JOINT PROPOSAL FOR RESEARCH IN NEUROSCIENCE SUBMITTED WITH GSC 30/31 (ANIMAL BIOLOGY AND PHYSIOLOGY)

1. The Growth of Neuroscience

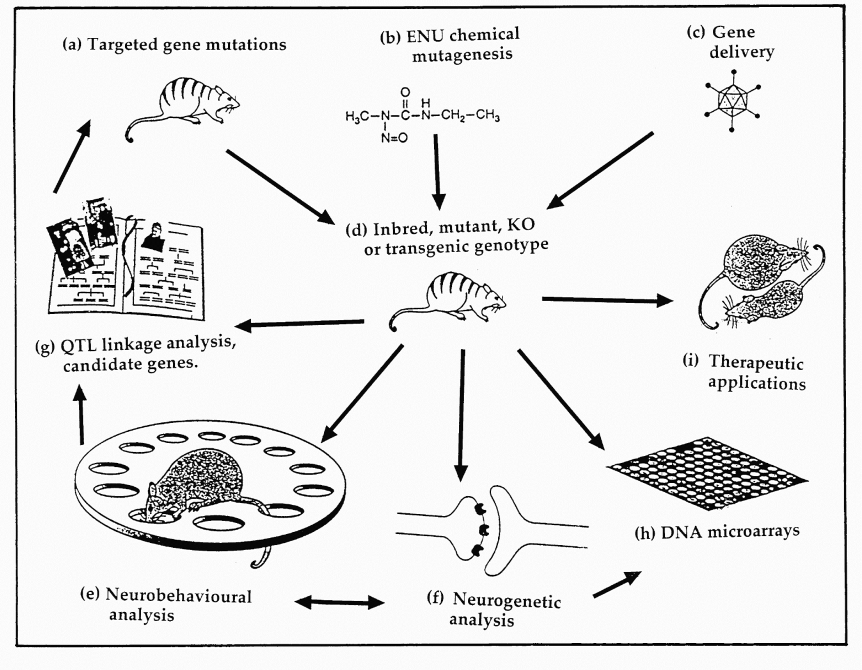
Neuroscience is one of the most rapidly growing areas of biological science, as documented by the 25-year growth of the Society for Neuroscience to more than 25,000 members. Canada has the third largest membership in the Society for Neuroscience (1,800 members), after the USA (18,400) and Japan (2,100). Canada also ranks third in the number of high impact papers published in Neuroscience after the USA and England (data from the ISI database: High impact papers in neuroscience, 1981-1998). Thus Canada is a world power in neuroscience research. To maintain this strength and extend it in the future, NSERC's neuroscience researchers who are mostly funded through GSC 30/31 and GSC12, require additional funds.

2. Neuroscience as a multidisciplinary science.

Neuroscience is by definition a multidisciplinary science, one which is represented by no single GSC, and is thus an appropriate area for a joint proposal for this reallocations exercise. Neuroscience involves a core group of academic disciplines including the preclinical areas of anatomy, physiology, pharmacology, and biochemistry, together with behavioural scientists in psychology as well as biologists from many academic departments. Neuroscientists hold appointments in many areas of the life and physical sciences, as well as clinical appointments. Within NSERC, most neuroscience grant holders apply to GSC12 and GSC30, but other applicants to GSC30/31 also work on nervous systems. Neuroscientists in different disciplines use different techniques and different animal species, including humans, to ask a variety of types of questions about the function of the brain. Thus, for example, molecular neuroscientists use cellular and molecular biological methods to investigate questions about gene function in neurons; electrophysiologists use electrical recordings from single cells and ion channels; neurochemists and neuropharmacologists examine the expression and metabolism of different neurotransmitter systems, or neuropeptides and their receptors; neurogeneticists study the genetic bases of neural development and function; neuroanatomists look at cellular structures and connections in the brain; behavioural neuroscientists analyse the behaviours of intact animals and their neurochemical, neuroanatomical or neurophysiological correlates in the brain, and cognitive neuroscientists use neuroimaging techniques such as fMRI and PET scans to examine the structural and functional activity of the brain in humans and other mammals. Increasingly, however, these categories merge in modern studies, an individual laboratory typically adopting a range of experimental approaches.

There are two outcomes of this technical diversity which put pressure on Canadian neuroscientists. First, to become trained as a neuroscientist, it takes a long period of study at graduate and postdoctoral levels; and, second, it is no longer possible for one laboratory to provide all the resources necessary to undertake a single experiment in neuroscience. As a remedy, many contemporary neuroscience publications integrate collaborations encompassing the activities of several laboratories. For example, the attached Figure shows a behavioural study of learning and memory in mutant mice created using mutagenesis or gene insertion techniques. One can study the correlation between neuroanatomical or neurochemical changes and behaviour in these mutant mice; search for genes involved in the behaviour by undertaking a Quantitative Trait Loci (QTL) analysis (in the fruit fly *Drosophila melanogaster*, the comparable procedure would be to map the gene by deficiency mapping and other fly crossing methods); and then search for genes using gene chips (in *Drosophila* these and complementary mapping methods using fly crosses work with particular efficiency). Finally, when a neural abnormality is discovered, genetic engineering, or pharmacological or other interventions can be used for "brain repair", to restore normal function and thus offset the effects of brain damage. Alternatively, the creation of transgenic animals, in which the normal gene is reinserted into the genome of an animal mutant for the gene, and its expression targeted to the affected brain region, can be used to rescue the mutant phenotype and thereby confirm that the action of the mutation is specific to the gene. This example uses mice, but the fruit fly *Drosophila*, zebrafish, or nematode *Caenorhabditis*, could all substitute without altering the multidisciplinary research approach to genes, brains and behaviour. Manipulating the

Genetic, molecular biological, neural and behavioural integration in neuroscience research. (From J. N. Crawley. Supplement to *Trends Neurosci*. (Suppl) Dec. 2000).



mouse genome is now practical via targeted gene mutations (a), ENU chemical mutagenesis (b), or gene delivery by viral vectors (c), each of which can produce mutant, knockout (KO), and transgenic mice (d) in inbred mice. Two approaches reveal how genes generate the morphogenesis and function of the brain, and how behaviour results therefrom: 1) genes for neurotransmitter receptor subtypes are inserted or deleted by targeted mutations (a) and the mouse (d) evaluated using behavioural (e) and neural (f) phenotyping methods. 2) behavioural (e) and neural (f) phenotyping of a mutant mouse (d) uncover neurobehavioural abnormalities. Mapping strategies from fly crosses in *Drosophila*, and QTL analyses (g) then map chromosomal loci linked to the neurobehavioural abnormality. Precise QTL or complementation maps identify candidate genes, which are then cloned. Clone genes are inserted or deleted using targeted gene mutations (a) and the resulting genotype (d) retested behaviourally (e) or neurally (f). DNA microarrays, or "chips" (h), also enable direct comparison between gene expression in normal and abnormal mice in arrays of up to 12,000 genes. As before, genes underlying neural abnormalities are identified and then either mutated or deleted from the genome using targeted mutations (a). Pharmacological or genetic rescue (i) of identified mutant genes is conducted using neural and behavioural analyses.

3. New areas of research.

The growth of neuroscience, its recruitment of new scientific areas and adoption of new methodologies, all spawned many new research areas: neuroimmunology, neurogenetics and neuroimaging, which have all advanced from their infancy two decades ago. About 80% of all our genes express in the nervous system. The emergence and dominance of molecular biological approaches therefore means that to study the action of a gene one almost invariably has to study the brain. Thus, neuroscience recruits molecular biologists, augmenting the strong currents of neurogeneticists and those adopting a more systems-level of analysis (such as vision science, or motor neuroscience). All such research requires substantial interdisciplinary knowledge and skills, and to promote them at a competitive level we need to facilitate cross-disciplinary research programs, and to support the increased laboratory resources required to implement these.

One emerging area of growth that is keenly anticipated arises from the release of genomic databases in a number of genetically manipulable species. Our grant holders work on all these as model genetic organisms, and their work, as well as that of other applicants anticipated, will increasingly be directed towards elucidating the phenotypes of mutant genes that perturb brain development and/or function. This is a monumental challenge, far exceeding the scope and range of methods required to sequence the genome in the first place. It may take a year of multi-team study to elucidate just one gene. Yet, this approach offers the ultimate causal explanation of neural

function, in gene transcription, insofar as gene action programs the development of the brain and the consequent emergence of behaviour. To give but one example, there are no fewer than 144 genes from 65 families of gene homologues to vertebrate genes that alone encode proteins required to release neurotransmitter in *Drosophila*, the function of which all need systematic examination.

4. Vision.

Our vision is to create a Canadian neuroscience initiative which will allow students and research workers to collaborate across the country, enabling them to move effortlessly over a seamless scientific landscape. The Neuroscience initiative will involve GSCs 12 and 30/31 and will promote training, technical advances and research facilities in participating laboratories. We envision a web of expertise across Canada supported by high-level technical facilities, animal resources and trained technicians who will be able to support the application of specialized techniques to joint projects. In the example above, a lab with expertise in behavioural neuroscience for the behavioural testing of mice; and a lab with expertise in QTL analysis; one with facilities for the reading of DNA chips; and one with molecular biological skills to create probes or transgenes; could all collaborate. A student in one lab could then use the other labs to test mice (zebrafish, fruitflies, etc.), analyse brain chemistry, analyze gene activity and conduct selective breeding assays to discover new QTLs. The student could have behaviour as a starting point, or could start with an unknown gene and elucidate its function. Often these collaborating laboratories will be in different departments at one university, but they could equally often work at different universities in Canada.

5. Strategy.

Applicants will indicate on their grant applications that they wish to be a part of the Neuroscience initiative and list their laboratory's expertise and qualifications in support of such collaborations. They will then list the colleagues with whom they collaborate, the types of collaborations in which they participate, and the students that they are training in the participating labs. Extra funds from the collaborative reallocation will be assigned to these specific collaborations, in addition to the funding given for their individual research project. A specific system of reporting will then be required in the next renewal application, to indicate the way in which these supplementary funds were used for collaborative purposes. As an example, an applicant in Winnipeg may propose to collaborate with a colleague in Montreal who is sending a student for two months to Winnipeg to conduct a specific experiment, to subclone a gene or to inject a transgene into a zebrafish embryo, for example. The student then takes the data, the clone or the fish brain back to Montreal for further analysis. The travel, housing and host laboratory costs of this visit would then be an allowable expense from the Neuroscience initiative. A second example would be a cognitive neuroscientist from Halifax who goes to London, ON to test human subjects in the fMRI centre there. The costs of travel, housing, payment for subjects and rental time on the magnet would be allowable expenses. As a third example, a group of neuroscientists may organize and run a research workshop open to graduate students at Canadian laboratories, to gain experience in areas of research or on experimental systems not available to them. Comparable workshops have proved successful at many locations (Cold Spring Harbor, Woods Hole, etc.), and a similar workshop was previously run at the University of Toronto under the auspices of the NCE in Neural Regeneration. The costs of such a workshop would be an allowable expense from the Neuroscience Initiative.

6. Specific Practical Proposals.

We seek funding for a number of finite practical objectives associated with the interdisciplinary needs of training, and with the increased costs of pursuing neuroscience research in the post-genomic era.

1. Costs for training. Our grant holders need to have increased technical support for equipment and techniques that are required for their research, and the training required to use these new procedures. Some, such as HPLC, cell injection, and cell culture methods, have wide currency in industrial, commercial and government laboratory settings throughout the country. Our students will need this technical support for their research, technicians to show them how to use equipment and collect data. This assistance is needed to offset the present trend for graduate students to work as unpaid technicians in their supervisor's laboratory, rather than as independent trainee scientists engaged on their own project. We also seek travel and housing costs (if applicable) for the collaborative visits needed to send students to work in other laboratories and for training courses. We propose a budget of $125,000 per year.

2. Costs for animal models. We also seek support for the costs of procuring and maintaining animals. Our grant holders need to run transgenic mouse facilities, zebrafish aquaria and other animal facilities. They will also

need to work on the genetic model organisms of the future, once the focus on the big four (mouse, zebrafish, *Drosophila*, *C. elegans*) has been widened to include those species with published genomic databases. Unlike species such as *Drosophila* or *C. elegans* which are small in size, with a short generation time, originally selected because they were well suited to the creation of genetic mutants that could be kept in simple containers (*Drosophila* in milk bottles; *C. elegans* in Petri dishes), the next group of model species may not be so amenable to laboratory culture. They may include animals such as Urochordates, for example, which will require marine holding facilities, and rats, which are more expensive than mice. We propose a budget of $175,000 per year.

3. Costs for new technologies, especially in imaging. Given that the brains of new model species are often small, selected for their genetic and not their experimental convenience, there is an increasing need to build expertise in various methods to image the functioning brain. Such methods as optophysiological recordings of either voltage or calcium changes in live intact systems of neurons, will be essential. We request supplementary funding to support the acquisition and maintenance of new infrastructure for these methods. At the other end of the scale, to cover brain analyses at the systems level, we also need increased support for imaging of the human brain in cognitive neuroscience studies aimed at revealing the neural activity underlying human perceptual and cognitive functions. This requires additional support for the hourly running costs of large installations such as those for fMRI, PET, and MEG. We propose a budget of $100,000 per year.

7. Budget.

We submit this joint proposal as a type 1 proposal, with an annual budget of $400,000 ($1.6M over 4 years for both Psychology and AB&P). Researchers who propose collaborative neuroscience projects, or who incur additional costs for new animal models or new technologies in neuroscience, are eligible for additional funds. We do not prescribe in detail how this funding should be allocated within the three different pools, but the following may serve as an example. An annual budget of $125,000 for training could provide 25 supplementary collaborative grants averaging $5,000. A budget of $175,000 per year for animal costs could provide 20 grants averaging $8,750. A budget of $100,000 per year for imaging costs could provide 25 grants of $4,000 for new technologies, such as those for optophysiology and imaging. These supplements would be awarded across GSC12,30,31 in approximate proportion to the individual budget of each committee. Depending on their justified needs, applicants could be eligible for one or more supplements. Those with a particular need to conduct collaborative research could, for example, apply to the joint neuroscience budget for additional support to cover the costs of trainees involved in collaborative training in joint proposals.

At the GSC level, we suggest that each application in neuroscience be evaluated first for operating grant funds and, if approved, go on to be considered for a supplement from the neuroscience joint budget. The amount of supplemental funds would be distributed based on the rankings of the approved grants, the extra funding recommended and the total supplemental budget. For example, a GSC might award an applicant a $40,000 operating grant and recommend that $7,000 be added from the supplemental neuroscience budget. Based on the funds available, a proportion of this $7,000 would then be added to the grant. Applicants returning to their GSC in later years would then be required to indicate the impact of the supplement on their productivity.