therapeutic failure and unanticipated toxicity due to too much or too little metabolism of a drug. In addition to clinically used drugs, researchers of the Pharmacogenetics Section, led by Drs. Rachel F. Tyndale and Edward M. Sellers, explore the role that genetic variation in drug-metabolizing enzymes can have on metabolism of drugs of abuse. The section investigates how such genetic variation can alter the risk for specific drug dependencies and alter the amount of a drug used by dependent individuals, and focuses on identifying high-risk individuals and developing novel treatment approaches. A second line of research investigates the expression and regulation of drug-metabolizing enzymes in the brain. These enzymes can alter drug levels in the immediate vicinity of drug targets such as receptors and transporters. They are also responsible for creating toxic byproducts that may lead to neurotoxicity. CYP enzymes in the brain are both genetically variable (they exist in some people and not in others) and environmentally regulated (the levels and distributions in the brain can be altered by drugs of abuse).

Research from the section has demonstrated a number of actions that metabolic variations can have on pharmacology and dependence risk profile of specific drugs. For example, genetic variation in an enzyme could alter activation of a drug to a more potent drug metabolite of similar pharmacology (e.g., codeine activation to morphine by CYP2D6). It can also create differences in metabolic patterns via variant alleles (e.g., methamphetamine is metabolized to different toxic metabolites by some people). Genetic variations can alter metabolism of drugs in which the parent drug and metabolite have similar effects but different durations of action (e.g., flunitrazepam and CYP2C19) and it can alter the activation of a drug to a metabolite that has different pharmacology (e.g., dextromethorphan to dextrorphan via CYP2D6). Variable drug metabolism can also convert an active parent drug to an inactive metabolite (e.g., nicotine to cotinine by CYP2A6).

The Pharmacogenetics Section investigates these variations using abuse liability, epidemiological, genetic, biochemical and therapeutic intervention studies. The Pharmacogenetics Section accomplished the following research goals during 2001.

New Publication in Pharmacogenomics

Dr. Tyndale, with Werner Kalow and Urs A. Meyer, co-edited the book, *Pharmacogenomics* (Drugs and the Pharmaceutical Sciences series, Marcel Dekker Inc., New York, 2001), outlining current pharmacogenomic techniques and applications. This book includes techniques used by both academic and industrial laboratories, for both small-scale and high-throughput requirements.

Enzyme Variations, Medications and Drug Metabolism

In collaboration with Dr. Deborah Mash of Miami (Professor, University of Miami School of Medicine), we showed that ibogaine, a drug being tested for addiction treatment, is metabolized by the genetically polymorphic enzyme CYP2D6. Treatment dose and outcomes are altered by this genetic variation — rapid metabolizers need larger doses and get better therapeutic outcomes.

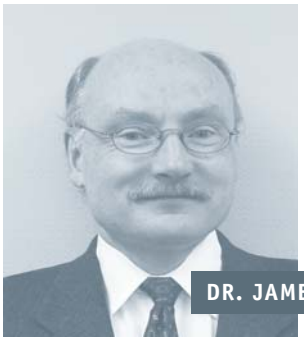
The section has also collaborated with Dr. Allan Okey (Professor, Department of Pharmacology, University of Toronto) to investigate genetic variation in the aryl hydrocarbon receptor. This receptor, which is altered by smoking, regulates an enzyme involved in metabolizing antipsychotic drugs. In collaboration with researchers in Seattle, we determined the contribution of two genetically variable enzymes (CYP2C19 and CYP3A4) to the metabolism of flunitrazepam (Rohypnol®), a drug of abuse.

We also characterized other genetic variants of hepatic enzymes, including differences in drug metabolism for two genetic variants of CYP2D6. CYP2D6 is responsible for the metabolism of codeine and amphetamine as well as a number of clinically used drugs. We have also worked collaboratively to establish the frequencies of these genetic variants among different ethnic populations (e.g., CYP2C9).

Smoking Research

In the area of smoking research, we identified and characterized inhibitors of CYP2A6, the genetically variable enzyme that inactivates nicotine and alters smoking behaviour. These inhibitors can be used to decrease nicotine metabolism *in vivo* and to decrease smoking. We also identified the genetic variant CYP2A6*2 as being fully deficient for nicotine metabolism.

In addition, we used animal models to show that ethanol can increase the enzyme that metabolizes nicotine (CYP2B1) in the liver, and that nicotine can increase one of the enzymes that metabolizes ethanol (CYP2E1) in the liver. This work merges well with the group's ongoing investigations of the effects of ethanol on one of ethanol's target receptors, the $GABA_a$ receptor. Using a number of paradigms, we showed that ethanol can alter ethanol metabolism and $GABA_a$ receptor regulation (in collaboration with Richard W. Olsen, UCLA, and Jose Nobrega and Denise Tomkins, CAMH).



Psychiatric Neurogenetics

NEUROSCIENCE RESEARCH DEPARTMENT

DR. JAMES L. KENNEDY, HEAD

Genetic factors play a role in causing schizophrenia, bipolar affective disorder, anxiety disorders, alcoholism, eating disorders, autism, impulse control disorders, and some dementias. Researchers in the Psychiatric Neurogenetics Section, headed by Dr. James Kennedy, actively search for the abnormal genes involved in the cause, expression, treatment, and possible cure of these disorders. The section houses one of the world's most comprehensive collections of interview data and DNA samples from patients with psychiatric disorders, and from their families, allowing researchers to pursue diverse lines of inquiry and make extensive comparisons among mental illnesses. Aided by technology in molecular genetics, Psychiatric Neurogenetics researchers work to further our understanding of psychiatric disorders.

PSYCHIATRIC EPIGENETICS

Epigenetics deals with regulation of gene activity. It postulates that dysregulation of normal sequence genes may be as detrimental to a cell as the DNA mutations that have been the primary target of traditional genetic linkage and association studies.

Epigenetic theory unifies a wide variety of biological and psychological theories, as well as empirical findings, that pertain to major psychosis. Over the past few years, we have been intensively investigating the role of epigenetic factors in major psychiatric illness. We performed an in-depth theoretical analysis of the epigenetic mechanisms that were assumed be operating in major psychosis (Petronis, 2001).

The epigenetic principles suggested for schizophrenia and bipolar disorder can be extrapolated and applied to a wide variety of other complex non-Mendelian disorders, such as diabetes, multiple sclerosis, rheumatoid arthritis and psoriasis, among others (Petronis, 2001).

In the laboratory, we have demonstrated that even genetically identical organisms such as monozygotic twins exhibit numerous epigenetic differences (Petronis et al. in press; submitted). This finding provides the basis for a series of new explorations in phenotypic discordance (non-identity for a disease) in identical twins, an unexplained phenomenon in human biology for more than 80 years.

Psychiatric epigenetics is an innovative development in psychiatric research, and to date, we represent the only group in the world fully dedicated to this development. With the support of the CAMH Foundation we are continuing to build a comprehensive CAMH Epigenetics Research Program in psychiatric and other human complex diseases.

PSYCHIATRIC GENETICS

Three general strategies are used in the lab. In one strategy, investigators scan all the human chromosomes, using a wide array of DNA markers, in the hope of discovering a disease gene without knowing the brain processes involved. In the second, researchers test the structure of genes known or believed to be involved in the brain function in psychiatric diseases, such as dopamine receptor or serotonin receptor genes in schizophrenia. The third strategy analyses regulation of genes; dysregulation of normal genes may increase the risk for a disease.

Candidate Genes and Attention Deficit and Hyperactivity Disorder

We have investigated numerous candidate genes for their role in neuropsychiatric disorders. In collaboration with Dr. Cathy Barr (Research Scientist, the Toronto Western Hospital), we analysed genetic variation in the genes encoding adrenergic and dopamine receptors in people who have attention deficit and hyperactivity disorder (ADHD) (Barr et al. 2001, a; b; c). Our results were consistent with those from other groups showing the dopamine transporter locus as a candidate gene for ADHD.

Phenotype for Bipolar Disorder

In bipolar disorder we examined the second messenger G-protein beta 3 subunit gene that may be involved in the action of lithium. The bipolar patients were biochemically characterized in terms of calcium homeostasis by Dr. Jerry Warsh's group. This resulted in an innovative phenotype to examine in bipolar disorder (Corson et al. 2001).

Molecular Mechanisms of Major Psychosis

With Dr. Carlos Pato (Professor of Psychiatry, State University of New York at Syracuse), we detected further evidence that the alpha 7-nicotinic receptor gene (CHRNA7) contributes to the risk of being affected with schizophrenia (Xu et al. 2001). In this study, we found that only paternal CHRNA7 is a risk factor to schizophrenia. This finding indicates the importance of epigenetic regulation in understanding the molecular mechanisms of major psychosis.

Trinucleotide Repeats in Portuguese Schizophrenia and Bipolar Patients

We continue to investigate the intriguing finding, first described by Vincent et al (2000), that unstable DNA in the form of trinucleotide repeats is increased in Portuguese schizophrenia and bipolar patients from the Azores Islands.

Serotonin System Genes and Bulimia Nervosa

In collaboration with Drs. Robert Levitan, Alan Kaplan, and Sid Kennedy, we evaluated the impact of genetic variation of the serotonin system genes (serotonin-1B receptor, HTR1B) on the body mass index in women affected with bulimia nervosa (Levitan et al. 2001). According to our data, we identified a possible association between HTR1B genetic polymorphism and body mass index. This finding may shed light on why, in response to dieting, some patients with bulimia nervosa are able to lose significant amounts of weight, whereas others have a natural limitation to their weight loss. Pending replication in a larger sample, these findings point to a possible genetic factor of fundamental importance to the bulimia nervosa population.

PSYCHIATRIC PHARMACOGENETICS

Although in its infancy, psychiatric pharmacogenetics will in the future aid clinical practice in the prediction of response and side-effects and minimize the current "trial and error" approach to prescribing medications.

Antidepressant-Induced Mania

Our group was the first to detect evidence that genetic variation in the serotonin transporter gene may account for abnormal response to medication in people with bipolar disorder (Mundo et al., 2001). We investigated "antidepressant-induced mania," a common side-effect in treatment of depression.

We found that carriers of a "short" version of the serotonin transporter gene exhibit a significantly higher probability of switching to a manic phase of the disease in comparison to people who carry a "long" serotonin transporter gene. A genetic test can help us identify patients at risk for the development of this potentially dangerous side-effect — a finding that will considerably improve the clinical management of bipolar disorder.

Antipsychotic Response and Side-Effects

Individual people with schizophrenia vary widely in their response and side-effects to antipsychotic medications. Using one of the best characterized samples for antipsychotic treatment response and side-effects in the world, we continued our lines of pharmacogenetic research: response to the atypical antipsychotic, clozapine; weight gain induced by clozapine; and antipsychotic-induced tardive dyskinesia, a debilitating motor system disease characterized by abnormal and involuntary movements.

We have published numerous studies examining clozapine response and DNA sequence variation across several key receptors from the serotonin and dopamine systems (Masellis et al., 2001; Ozdemir et al., 2001a, b). We evaluated the role of polymorphisms in dopamine D3 receptor (DRD3) and CYP1A2 genes for propensity to develop tardive dyskinesia in patients with schizophrenia. Combining pharmacogenetic analysis of pharmacokinetic and pharmacodynamic targets for antipsychotics should improve our ability to identify subpopulations that differ in drug safety profile.

Weight gain is a serious side-effect of antipsychotic therapy. Clozapine, in particular, has the highest propensity of all antipsychotics to lead to increased weight. Because of this, we have tested if and how DNA sequence variants of the genes encoding brain receptors and the molecules involved in energy utilization are associated with susceptibility to weight gain (Basile et al., 2001).

To analyse patient susceptibility to clozapine-induced weight gain, we tested 10 genetic polymorphisms across nine candidate genes, including the serotonin 2C, 2A, and 1A receptor genes (HTR2C/2A/1A); the histamine H1 and H2 receptor genes (H1R/H2R); the cytochrome P450 1A2 gene (CYP1A2); the beta3- and alpha-adrenergic receptor genes (ADRB3/ADRA1A); and tumor necrosis factor alpha (TNF-alpha). We collected prospective weight gain data for 80 patients with schizophrenia who completed a structured clozapine trial. We observed promising trends for ADRB3, ADRA1A, TNF-alpha, and HTR2C.

This work is the first to provide a detailed methodological analysis of the literature on the obesity-related pathways and to develop rationale for other molecular genetic studies in this field of pharmacogenetics.

Smoking and Nicotine Dependence
NEUROSCIENCE RESEARCH DEPARTMENT

DR. WILLIAM CORRIGALL, HEAD

The long-range goal of the Smoking and Nicotine Dependence Research Section is to better understand the brain mechanisms involved in nicotine addiction and to use this knowledge to test neurochemical targets to develop medications that can help in tobacco-use cessation. The experimental design of our ongoing studies recognizes that nicotine, the primary psychoactive agent in tobacco smoke, maintains voluntary self-administration in laboratory animals. This behaviour is a core element of addiction. Using a rat model of this self-administration behaviour, we have previously shown that nicotine maintains self-administration behaviour by acting on certain brain substrates. At present, our studies have two main directions.

Neurochemistry of Nicotine Addiction

In this project, we have previously shown that voluntary self-administration of nicotine depends on the action of the drug in two areas: 1) neurons in the ventral tegmental area (VTA) of the midbrain that use the neurochemical dopamine as transmitter (these neurons have been shown to be a critical pathway in drug reinforcement processes in general) and 2) a non-dopamine system projecting to the VTA from an area in the brainstem called the pedunculopontine tegmental nucleus (PPTg). This area may be particularly involved in nicotine addiction. The action and reinforcement of nicotine in these brain regions is influenced by neurochemical systems present there. These findings have been made through micro-pharmacological manipulations of the VTA and PPTg in animals trained to self-administer nicotine.

Under the leadership of Dr. Shafiq Rahman (Research Scientist, CAMH), our research focus has moved to examine the characteristics of the neurochemical release of dopamine in laboratory animals during both voluntary self-administration and experimenter-administration of nicotine. This research relies on a technique known as *in vivo* microdialysis coupled with neurochemical detection, which allows us to sample small amounts of neurochemicals as they are released focally in the brain reward circuits. The amount of transmitter release is then quantified electrochemically. The unique strength of these studies is the combination of *in vivo* microdialysis with nicotine self-administration, a union that will allow us to make discoveries about brain mechanisms in nicotine-reinforced behaviour.

In vivo microdialysis procedures allow us to study the extracellular dopamine concentrations in the mesolimbic dopamine system during nicotine exposure in animals trained for nicotine self-administration. As control conditions, we are also measuring dopamine concentrations during nicotine self-administration and food-maintained responding. These experiments allow us to determine nicotine-specific effects on the dopamine system, apart from the response of this brain system to other drugs (e.g., heroin, cocaine) or behaviour motivated by natural reinforcers (e.g., food).

Additionally, we are characterizing the dopamine concentration during nicotine self-administration maintained on schedules of reinforcement that require animals to do different amounts of work to obtain their drug. In this way, we can elucidate the relationship between the behavioural output for a drug and changes in dopamine concentration.

Similar procedures help us monitor the changes in dopamine concentration in the mesolimbic dopamine system following systemic administration of nicotine coupled with microinfusions of cholinergic, GABA-ergic, glutamatergic, and opioid compounds into the VTA and the PPTg. These compounds have been shown to modify nicotine self-administration. Our studies in this area will explain the mode of action of these compounds on the midbrain dopamine system.

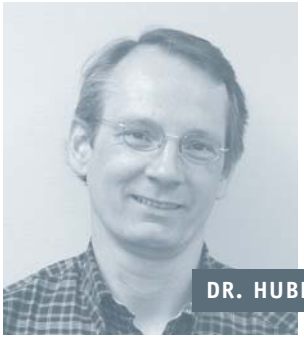
The results of these studies will help us understand brain mechanisms involved in nicotine addiction. In particular, the research will uncover mechanisms within the mesolimbic dopamine system, possibly new mechanisms that are mesolimbic-dopamine independent. Information of this kind can support initiatives to develop medication as well as help identify risk factors for nicotine addiction.

Drug Self-Administration in Animals/Pre-clinical Medication Development

We are testing particularly relevant neurochemical agents for their ability to reduce nicotine self-administration when they are administered systemically. This year, one set of experiments began examining agonists for GABA receptors. GABA is the brain's main inhibitory transmitter. We have previously found that GABA agonists delivered into the VTA or PPTg attenuated nicotine self-administration, and did so preferentially, compared to the self-administration of cocaine. In addition, anatomical data showed that nicotine may directly target GABA-containing neurons in the PPTg. For these reasons, we are exploring the efficacy of GABA agonists delivered systemically to selectively reduce nicotine self-administration. GABA agonists are also being used in human experimental studies of drug use, including tobacco smoking, as a potential target for medication development.

A similar rationale gives evidence that a particular serotonergic target may also afford a pharmacological access point to nicotine reinforcement.

In addition, we are examining whether high-dose nicotine replacement might be a useful smoking cessation approach. Our animal model is a useful means to address this issue — we can examine the effects of sustained high-dose delivery of nicotine to the experimental animals, and we can measure the effects of high-dose delivery on nicotine self-administration and relapse after removal of the drug.



Transgenic Facility
NEUROSCIENCE RESEARCH DEPARTMENT

DR. HUBERT H.M. VAN TOL, HEAD

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 have given us new opportunities 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 is also equipped to help scientists create their own transgenic mice strains or to employ viral-mediated gene transfer experiments. This year, the facility lent its services to researchers in the Molecular Neuroscience, Biopsychology and Neuroimaging sections.