Animal Care Committee responsibilities

CCAC

ACC 101 @ CCAC National Workshop, May 13, 2010
Animal Care Committees

- The keystone of the Canadian system is the local, institutional Animal Care Committees (ACCs)
- Their composition, authority and functioning are defined in the *CCAC policy statement: terms of reference for animal care committees* (2006)
- The ACC reports to the senior institutional administrator responsible for animal care and use (VP Research, President, CEO, etc.)
ACC composition

- Scientists and/or teachers with experience in animal use
- Institutional member who does not use animals
- Experienced veterinarian(s)
- Community representative(s)
- Technical staff representative (Manager)
- Student representative (where students are present)
- ACC coordinator
- Others as needed (person(s) responsible for health and safety/biosafety, biostatistician, ethicist, public relations liaison)
Responsibilities of the ACC

Described in the terms of reference and in the CCAC Animal Care and Use Program Review Form (2008)

Main responsibilities are protocol review, approval and follow-up (post-approval monitoring)

Other responsibilities include working with the administration to ensure that appropriate:

- facilities are being used, and are well maintained and managed;
- veterinary and animal care services are in place;
- continuing education and training programs are in place;
- occupational health and safety and crisis management programs are in place.
Protocol review

The ACC must review all animal use proposals before they begin, based on the CCAC guidelines on: animal use protocol review (1997), the CCAC policy statement on: terms of reference for animal care committees (2006) and other relevant guidelines and policies.
Tools for Protocol Review

- An animal use protocol form
- CCAC guidelines and policies and associated documents
- Institutional policies and standard operating procedures (SOPs)
- The expertise, judgment and common sense of committee members
- Additional resources and expertise as necessary
Animal Use Protocol Form

- Each institution must develop an animal use protocol form that suits the nature and culture of the institution, while including all elements required by the CCAC for a complete ethical review (point 3 c) of the CCAC terms of reference for ACCs for new protocols, and point 3g) for protocol renewals)

- All elements must be presented by scientists/teachers in a language that is easily understood by all ACC members, including community representatives
General Identification

- Project title and descriptive keywords, or brief protocol description
- Author and all personnel who will handle animals (students, staff)
- Training and qualifications
General Identification

- Departmental affiliation
- Proposed start date
- Proposed end date
- Lay summary
General Identification

- Funding source(s) and status of funding approval
- Peer review of scientific merit for research projects
- Pedagogical merit for teaching protocols
- Current regulatory guidelines for testing protocols
Specific Information

- Use of hazardous agents
  - Institutional approval of this use
- Categories of invasiveness
- Purpose of animal use
Specific Information

Alternatives:
- Replacement
- Reduction
- Refinement

Species and numbers of animals to be used, and justification thereof
Procedures

- A description detailing the procedures that are carried out on the animals
- Anesthesia and analgesia, including:
  - dosages
  - methods of use
- Justification for not using anesthesia or analgesia, if relevant
Endpoints

A description of the endpoint(s) of the experimentation, and of the persons/procedures in place for monitoring animals and applying endpoints
**Field work**

- A description of capture, restraint, transportation and/or housing of animals used in field studies.

- Any other information pertinent to field studies, such as capture permits, capture of non-target species, ecological impacts and potential injuries or mortality during capture or transportation, if relevant.
Fate of the animals

- The method of euthanasia, if used
  - justification for any physical euthanasia methods
  - or for any methods that deviate from those described in the most recent CCAC guidance on euthanasia
- A description of the fate of the animals if they are not to be euthanized
Any other relevant information

- Any other information considered important or necessary and pertinent
  - including information or results derived from any relevant previous protocols
Thank You!

Canadian Council on Animal Care
Conseil canadien de protection des animaux
1510 – 130 Albert
Ottawa, ON K1P 5G4
www.ccac.ca
Animal Use Protocol – Research

Protocol #: 
Investigator #: 
Approval End Date: 

☐ Pilot  ☐ New Application  ☑ Renewal of Protocol # 25

Title: Birth Complications and the Central Nervous System
(must match the title of the funding source application)

1. Investigator Data:

   Principal Investigator: Brian Smith  
   Office #: 613-238-4031
   Department: Psychiatry  
   Fax#: 613-238-2837
   Address: University Hospital  
   Email: Bsmith@XYUniversity.ca

2. Emergency Contacts: Two people must be designated to handle emergencies.

   Name: Brian Smith  
   Work #: 238-4031  
   Emergency #: 123-4567
   Name: Evelyn Brown  
   Work #: 238-4031  
   Emergency #: 765-4321

3. Funding Source:

   External ☑  Internal ☐
   Source (s): CIHR  
   Peer Reviewed: ☑ YES  ☐ NO**
   Status: ☑ Awarded  ☐ Pending
   Funding period: April 2001 – March 2005
   Source (s): __________
   Peer Reviewed: ☐ YES  ☐ NO**
   Status: ☐ Awarded  ☐ Pending
   Funding period: __________

** All projects that have not been peer reviewed for scientific merit by the funding source require 2 Peer Review Forms to be completed. E.g. Projects funded from industrial sources.

   Proposed Start Date of Animal Use (d/m/y): __________  or ongoing ☑

   Investigator’s Statement: The information in this application is exact and complete. I assure that all care and use of animals in this proposal will be in accordance with the guidelines and policies of the Canadian Council on Animal Care and those of XY University. I shall request the Animal Care Committee’s approval prior to any deviations from this protocol as approved. I understand that this approval is valid for one year and must be approved on an annual basis.

   Principal Investigator’s signature: __________  Date: __________

Approval Signatures:

Chair, Animal Care Committee: __________  Date: __________

University Veterinarian: __________  Date: __________

Approved Period for Animal Use

Beginning: __________  Ending: __________

☐ This protocol has been approved with the modifications noted in Section 12.
4. Research Personnel and Qualifications: List the names of all individuals who will be in contact with animals in this study (including the Principal Investigator) and their employment classification (investigator, technician, research assistant, undergraduate/graduate student, fellow). Indicate any training received (e.g. workshops, lectures, etc.). The PI certifies that all personnel listed here have suitable training and/or experience, or will be provided with the specific training which qualifies them to perform the procedures described in the protocol. Each person listed in this section must sign to indicate that s/he has read this protocol. (Space will expand as needed.)

<table>
<thead>
<tr>
<th>Name</th>
<th>Classification</th>
<th>Training Information</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brian Smith</td>
<td>PI</td>
<td>Rat workshop</td>
<td></td>
</tr>
<tr>
<td>Evelyn Brown</td>
<td>Technician</td>
<td>Rat and mouse workshops</td>
<td></td>
</tr>
<tr>
<td>Ming Zhu</td>
<td>Graduate student</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Enter the first name, press 'enter', then the 2nd name... complete the first column, then the 2nd, then the 3rd
** If an undergraduate student is involved, the role of the student and the supervision received must be described.

5. Summary (in language that will be understood by members of the general public)

a) RATIONALE: Describe, in a short paragraph, the overall aim of the study and its potential benefit to human/animal health or to the advancement of scientific knowledge.

It is known that birth complications and maternal infections during pregnancy are associated with later development of schizophrenia. Many birth complications, including birth by Caesarean section, involve insufficient oxygen delivery to the fetus (hypoxia). To date, the long term consequences of birth hypoxia and maternal infection on brain development and behavior in offspring have not been examined in great detail. This is the subject of our studies.

b) SPECIFIC OBJECTIVES OF THE STUDY: Summarize in point form the primary objectives of this study.

1. To assess effects of birth hypoxia on neurotransmitter biochemistry and brain morphology.
2. To assess the effects of birth complications on behavior related to brain dopamine transmission, i.e., amphetamine- and apomorphine-induced locomotion and acoustic startle and stress induced locomotion.
3. To assess the role of plasma hormones (catecholamines, testosterone) at birth in birth hypoxia-induced changes in amphetamine-induced locomotion.

c) PROGRESS REPORT: If this is a renewal of an ongoing project, BRIEFLY summarize what was accomplished during the prior approval period and indicate if and how the current goals differ from those in the original application.

We have completed several of the specific experiments from our original proposal, as described. 1. To complement experiments in the rat, we have developed and completed experiments with a guinea pig model of birth hypoxia during C-section. We have demonstrated that C-section birth also produces long term enhancement of amphetamine-induced locomotion in the guinea pig. 2. We have shown that among 3 rat strains, the long term effects of C-section birth on dopamine (DA)-mediated behavior differ markedly. 3. We have demonstrated long term effects of C-section birth and birth hypoxia on DA receptors, DA transporters and bFGF-immunoreactive neurons in the rat and showed that these birth complications interact with stress at adulthood to affect these brain measures. 4. We have shown that male and female rats respond differentially to C-section birth. Male C-sectioned rats lack the plasma catecholamine surge that normally accompanies a vaginal birth and also show increased nucleus accumbens DA levels as adults, in contrast to females. 5. We have examined effects of maternal endotoxin exposure during pregnancy (E18 and E19) alone or combined with perinatal hypoxia, on DA-mediated behavior in rat offspring.

d) SUMMARY OF PROCEDURES FOR ANIMAL USE REPORT TO THE CCAC: Using KEY WORDS ONLY, list the procedures used (e.g. anaesthesia, breeding colony, injection IP, gavage, drug administration, major survival surgery, euthanasia by exsanguination, behavioural studies). Refer to Appendix 1 of the Guidelines for a more complete list of suggested key words.

Isoflurane/nitrous oxide inhalation anesthesia, Caesarean section delivery, cross-fostering, behavior, locomotion, acoustic startle, tail pinch stress, subcutaneous and intraperitoneal drug injection, physical euthanasia with/without anesthesia, post-mortem brain neurochemistry.
6. Animals To Be Used

a) Purpose of Animal Use (Check one):
1. ☐ Studies of a fundamental nature/basic research
2. ✗ Studies for medical purposes relating to human/animal diseases/disorders
3. ☐ Regulatory testing
4. ☐ Development of products/appliances for human/veterinary medicine

b) Will the project involve breeding animals? NO ☐ YES ☐
Will the project involve the generation of genetically altered animals? NO ☐ YES ☐
Will field studies be conducted? NO ☐ YES ☐

c) Description of Animals

<table>
<thead>
<tr>
<th>Species</th>
<th>Sp/strain 1</th>
<th>Sp/strain 2</th>
<th>Sp/strain 3</th>
<th>Sp/strain 4</th>
<th>Sp/strain 5</th>
<th>Sp/strain 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplier/Source</td>
<td>rat</td>
<td>rat</td>
<td>Offsprng from pregant mothers</td>
<td>Sprague Dawley</td>
<td>Sprague Dawley</td>
<td></td>
</tr>
<tr>
<td>Strain</td>
<td>Sprague Dawley</td>
<td>Sprague Dawley</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age/Wt</td>
<td>Timed pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># To be purchased</td>
<td>132</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># Produced by in-house breeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># Other (e.g. field studies)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># needed at one time</td>
<td>15-19</td>
<td>50-120</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># per cage</td>
<td>1</td>
<td>As per CCAC guidelines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL#/YEAR</td>
<td>132</td>
<td>418</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Quality Control Assurance: To prevent introduction of infectious diseases into animal facilities, a health status report or veterinary inspection certificate may be required prior to receiving animals from all non-commercial sources or from commercial sources whose animal health status is unknown or questionable. Quarantine and further testing may be required for these animals.
7. Justification of Animal Usage

a) Please justify the number of animals requested for each species described in the table 6c above, BASED ON THE EXPERIMENTAL OBJECTIVES OF THE PROJECT. Include information on experimental and control groups, # per group, and failure rates. Also justify in terms of statistical requirements, productivity, etc. For breeding, specify how many adults are used, number of offspring produced, and how many offspring are used in experimental procedures. The numbers of animals are for one year only, not the length of funding. Use the table below when applicable (space will expand as needed).

For objectives 1, 2 and 3, our experiments generally involve 5 groups varying in terms of birth condition: vaginally born and Caesarean section from decapitated or isoflurane anesthetized dams with or without 15 min of added hypoxia. Our experience has been that we require 10 animals per group to generate reliable results. Thus we require 50 offspring per expt and will run 5 expts/year = 250 offspring. In each expt, each of the 5 groups of pups must be generated from at least 3 different dams to ensure effects in the offspring are not due to one aberrant dam; thus each expt requires 5 x 3 = 15 pregnant dams x 5 expts = 75 pregnant dams.

Thus in total for all objectives we require 75 pregnant female rats, and will retain 250 rat offspring for testing.

<table>
<thead>
<tr>
<th>Test Agents or Procedures e.g. 2 Drugs</th>
<th># of Animals and Species Per Group e.g. 6 rats</th>
<th>Dosage and/or Route of Administration e.g. .03, .05 mg/kg IM, IP (4 variables)</th>
<th># of endpoints e.g. 1, 7, 10 days (3 variables)</th>
<th>Other variables (i.e. sex weight, genotypes, etc.) e.g. Male, Female groups (2 variables)</th>
<th>Total number of animals per year e.g. 2 x 6 x 4 x 3 x 2 = 288</th>
</tr>
</thead>
</table>

* For the above table, enter the first agent/procedure, press ‘enter’, then the 2nd agent... complete the first column, then the 2nd, then the 3rd...

b) Please justify the need for live animals versus alternate methods (e.g. tissue culture, computer simulation). We are assessing long term effects of birth hypoxia and maternal infection on behavior and brain biochemistry in offspring. Live animals are therefore required.

c) Describe the characteristics of the animal species selected that justifies its use in the proposed study (consider characteristics such as body size, species, strain, data from previous studies or unique anatomic/physiological features).

The rat is the species upon which most previous evidence has been gathered, and the rat is also most amenable to long term studies for behavioral testing.
8. Animal Husbandry and Care
   a) Special cages: NO ☒ YES ☐ Specify:
   b) Is there any component to the proposed procedures which will result in immunosuppression or decreased immune function (e.g. stress, radiation, steroids, chemotherapeutics, genetic modification of the immune system)?
   c) Multiple institution facility housing: NO ☒ YES ☐
      Indicate all facilities where animals will be housed: Building: University Hospital Room No: 232
      Indicate area(s) where animal use procedures will be conducted: Building: University Hospital Room No: 236
      If animal housing and animal use are in different locations, briefly describe procedures for transporting animals:

9. Standard Operating Procedures (SOPs)
   Complete this section if you plan to use any of the ACC SOPs listed below. IT IS ACC POLICY THAT THESE SOPS BE USED WHEN APPLICABLE. Any proposed variation of the SOPs must be described and justified. The completed and signed SOP form must be attached to the protocol.
   Check all SOPs that will be used:
   Blood Collection (UACC#1) ☐ Production of Monoclonal Antibodies (UACC#7) ☐
   Anaesthesia (rodents) (UACC#2) ☐ Production of Polyclonal Antibodies(UACC#8) ☐
   Analgesia (rodents/larger species) (UACC#3) ☐ Collection of Amphibian Oocytes (UACC#9) ☐
   Breeding (transgenes/knockouts) (UACC#4) ☐ Rodent Surgery (UACC#10) ☐
   Transgenic Generation (UACC#5) ☐ Neonatal Rodent Anesthesia and Euthanasia (UACC#11) ☐
   Knockout/In Generation (UACC#6) ☐ Stereotaxic Survival Surgery in Rodents (UACC#12) ☐

10. Description of Procedures
   a) FOR EACH EXPERIMENTAL GROUP, DESCRIBE ALL PROCEDURES AND TECHNIQUES IN THE ORDER IN WHICH THEY WILL BE PERFORMED - surgical procedures, immunizations, behavioural tests, immobilization and restraint, food/water deprivation, requirements for post-operative care, sample collection, substance administration, special monitoring, etc. IF A PROCEDURE IS COVERED BY AN SOP, WRITE "AS PER SOP", NO FURTHER DETAIL IS REQUIRED.
   Objectives 1,2 and 3
   Pregnant rat dams are decapitated rapidly without anaesthetic. The entire uterus is removed to a 37°C water bath for 10-20 minutes of hypoxia. Following this, the pups are delivered and raised by a foster dam. (Some of the foster dam’s pups are retained as controls, excess controls are sacrificed by decapitation.) Pups are marked by subcutaneous injection of approximately .01 ml of India ink into the surface of a paw. Pups are weaned at 21 days of age and grown up to 3 months. (Concerning the use of subcutaneous India ink to permanently mark neonatal animals, this has been an ongoing process in which we have tried numerous solutions and have now settled on subcutaneous India ink, with the approval of University
Hospital Animal Centre, for several years now. In our initial studies we used toe clipping, also approved by University Hospital Animal Centre, but were then asked to find an alternative means to mark newborn animals. Surface marking is not acceptable as the animals are vigorously cleaned by the mother and their fur eventually grows over their skin. Similarly subcutaneous injection of ink in a position other than the paw is not feasible since fur grows making the marking non-visible. We tried ear clipping but this requires a large clip in the neonatal ear since the ear grows rapidly and small clips grow over.

We have now settled on subcutaneous India ink, which is non-irritant since we have never had inflammation or infection after marking hundreds of animals, and allows for permanent reliable marking of animals with a single procedure.

In some experiments, instead of being immediately decapitated the pregnant rat dam is anesthetized using an Isoflurane/Nitrous Oxide Anesthesia System. Using this system, animals breathe unassisted without intubation. For our experiments, analgesia in the pregnant dam is induced by 60% NO2 in 02 for 2 min. (NO2 is often used as an analgesic during the first stage of human parturition and is widely used as an adjuvant to other general anesthetics; we wish to model the general anesthesia most often used in a human C-section birth.) Following this, isoflurane at 2.5% will be added for 8 min to induce anesthesia and then reduced to 2.0%, which is 1.3 x the MAC value in the rat. Under this anesthesia, the uterus is removed via abdominal incision and the pups submitted to 10 or 15 min of hypoxia, as above, and delivered. The dam will be sacrificed by decapitation. The pups will be fostered and grown to 3 months as described above.

At 3 months of age various experimental manipulations are performed as follows:

1) Some animals will be decapitated and brains taken for biochemical and histological measures.
2) Some animals will be anesthetized using pentobarbital (60 mg/kg, ip) and sacrificed by intracardiac perfusion with saline followed by 4% paraformaldehyde.

The following behavioural tests will be performed on some of the animals.
3) Performance of some animals will be tested in an acoustic startle paradigm. Each animal is placed for a 22 min session in a standard acoustic startle chamber, which measures the force transduced when the animal startles following an acoustic stimulus. Background noise of 70 dB is played throughout the session. Acoustic stimuli consist of a 30 msec pulse at 120 dB sometimes preceded by pre-pulses from 73 to 85 dB. Forty trials of acoustic stimuli are given over the 22 min session with an average 15 sec inter-trial interval. Following the session the animal is returned to its home cage. In some expts animals receive an sc injection (volume of 1 ml/kg) of apomorphine (0.1-0.4 mg/kg), of amphetamine (0.5-3.0 mg/kg) or of saline immediately prior to testing.
4) Some animals will receive sc injections of saline or of amphetamine (0.5-2.0 mg/kg; 1 ml/kg). Immediately following this, the animal will be placed in a behavioural apparatus which monitors locomotor activity for 1h (by interruption of one of 2 photocell beams shone across the bottom of the box). The animal is then returned to its home cage. In some experiments animals will receive repeated tail pinch stress prior to testing for locomotor activity. For repeated tail pinch stress, the animal is placed singly in a holding cage and a plastic clothes pin is placed snugly at 1 cm from the base of the tail for 15 min daily on each of 8 consecutive days. At the end of the 15 min tail pinch session the clothes pin is removed and the animal is either returned to its home cage or placed in the locomotor box for 2h of locomotor testing.
5) In one expt testing effects of neonatal testosterone on brain development, neonatal rats will receive a subcutaneous injection of 200 µg of testosterone propionate in 0.1 ml peanut oil, or 0.1 ml peanut oil alone, on the first and 2nd days after birth, before being grown to adulthood and behaviorally tested as above. A separate group of rat pups will be decapitated immediately after birth and trunk blood taken for measurement of plasma testosterone.

Animals are sacrificed by decapitation following the behavioural experiments when their brains are required for histology, or by CO2 inhalation if brains are not required.
b) Field Studies – Provide all relevant details. Procedures to be conducted (e.g. surgery, blood collection, tagging etc.) should be described above.

Method of capture/restraint, duration of captivity, potential injury/mortality, monitoring frequency:

Transportation and/or housing of animals in the field:

Special handling required:

Capture of non-target species, potential injury/mortality:

Will captured animals be released at or near the capture site YES □ NO □

If not, specify if they will be relocated to other locations and/or populations.

Describe any potential ecological disruption this study may cause:

It is the responsibility of the investigator to obtain all necessary permits for work with wildlife. Copies of these permits must be forwarded to the Research Ethics Officer (Animal Studies) when they are obtained.

c) Pre-Anesthetic/Anesthetic/Analgesic Agents: List all drugs that will be used to minimize pain, distress or discomfort. Table will expand as needed. (*complete 1st column pressing ‘enter’ after each species, then 2nd column…)

<table>
<thead>
<tr>
<th>Species</th>
<th>Agent</th>
<th>Route</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat</td>
<td>nitrous oxide</td>
<td>Inhalation</td>
<td>12 min</td>
</tr>
<tr>
<td>rat</td>
<td>isoflurane</td>
<td>Inhalation</td>
<td>once</td>
</tr>
<tr>
<td>rat</td>
<td>pentobarbital</td>
<td>ip</td>
<td>once</td>
</tr>
</tbody>
</table>

d) Administration of non-anesthetic substances: List all non-anesthetic agents under study in the experimental component of the protocol, including but not limited to drugs, infectious agents, viruses (table will expand as needed). (*complete 1st column pressing ‘enter’ after each species, then 2nd column…)

<table>
<thead>
<tr>
<th>Species</th>
<th>Agent</th>
<th>Route</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat</td>
<td>amphetamine</td>
<td>sc</td>
<td>once</td>
</tr>
<tr>
<td>rat</td>
<td>apomorphine</td>
<td>sc</td>
<td>once</td>
</tr>
<tr>
<td>rat</td>
<td>lipopolysaccharide</td>
<td>ip</td>
<td>twice</td>
</tr>
<tr>
<td>rat</td>
<td>endotoxin</td>
<td>ip</td>
<td>twice</td>
</tr>
</tbody>
</table>

e) Endpoints: 1) Experimental – for each experimental group indicate survival time.

2) Clinical – describe the conditions, complications, and criteria (e.g. >20% wt. loss, tumor size, vocalizing, lack of grooming) that would lead to euthanasia of an animal before the expected completion of the experiment (specify per species and project if multiple projects involved).

Pregnant rat dams: survive until birth of pups.
Surrogate dams: survive until weaning of pups.
Rat offspring: Some survive until 3 months of age, when they are sacrificed for brain biochemical measures; some undergo behavioral testing for several days at 3 months of age, and are sacrificed following testing.

Specify person(s) who will be responsible for animal monitoring and post-operative care
Name: Evelyn Brown Phone#: 238-4031

f) Method of Euthanasia – According to CCAC guidelines, justification must be provided for use of any physical method of euthanasia without prior use of anaesthesia (justify here):
In some experiments pregnant rat dams are decapitated without anesthesia and the pups born by rapid C-section from the decapitated dam. Birth in this way is compared to C-section from an isoflurane anesthetized dam. This experiment is necessary in order to test effects of the C-section birth alone, without the confound of anesthetic effects, on brain development in the offspring. Anesthetic agents may contribute to respiratory depression in the newborn thus contributing to brain hypoxia, which may have effects on brain development. Also in some cases anesthetic agents can be neuroprotective agents since they decrease cerebral metabolism. Thus, in order to study effects of C-section birth alone, on brain development, it is necessary to perform C-section from an unanesthetized dam in some experiments. We have been performing this type of experiment for at least 8 years, with approval from the Animal Care Committee. Thus we have amassed a large amount of data using this paradigm, and our further studies require comparison to our previous work.

Specify Species

- anaesthetic overdose, list agent/dose/route:
- exsanguination with anaesthesia, list agent/dose/route:
- decapitation without anaesthesia
- decapitation with anaesthesia, list agent/dose/route: NO2 60% and isoflurane 2.0% via inhalation
- cervical dislocation
- CO2 chamber
- other (specify) intracardiac perfusion with 4% paraformaldehyde under pentobarbital anesthesia (60 mg/kg ip)
- not applicable (explain)

11. Category of Invasiveness:

Categories of Invasiveness (from the CCAC Categories of Invasiveness in Animal Experiments). Please refer to this document for a more detailed description of categories.

Category A: Studies or experiments on most invertebrates or no entire living material.

Category B: Studies or experiments causing little or no discomfort or stress. These might include holding animals captive, injection, percutaneous blood sampling, accepted euthanasia for tissue harvest, acute non-survival experiments in which the animals are completely anaesthetized.

Category C: Studies or experiments involving minor stress or pain of short duration. These might include cannulation or catheterizations of blood vessels or body cavities under anaesthesia, minor surgery under anaesthesia, such as biopsy; short periods of restraint, overnight food and/or water deprivation which exceed periods of abstinence in nature; behavioural experiments on conscious animals that involve short-term stressful restraint.

Category D: Studies or experiments that involve moderate to severe distress or discomfort. These might include major surgery under anaesthesia with subsequent recovery, prolonged (several hours or more) periods of physical restraint; induction of behavioural stress, immunization with complete Freund's adjuvant, application of noxious stimuli, procedures that produce pain, production of transgenics (in accordance with University policy).

Category E: Procedures that involve inflicting severe pain, near, at or above the pain threshold of unanaesthetized, conscious animals. Not confined to but may include exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs or chemicals at levels that may markedly impair physiological systems and which cause death, severe pain or extreme distress or physical trauma on unanaesthetized animals. According to University policy, E level studies are not permitted.

12. Reviewer’s Modifications (to be completed by ACC only): The Animal Care Committee has made the following modification(s) to this protocol during the review process. Please make these changes to your copy. You must comply with the recommended changes as a condition of approval.