Comparative Medicine
Copyright 2004
by the American Association for Laboratory Animal Science

Vol 54, No 5
October 2004
Pages 486-496

Overview

Animal Models of Ischemic Stroke: Balancing Experimental Aims and Animal Care

Sharon M. Graham, DVM,[1] Louise D. McCullough, MD, PhD,[2] and Stephanie J. Murphy, VMD, PhD[1,†,*]

Animal models of ischemic stroke are examples of an induced model that can present challenges from the perspectives of protocol review and animal management. The review presented here will include a brief summary of the current state of knowledge about clinical stroke; a general synopsis of important unanswered research questions that justify use of animal stroke models; an overview of various animal models of ischemic stroke, including strengths and limitations; and a discussion of animal care issues relative to ischemic stroke models. Good communication and interactive education among primary investigators, laboratory animal veterinarians and caretakers, and institutional animal care and use committee members are critical in achieving a balance between research objectives and animal care issues when using animal stroke models.

Stroke, or cerebral vascular accident, is defined as loss or alteration of bodily function that results from an insufficient supply of blood to part of the brain (3). It is a disease process that can strike abruptly, with severe consequences. On average, someone in the United States has a stroke every 45 sec, and death can occur in up to 50% of such events that happen outside a hospital setting (2). Stroke is ranked third behind cancer and heart disease as a cause of death in the United States, and is a leading cause of serious, long-term disability (2). Among American stroke survivors surveyed in 1999, over a million adults reported limitations in daily activities, functional disabilities, and greater dependence on caregivers, resulting in changes in everyday living and quality of life (2). Costs for medical care, therapy, lost productivity, and disability exceed $50 billion per year (2).

In humans, stroke is a diverse disease in terms of causes, manifestations, and anatomic sites of ischemia. Three types of stroke are generally seen in clinical patients: ischemic, intracerebral hemorrhage, and subarachnoid hemorrhage. The most common type of stroke is ischemic stroke, accounting for 88% of cases (2). A clot or other blockage in an artery leading to the brain generally causes ischemic stroke. Blood clots or vascular stenosis from plaque formation are common causes of arterial blockage. The arterial obstruction leads to local loss of oxygen and nutrients, resulting in cell death or infarction injury.

Hemorrhage within or around the brain can also cause decreased blood flow and disruption of the blood-brain barrier that protects normal chemical balance in brain tissue, again leading to cell death. Hemorrhage can be secondary to trauma, congenital malformation, aneurysm, or disease. Intracerebral hemorrhage is a type of stroke caused by sudden rupture of an artery within the brain that is often related to hypertension. Blood is then released into the brain, compressing brain structures. This type accounts for 9% of stroke cases (2). Subarachnoid hemorrhage also is a type of stroke caused by sudden rupture of an artery, but it differs from intracerebral hemorrhage in that location of the rupture leads to blood filling the space surrounding the brain rather than inside of it. About 3% of clinical stroke cases fall into this category (2).

Research Questions Addressed by Use of Stroke Models

Many of the general research questions regarding stroke could apply to any major disease currently under study. Clinical and experimental efforts are focused on the following areas: primary and secondary prevention (161), treatment and management of stroke (161), and underlying mechanisms of ischemic cerebral pathophysiology and neuroprotection (164). Particular to stroke research, clinicians and scientists are interested in redefining and extending the therapeutic window for pharmacologic intervention that is currently limited to a few hours after stroke onset. In many stroke studies, the period between early intervention and primary outcome assessment (usually 3 months after stroke) is also largely unexplored, even though events that occur during this period may appreciably impact final patient outcome (32).

Exploring sex-specific differences in brain injury and the role of sex steroids in these differences has become an emerging area of stroke research (112). Recent emphasis on women's health issues has caused investigators to refocus and view hormonal cycles, menopausal physiology, and hormone replacement therapy (HRT) as important to outcome in many diseases including stroke (36, 70). Historically, animal vascular and neurologic disease models have almost exclusively involved study of males. Use of males was justified as a means of reducing experimental variability caused by female hormone cycling and was based on the assumption that

Received: 8/05/04. Revision requested: 9/13/04. Accepted: 9/23/04.
[1]Department of Anesthesiology and Peri-Operative Medicine, Oregon Health and Science University, Portland, Oregon 97239 and [2]Department of Neurology and Neuroscience, University of Connecticut Health Center, Farmington, Connecticut 06030.
[†]Present address: Oregon Health and Science University—West Campus, 20000 N.W. Walker Road, Mail Code: OGI, Beaverton, Oregon 97006.
[*]Corresponding author.

Table 1. Animal models of ischemic stroke[a,b]

Ischemic Stroke Model	Animal Species
Complete global cerebral ischemia	
• Decapitation	Rat
• Aortic and vena caval occlusion	Dog
• Neck tourniquet or cuff inflation	Nonhuman primate, dog, cat, rat
• Cephalic artery occlusion (neck, thorax)	Nonhuman primate, cat
• Cardiac arrest ± cardiopulmonary resuscitation	Nonhuman primate, dog, sheep, pig, rat, mouse
• Bilateral common carotid artery occlusion (CCAO)	Gerbil
Incomplete global cerebral ischemia	
• Hemorrhage/hypotension	Cat, pig
• Hypoxia-ischemia	Dog, cat, sheep, pig, rat, mouse
• Intracranial hypertension ± unilateral CCAO	Rat
• 2-Vessel occlusion ± hypotension	Rat, mouse
• 4-Vessel occlusion	Rat
• Unilateral CCAO	Gerbil
Focal cerebral ischemia	
• Middle cerebral artery occlusion (MCAO)	Nonhuman primate, dog, cat, rabbit, guinea pig, rat, mouse
• MCAO + ipsilateral CCAO	Dog, rat
• MCAO + bilateral CCAO	Rat
• Spontaneous brain infarction	Spontaneously hypertensive rats (SHR)
Multifocal cerebral ischemia	
• Autologous or heterologous blood clot embolization	Rat
• Microsphere embolization	Rabbit, rat
• Photochemical-initiated multifocal embolization	Rat

[a]Adapted from reference 112.
[b]Based on information from the following references (see text): 10, 44, 55, 95, 101, 164, 167.

mechanisms of cell injury or treatment effects observed in males would also apply to females. In recent years, it has been recognized that disease conditions and responses to therapy may be different between genders, and women must be incorporated into clinical trials and human research. Reversing the "male-only" approach in experimental, animal-based studies has been slower. Much remains to be explored about stroke in women, such as the effects of age and reproductive status, gender-specific risk factors, and the importance of exogenous sex steroid availability (i.e., contraceptives, HRT).

Identification and characterization of genetic and molecular components of stroke also will be critical to our future understanding, diagnosis, and treatment of this disease. Ongoing work in human and selected animal genome projects has already led to identification of candidate genes for stroke susceptibility (28, 145), as well as sex differences in molecular mechanisms of ischemic damage (105). Animal stroke models will, therefore, be essential for allowing investigators to examine which genes and proteins are affected in response to stroke. Use of genetically engineered mice will be particularly useful in further understanding the complexities of ischemic pathophysiology and in designing new neuroprotective and therapeutic strategies.

Overview of Animal Models

Most animal stroke models are based on ischemic stroke (Table 1), but experimental stroke models of intracerebral and subarachnoid hemorrhage have been described as well (4, 90, 107, 119).

Why use animal models of stroke? The presence of intact cerebral vasculature is essential to the study of abnormal brain perfusion. Because of the complexity of the brain and its response to injury, use of in vitro systems alone cannot thoroughly evaluate cerebral ischemia and its consequences. Preclinical and translational research into the causes, pathogenesis, and therapeutic management of stroke therefore use animal models in addition to other in vitro techniques and models, such as tissue culture and brain slices.

Many animal stroke models have been developed and characterized (4, 10, 44, 55, 90, 95, 101, 107, 153, 164), but no one model alone may fully mimic human stroke because of the heterogeneity of human clinical disease (32). Some of the limitations of these models can include physiologic variability and, depending on the model and research question, substantial mortality in acute and chronic survival studies. Principal investigators must also address the variable injury or infarct size that can be seen in some animal models. Completeness of the vascular occlusion may be unclear at times as well. Nonetheless, experimental animal models of stroke allow investigators to carefully recreate specific aspects of human stroke and to study pathophysiologic and neuroprotective mechanisms as well as therapeutic responses under controlled conditions and in ways that cannot be done easily or at all in clinical patients and in human subjects (32). More rigorous histopathologic, biochemical, and physiologic measurements also can be done in animals. Finally, animal models allow investigators to study immediate and early ischemic events, events that can be difficult to examine in human patients because of the variable time delays in early recognition of a stroke and initial therapeutic intervention.

Stroke models by species. One way to characterize animal stroke models is in terms of the animal species used. Models defined in this way can be placed into two general categories, nonrodent and rodent stroke models. Nonrodent models offer several distinct advantages over rodent models to the principal investigator. For example, in these models, concurrent and multiple measurements (i.e., blood gas values, blood glucose concentration) can be done over time in the same animal as well as more sophisticated and complex physiologic monitoring, such as continuous direct blood pressure measurements, with minimal animal distress and research complications. Even though regional imaging technology is available for rodents and is continually being improved (79, 85), many nonrodent stroke models are more amenable to regional imaging techniques. Similarly, regional cerebral blood flow (CBF) and metabolism can be more easily determined in nonrodent models. Lastly, similar to that in humans, many of these nonrodent models have a gyrencephalic or convoluted brain.

Some of the limitations of nonrodent stroke models are similar to some of the challenges faced for other surgical or disease models using these particular animal species. For example, species-specific anesthesia requirements may affect outcome measures such as infarct size, blood pressure, or CBF. Public animal welfare concerns tend to focus more on nonrodent species, such as nonhuman primates, cats, and dogs, than on rodents. Cost and labor are other difficulties often cited by researchers using large-animal stroke models, although the cost of using genetically engineered mice for stroke research applications, as well as the work and time involved in using and maintaining such genetically modified mice, can approach and even exceed that of large-animal experiments.

Some of the benefits of using rodent models in stroke research include smaller brain size, allowing more extensive and comprehensive evaluation of the entire brain without excess cost, time, and labor. Rats in particular are useful for stroke research be-

cause of their anatomic similarity to the human anatomy of cranial circulation (176). Commercially available inbred rodent strains are genetically homogeneous, allowing researchers to minimize confounding effects due to a heterogeneous background. Use of genetically engineered mice is particularly efficacious in further understanding the complexities of ischemic pathophysiology and in designing potential new preventative, neuroprotective, and therapeutic drugs and interventions. Although transgenic and gene-targeted mice can be expensive, normal rodent strains and stocks are reasonable in terms of purchase and maintenance costs compared with those for nonrodent, larger animals. Furthermore, investigators have begun to look at functional outcomes in experimental stroke by evaluating a battery of behavioral, cognitive, and sensorimotor tests. A number of neurosensory and motor behavior outcomes have been well described and standardized for rodents (22) and can be applied to stroke paradigms (47, 59, 89). As mentioned previously, the public tends to have fewer animal welfare concerns regarding rodents in stroke research.

Some of the difficulties in the use of rodent models of stroke have already been discussed. Unlike humans, rodents have lissencephalic or unconvoluted brains. Due to size and blood volume limitations, physiologic monitoring can be more demanding in rodents. Lastly, concurrent and multiple measurements over time may be limited or impossible. Experimental studies involving rodents may have to include nonsurvival animal cohorts for physiologic measurements in addition to separate survival groups for infarct and functional assessments.

Stroke models by type of ischemic insult. Another way to characterize animal ischemic stroke models is by type of ischemic cerebral injury created. Models defined in this way can be placed into one of three categories: global, focal, or multifocal cerebral ischemia (Table 1). In global cerebral ischemia, CBF is reduced throughout the brain. In complete global ischemic models, global flow has ceased completely for a period. In incomplete models, CBF has been sufficiently reduced so that normal metabolism and function cannot be adequately maintained. Table 1 lists examples of complete and incomplete global cerebral ischemic models.

It is important to specifically mention the gerbil, as this species is widely used as a model of global cerebral ischemia and was one of the earliest models to be used by stroke researchers (75). Gerbils have a unique and convenient vascular anatomy in that they do not have a posterior communicating artery, resulting in an incomplete Circle of Willis and no redundant circular vascular supply at the ventral aspect of the brain (88). Complete global cerebral ischemia can, therefore, be induced by bilateral occlusion of the common carotid artery (33). A less severe, incomplete model of global stroke involves unilateral common carotid artery occlusion. Animal species other than the gerbil have a complete Circle of Willis and, therefore, bilateral or unilateral occlusion of the common carotid artery alone cannot be relied on to induce complete or incomplete global stroke, respectively (Table 1). For example, incomplete global cerebral ischemia in rats can be induced by four-vessel occlusion (bilateral vertebral and common carotid arteries [126-129]), two-vessel occlusion (bilateral common carotid arteries) combined with systemic hypotension (48, 49, 69, 103, 121, 148, 149), or unilateral common carotid artery occlusion combined with hypoxia (87, 132, 141, 142, 167).

The relative simplicity of the surgical procedure performed in gerbils, compared with rat models of incomplete cerebral global ischemia, as well as the ability to study animals in a short period are some of the major advantages of the gerbil global ischemia model. However, the model can be quite variable in that some gerbils may be resistant to stroke induction. Use of rat models involving common carotid and other artery manipulations can also yield considerable variability in results between laboratories (55, 102, 164). Another confounding variable to ischemic outcome is the natural susceptibility of gerbils to seizures. Seizures triggered by handling, startling, or environmental change can occur in approximately 20% of the gerbil population (71, 92, 162). Some of the disadvantages discussed for rodent models, such as limited physiologic monitoring and sampling, also are associated with gerbils.

In contrast to those in the gerbil and rat, effective models of global cerebral ischemia in mice have proven more difficult due to high mortality and frequent complications such as seizure (164). Investigators have attempted to adapt rat and gerbil global ischemia models to the mouse with limited success. However, cardiac arrest followed by cardiopulmonary resuscitation (24, 81, 118) and bilateral common carotid arterial occlusion combined with controlled ventilation (110) are examples of complete global ischemic models that have been developed in the mouse with some success.

Reduction in blood flow to a specific brain region is observed in focal stroke models. Most focal stroke models involve middle cerebral artery occlusion (MCAO) (51, 164). Injection of preformed fibrin or blood clots (11, 37, 179-181), photochemical thrombosis (172), electrocoagulation (157, 160, 166), surgical vascular clips (23, 34, 53, 155, 177), ligatures (35, 42, 97), cuff inflation (46, 152, 173), topical application of the vasoconstrictor peptide endothelin 1 (54, 96, 100, 147, 169), or introduction of an intraluminal filament (58, 82, 91, 165) are examples of some of the many methods used to induce MCAO in animal models. Middle cerebral artery occlusion results in brain injury (Fig. 1) and reduced CBF (Fig. 2) in the caudoputamen and the cortex, but the degree and distribution of blood flow depends on duration of occlusion, site of occlusion along the middle cerebral artery, and amount of collateral blood flow into the middle cerebral artery territory (164). Various transient and permanent MCAO models exist and involve either the proximal or distal part of the vessel (159, 164). Many of these models have been used extensively because of their supposed relevance to human thromboembolic stroke (52, 61, 159).

A patchy pattern of reduced CBF is seen in multifocal cerebral ischemic models (101). In these models, multiple sites of ischemia result from the use of embolization of autologous or heterologous blood clots (164), microspheres (78), or photochemically induced thrombi (172). Embolic models more closely resemble clinical vascular dementia than do MCAO models, even though the multiple ischemias are confined to one hemisphere (115). Multifocal ischemia models have limited applications, but can provide a unique opportunity to investigate potential antiplatelet and thrombolytic regimens (101). Although these models are easy to study, the main disadvantages are inconsistency in the location of the infarctions, difficulty in detecting where insults are relative to infarction areas, and possible microvascular injury with photochemical reactions (101, 164).

Animal Care Issues

The pathogenesis of stroke is multifaceted, with overlapping

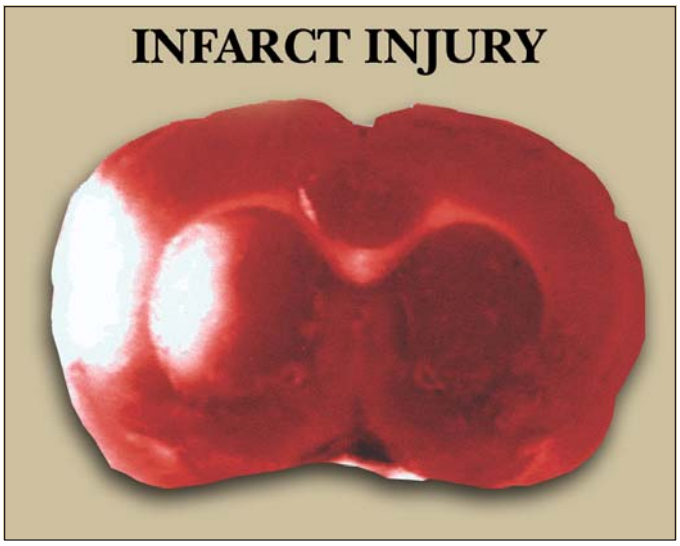

Figure 1. In rodent models of stroke, infarct injury outcomes are often determined using 2,3,5-triphenyltetrazolium chloride staining (12). The brain is harvested and sliced into 2-mm-thick coronal sections, which are then placed into a 1% 2,3,5-triphenyltetrazolium chloride solution in saline and incubated at 37°C for 30 min. Viable brain areas appear red as a result of the reduction of 2,3,5-triphenyltetrazolium chloride by mitochondrial enzymes, whereas injured areas appear white. Stained sections are then fixed in neutral-buffered 10% formalin. The rat brain section shown is representative of the type of injury (caudoputamen, cortex) observed in middle cerebral artery occlusion induced by insertion of an intraluminal filament. Adapted from cover of the *Contemporary Topics in Laboratory Animal Science*, volume 40 (2), featuring work from reference 113.

and interactive mechanisms (16, 62, 143). Many of the pathways involved have been worked out on the basis of results of studies on animal models. In addition to the animal species selected, the type of ischemic insult chosen, and the time point being studied, the research hypotheses being explored also will impact animal care issues associated with stroke models. This section will involve animal care issues pertinent to experimentally induced ischemic stroke models.

Housing and handling. Housing and handling conditions are important issues in modeling ischemic brain injury in animals (144). For many studies, animals after surgery are often singly housed so that investigators can monitor and follow recovering individuals more closely. These animals may also require separate housing due to decreased ambulatory abilities from neurologic deficits, rendering them more susceptible to undesired attention or attack from cage-mates. However, individual housing may be an issue for more social species, such as rodents, dogs, and macaques, which benefit from physical contact with compatible conspecifics (64). In the case of nonhuman primates, federal guidelines dictate that research facilities develop and document a plan for environment enhancement to promote psychological well-being in nonhuman primates (31). Such plan must include and address compatible social grouping as well as environmental enrichment (31). For a stroke research protocol, any exemption from participation in all or part of an institutional environmental enhancement plan for nonhuman primates must be scientifically justified by the investigator and reviewed and approved by the institutional animal care and use committee (IACUC) (31).

Most animals on stroke studies are housed in simple, non-enriched environments, or "standard" caging and housing conditions (31, 64), even though they are meant to model brain injury in people who typically live in complex environments before and after stroke (144). Many experimentally naïve laboratory animal species housed in complex environments will develop an increased number of neuronal synapses as well as other neuroanatomic differences from animals raised in standard caging environments (15). Unfortunately, little has been published about the effect of environmental enrichment on stroke outcomes in nonrodent species. Environmental enrichment for rodents used specifically in stroke studies has only recently received attention in the last decade. For example, some stroke studies have involved evaluation of the impact of environmental enrichment on functional assessments (17, 39, 50, 66, 68, 133), gene expression (40, 41), neuroprotection (175), neurogenesis and differentiation (17, 83), neuroplasticity (67, 68), and cell death (50) in rodents. In those studies, enriched environments consisted of larger cages with elevated horizontal and inclined boards and ladders, as well as objects such as wooden tunnels, blocks, or balls, running wheel, chain, or swing (17, 39-41, 50, 66-68, 83, 133). Overall, environmental enrichment in rodents influenced and affected all outcome measures evaluated in these studies. Therefore, depending on the specific stroke research hy-

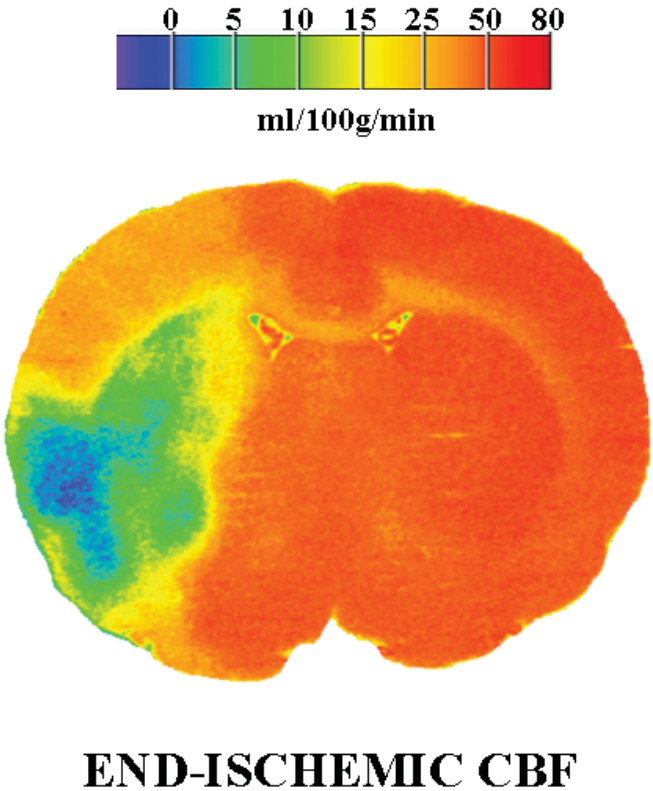

Figure 2. Regional cerebral blood flow (CBF) measurements can be made in rodent stroke models using quantitative [^{14}C]iodoantipyrine autoradiography (140). Prior to euthanasia, anesthetized animals are infused intravenously with [^{14}C]iodoantipyrine. During infusion, arterial blood samples are collected. The brain is frozen, cryosectioned into 20-µm-thick coronal sections, and thaw-mounted onto cover slips. Sections are then exposed to film to generate autoradiographic images. Rat autoradiographic brain section shown is representative of the type of regional CBF patterns observed at the end of 2 h of middle cerebral artery occlusion induced by insertion of an intraluminal filament. Decreased blood flow is seen in caudoputamen and cortex in the ipsilateral, or ischemic hemisphere. (*see* Fig. 1 for source).

pothesis being examined, such consequences from environmental enrichment may or may not be a potential confounding variable to experimental outcomes.

The current use of genetically engineered mice and rodents with spontaneous mutations in biomedical research may lead to specific care issues unique to these individual animals that will need to be incorporated into housing and stroke research protocols. For example, diabetes mellitus and hypertension are two well-known risk factors for stroke (2). Rodent models of both of these conditions have been used in gender-based stroke research (1, 26, 29, 123, 139, 163, 168). Diabetic animals require more frequent cage bedding changes due to increased urine output as well as daily insulin therapy and monitoring of urine and blood glucose values (163). Aged hypertensive rats can sometimes experience decreased appetite and weight loss due to progressing heart failure and the increased effort required to access pelleted feed (14). Supplementation with powdered feed in food jars can greatly improve eating and aid weight maintenance in these animals (14).

Because of experimental interventions, treatments, and functional assessments, animals may need to be handled extensively during experimental stroke studies. Depending on the experimental paradigm, acclimatizing animals to treatments and other experimental manipulations before and after stroke surgery can help to minimize stress and distress to the animal. Nonrodent species and rodent species other than laboratory rats and mice that have undergone experimentally induced stroke (i.e., gerbils) may also need to be housed outside of core animal facilities for longer than 12 h to provide intensive postoperative care and monitoring (*see* Peri-operative care section). Such housing requires justification in research protocols and prior IACUC approval (31). In the case of laboratory rats (*Rattus* sp. only) and mice (*Mus* sp. only), housing in satellite facilities for longer than 24 h requires justification and prior IACUC approval if any institutional research activities are conducted or supported by the Public Health Service (PHS), which includes the National Institutes of Health (NIH) (122). Any IACUC-approved animal housing outside of core animal facilities must adhere to federal housing guidelines appropriate for the animal species in question (31, 64, 122).

Animal numbers. The ongoing challenge in biomedical research is balancing the necessary use of animal subjects to generate unbiased data with the animal welfare theory of reduction, refinement and replacement (138). Current guidelines published by the Stroke Therapy Academic Industry Roundtable (STAIR) recommend use of neurobehavioral evaluations, functional tests, and other long-term outcome measures in animal stroke models (154). Therefore, some stroke investigations may require large numbers of animals so that multiple long-term histologic and functional measures can be evaluated. As mentioned previously, greater numbers are often needed for rodent studies when concurrent measurements over time are restricted or unfeasible due to size and blood volume limitations. As well, morbidity and mortality associated with experimentally induced stroke can be variable, depending on the model used.

Despite some of these issues and experimental requirements, stroke researchers attempt to design experimental protocols that involve use of the smallest number of animals possible while allowing sufficient statistical power to test the hypothesis of interest. Knowing ahead of time what is an expected success rate on the basis of previous experience with a specific model as well as performing a power analysis for various outcome measures can help to minimize animal numbers while in the planning stages of a project. For some stroke models and research areas, it is possible to maximize the amount of data collected from one experimental animal through careful planning. For example, in global cerebral ischemia models created by use of cardiac arrest, tissue from multiple organs can be collected so that collaborators may study peripheral consequences of reduced flow in other target organs such as kidney (24). Also, many stroke laboratories will routinely bank a variety of tissues for collaborator or future use when genetically engineered mice or other species of limited availability, such as nonhuman primates, reproductively senescent rodents, or aged animals are involved.

Peri-operative care. Animal stroke studies may require administration of an experimental agent or drug before, during, or after the ischemic insult (104, 111, 114). Frequency, administration route, and timing of experimental interventions will dictate how much and what type of peri-operative care will be needed for such treatments. Many of these cerebral ischemic models can require appreciable surgical manipulation of animals under anesthesia. Anesthetic choice has been documented to affect ischemic outcome in experimental stroke (77, 106). Moreover, there can often be wide variability in infarct outcome between surgeons due to variable levels of surgical expertise. Monitoring of brain and core body temperatures (25, 55, 60, 74, 108, 130, 178) and physiologic variables, such as blood gas values (21, 95, 150, 178), blood pH (146, 178), blood pressure (30, 125), and blood glucose concentration (55, 72, 73, 117, 171), also is essential when studying ischemic models. These parameters can be a source of infarct size variability if poorly controlled within and between treatment groups.

During the initial postoperative periods, "stroked" animals generally require intensive care, such as fluid, nutritional, and temperature support, especially in the first 24 to 48 h of long-term studies. After surgery, animals in general and "stroked" animals in particular are usually less adaptive to environmental changes due to possible loss of normothermic regulation and may require external heat sources, such as recirculating warm water blankets placed beneath a portion of their recovery cage, to maintain appropriate body temperature. Loss of normal feeding behaviors due to neurologic damage, possible visual deficits, facial paralysis, or general depression from surgery and anesthesia may require hand feeding or placing softened food pellets or powdered food near the animal to facilitate food intake. Prolonged surgical procedures such as MCAO can cause dehydration and may require peri-operative administration of fluids to correct imbalances. Baseline and postoperative functional assessments may also be required for postoperative care and for experimental outcome measures.

In rodent stroke models, poststroke complications can involve a combination of factors principally involving the respiratory, cardiac, and central nervous systems. Pulmonary edema and hypothermia secondary to prolonged anesthesia and positioning, hypovolemia from multiple physiologic sampling, or generalized shock syndrome, depending on the severity of the infarct and resulting cerebral edema, are some of the problems that may be seen in the immediate postoperative period and during recovery. Most animals are able to compensate and adapt to long-term neurologic deficits, such as hemiparesis and induced circling, as long as food and water are made easily accessible to these animals as described previously.

Typically, in nonhuman primate models, similar findings with temperature control and dehydration are seen during the immediate recovery period. Depending on the model or protocol used, administration of a long-acting analgesic, such as flunixin meglumine (99) or buprenorphine (169), may be used to reduce discomfort within the first 36 postoperative hours. Neurologic deficits, such as left-sided hemiparesis or abnormal grasp reflex, can lead to a necessary period of adaptation but usually does not lead to permanent loss of mobility or feeding habits (99, 169). Easy access to soft food and water (169) as well as hand-feeding by caregivers, using human baby formula cereals or sweetened oral rehydration solutions with added banana-flavored milkshake mixes, can aid in recovery during the first 48 h after surgery (99).

Pain and distress. The question often arises on how to assess pain and distress in animal subjects after stroke. How much pain do animals experience due to the stroke itself rather than experimental surgical manipulations? Limited experimental data exist that specifically address this topic, especially in global ischemic models, where selective neuronal cell loss occurs principally in the hippocampus and to a lesser degree in the striatum and cortex. Pain-sensitive structures within the brain are limited to cerebral and dural arteries; the 5th, 9th, and 10th cranial nerves; and parts of the dura at the base of the brain (19, 170). White matter, the ependymal lining of the ventricles, choroid plexus, and much of the cortex and its pia-arachnoid covering are not pain sensitive (19, 170). However, a secondary vascular lesion within the brain, particularly in thalamus, could possibly be a source of central pain (170). One could therefore assume that, in animal ischemic stroke models, observed clinical signs of pain are more likely related to surgical preparation and manipulation (i.e., incisions) than to neuronal damage.

Observational studies exist in human stroke literature, but often these studies center more on chronic pain than pain from the acute ischemic insult. For example, a common problem in clinical stroke is the management of poststroke spasticity and shoulder pain after hemiplegic stroke. Shoulder pain is a common complication after stroke that can limit the patient's ability to reach the maximal functional potential and impede rehabilitation. In a recent study of 85 patients with stroke, 54 of these patients (63.5%) were found to have shoulder pain, although these patients were far removed temporally from the acute event (8). This particular clinical consequence may not be an issue in experimental stroke models, as many investigational time points for survival are often measured in days (*see* Endpoints section).

Chronic hemisensory pain known as Déjerine-Roussy syndrome can develop after acute strokes, usually as a result of lesions in the non-dominant thalamus and rarely in the parietal lobe (5, 43, 116). In 40 to 60% of patients, the onset of central pain often occurs more than a month after stroke (57). Often post-stroke pain is refractory to treatment, but can be responsive to anti-epileptics or amitriptyline (120). Acute limb pain associated with stroke is a much less studied and rare event, and has been described in subjects with hemispheric, thalamic, and, less frequently, brainstem lesions (18, 27, 124, 134). This is distinguishable from the more common delayed central pain that develops weeks or months after a thalamic stroke as described previously (56). In such instances, pain often begins when sensory function is returning months after the acute event. Acute limb pain is usually short lived (several days) and dysesthetic or "burning" in quality (134).

A common patient complaint that usually follows or coincides with acute stroke is headache. This can develop due to vessel wall damage, as headache is a cardinal clinical feature of arterial dissections and can occur without a subsequent ischemic event. Headaches are more commonly reported after intracerebral and subarachnoid hemorrhage than after ischemic stroke. However, in a large clinical study, headache occurred in 32% of patients with ischemic stroke and 64.5% of patients with hemorrhagic stroke (9). Clinically, neurologists often do not treat these patients due to concerns of over-sedation. In addition, patients with large strokes may also have difficulty in communication that is attributable to aphasia or low arousal. Patients with subarachnoid or intracerebral hemorrhage are often treated with only acetaminophen (i.e., Tylenol), as narcotics make it difficult to assess clinical deterioration, and antiplatelet drugs and aspirin are contraindicated due to the increased risk of rebleeding. Consequently, pain after acute stroke and brain injury often remains untreated in human patients.

Many stroke scales and clinical assessment tools (65) such as the NIH stroke scale, used to evaluate human stroke patients have been adapted for use in large animals such as nonhuman primates (98, 99). Although modifications of these clinical assessment guidelines are designed to directly evaluate functional outcome in animals, they may be useful in indirectly evaluating pain and distress in that these factors may alter animal behavior and functional responses. To better assess animal pain and distress due to experimental stroke surgical manipulations, researchers must be aware of normal species-typical behavior and be able to recognize behavior patterns associated with the onset of pain and distress. Several behaviorally based scoring systems for general postoperative application (109, 131, 174) as well as one recently published guideline for specific application to a focal rodent stroke model (76) are available. However, such subjective scoring systems are limited, in that pain may not necessarily be the sole or even principal cause of behavioral and physiologic changes evaluated in "stroked" animals. Some responses may be due to subtle differences in animal handling, in the experimental environment, or to analgesic administration (135, 136). Despite these limitations, scoring systems can be helpful in establishing intervention criteria, such as early removal or euthanasia of an animal, especially in studies where analgesics cannot be given due to effects on outcome measures.

Use of analgesics, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids, in experimental surgical stroke models may be problematic depending on the time points being evaluated and the research questions being studied. For long-term studies (weeks to months), administration of analgesics in the initial postoperative period may not be a possible confounder. However, for short-term studies (hours to days), analgesics could alter outcome measures. For example, considerable evidence indicates that cerebral ischemia triggers inflammation, which in turn contributes to brain injury and worsening of neurologic outcome (45, 63). Results of a recent study in rats indicated that ibuprofen reduced striatal infarct size after focal cerebral ischemia (7). Consequently, use of NSAIDs or corticosteroids in animal stroke models could bias experimental results if investigators are examining inflammatory mediators, cytokine pathways, or subacute to acute time points. In addition, several laboratories have documented alterations in the opioidergic system in response to cerebral ischemia (20). The effect of analgesic

opioids on experimental ischemic brain damage is currently unclear, with beneficial (38, 158), detrimental (80), or no effects (151) on experimental stroke outcome being reported. The decision not to administer analgesics in experimental stroke protocols must be justified and pre-approved by the IACUC (31, 64).

Multiple surgeries and/or interventions. For many experimental stroke paradigms, multiple minor or major surgical procedures may precede the actual experimental stroke protocol. For example, use of telemetry to continuously evaluate parameters such as brain and body temperature, activity levels, and blood pressure will require surgery to implant transmitters days to weeks before an experimental ischemic insult (84). Whether this procedure will be considered major or minor surgery will depend on where the transmitter is placed. Implants for drug or agent delivery as well as placement of injection or sampling ports require similar considerations. Long-term indwelling catheters may be required and may be used in conjunction with a tether or jacket system to allow mobility of the animal. The rationale for many of these preplaced devices is to minimize handling and stress to the animal in the peristroke period. As mentioned previously, some studies will require multiple drug or agent administrations via oral or parenteral routes as well as continuous intravenous or bolus infusions (*see* Peri-operative care section).

Minor survival surgery is a procedure that does not expose a body cavity and causes little or no physical impairment (31, 64). Examples of minor procedures that may be used in stroke studies would be intrascapular subcutaneous drug/agent implants (1, 137) or telemetry transmitters (86, 93, 94, 156). Any procedure that penetrates or exposes a body cavity or causes substantial or permanent impairment of physical or physiologic function is considered major surgery (31, 64). For example, in stroke programs evaluating gender effects, ovariectomy of a female rodent would be defined as major surgery. By this definition, placement of a telemetry transmitter within the abdominal cavity would be another example of major surgery. Multiple major survival surgeries are not allowed for a single animal unless the investigator can provide scientific justification and receives prior approval from the IACUC (6, 31). Many stroke researchers will design studies so that multiple procedures can be done during one anesthesia episode (i.e., ovariectomy and hormone implantation in rodents) and will allow sufficient time for recovery and healing prior to experimentally induced stroke.

Endpoints. Death as an endpoint is rarely needed or justified for much of the stroke research currently being done. In permanent and transient ischemia studies, typical endpoints for acute stroke studies require hours to days, whereas chronic stroke studies may require weeks to months. Many studies will evaluate time points during ischemia itself or during the early reperfusion period (minutes to hours), with no need to have the animal recover from anesthesia (nonsurvival cohorts).

When considering any animal-based experimental protocol, a principal concern should be eliminating unnecessary animal discomfort. This can be done by establishing criteria for humane endpoints within a given stroke model on the basis of clinical assessment of physical and behavioral parameters before and after experimentally induced stroke (76). Though final approval must come from the IACUC when determining specific parameters on ending individual experiments due to pain or distress, clinical assessment of the "stroked" animal will guide the observer in choosing early termination of the experiment. For example, a moribund or comatose animal with labored respiration would be an immediate candidate for euthanasia using pre-approved guidelines. It is the more subtle clinical signs of stroke, such as circling or changes in vocalization (13), that require thorough understanding of the specific stroke model so as to differentiate between expected clinical outcome versus true anxiety or distress. A thorough knowledge of normal behavior and the progression of physical and behavioral changes secondary to the protocol is essential for all personnel involved with the monitoring and care of these animals (*see* Pain and distress section). Proper training is necessary to determine when such intervention is appropriate. This training can often be done in concert with institutional veterinarians and animal care staff to ensure a positive approach to animal care and welfare. Careful planning and establishment of specific protocols to handle these questions before they arise allow good animal care and good science.

Conclusion

Stroke is a clinical disease with substantial mortality, morbidity, and annual health-care costs. Current clinical and experimental research is focused principally on mechanisms of injury, prevention, and treatment. Many animal models of ischemic stroke are available, but no model in particular fully mimics the diversity of human clinical ischemic stroke. Nonetheless, these models are useful tools for studying specific injurious and protective mechanisms as well as preventative and therapeutic measures for cerebral ischemia. Animal care issues particularly relevant to ischemic stroke, such as pain and distress, must be considered when using these surgical animal models for research. A realistic balance between experimental aims and animal care can be achieved through discussion and interaction among researchers, laboratory animal veterinarians and caretakers, and IACUC members.

Acknowledgments

The authors thank Debra L. Hickman, Veterinary Medical Officer, Portland VA Medical Center, Portland, Oregon, and Richard J. Traystman, Professor, Department of Anesthesiology and Peri-Operative Medicine, and Associate Vice President for Research Planning and Development and Associate Dean of Research, Oregon Health and Science University, Portland, Oregon, for their review and comments on preliminary versions of this manuscript. This work was supported by National Center for Research Resources grant RR00163. Portions of this article were presented at the 2003 American College of Laboratory Animal Medicine (ACLAM) Forum in Ft. Myers, Florida, by Stephanie Murphy in May 2003.

References

1. **Alkayed, N. J., I. Harukuni, A. S. Kimes, E. D. London, R. J. Traystman, and P. D. Hurn.** 1998. Gender-linked brain injury in experimental stroke. Stroke **29:**159-166.
2. **American Heart Association.** 2003. Heart disease and stroke statistics—2004 update. American Heart Association, Dallas.
3. **American Stroke Association, a Division of American Heart Association.** 2004. American Stroke Association. [Online] Available at http://www.strokeassociation.org. Accessed 10/04/04.
4. **Andaluz, N., M. Zuccarello, and K. R. Wagner.** 2002. Experimental animal models of intracerebral hemorrhage. Neurosurg. Clin. North Am. **13:**385-393.
5. **Andersen, G., K. Vestergaard, M. Ingeman-Nielson, and T. S. Jensen.** 1995. Incidence of central post-stroke pain. Pain **61:**87-193.

6. **Animal and Plant Health Inspection Service, USDA.** Policy #14—Major survival surgery, single vs. multiple procedures. Last revision April 14, 1997.
7. **Antezana, D. F., R. E. Clatterbuck, N. J. Alkayed, S. J. Murphy, L. G. Anderson, K. Frazier, P. D. Hurn, R. J. Traystman, and R. J. Tamargo.** 2003. High-dose ibuprofen reduces striatal infarcts during middle cerebral artery occlusion in rats. J. Neurosurg. **98:**860-866.
8. **Aras, M. D., N. K. Gokkaya, D. Comert, A. Kaya, and A. Cakei.** 2004. Shoulder pain in hemiplegia: results from a national rehabilitation hospital in Turkey. Am. J. Physiol. Med. Rehabil. **83:**713-719.
9. **Arboix, A., J. Massons, M. Oliveres, M. P. Arribas, and F. Titus.** 1994. Headache in acute cerebrovascular disease: a prospective clinical study in 240 patients. Cephalalgia **14:**37-40.
10. **Ashwal, S. and W. J. Pearce.** 2001. Animal models of neonatal stroke. Curr. Opin. Pediatr. **13:**506-516.
11. **Atochin, D. N., J. C. Murciano, Y. Gursoy-Ozdemir, T. Krasik, F. Noda, C. Ayata, A. K. Dunn, M. A. Moskowitz, P. L. Huang, and V. R. Muzykantov.** 2004. Mouse model of microembolic stroke and reperfusion. Stroke **35:**2177-2182.
12. **Bederson, J. B., L. H. Pitts, S. M. Germano, M. C. Nishimura, R. L. Davis, and H. M. Bartkowski.** 1986. Evaluation of 2,3,5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats. Stroke **17:**1304-1308.
13. **Bederson, J. B., L. H. Pitts, M. Tsuji, M. C. Nishimura, R. L. Davis, and H. Bartkowski.** 1986. Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. Stroke **17:**472-476.
14. **Belanger, M. P., W. J. Wallen, and C. Wittnich.** 1999. Special feeding and care of senescent spontaneously hypertensive rats. Contemp. Top. Lab. Anim. Sci. **38(4):**7-11.
15. **Benefiel, A. C. and W. T. Greenough.** 1998. Effects of experience and environment on the developing and mature brain: implications for laboratory animal housing. ILAR J. **39:**5-11.
16. **Bhardwaj, A., N. J. Alkayed, J. R. Kirsch, and P. D. Hurn.** 2003. Mechanisms of ischemic brain damage. Curr. Cardiol. Rep. **5:**160-167.
17. **Biernaskie, J. and D. Corbett.** 2001. Enriched rehabilitative training promotes improved forelimb motor function and enhanced dendritic growth after focal ischemic injury. J. Neurosci. **21:**5272-5280.
18. **Bogousslavsky, J., F. Regli, J. Ghika, and J. J. Feldmeyer.** 1984. Painful ataxic hemiparesis. Arch. Neurol. **41:**892-893.
19. **Bonica, J. J., and J. D. Loeser.** 2001. Applied anatomy relevant to pain. *In* J. D. Loeser (ed.), Bonica's management of pain, 3rd ed. Lippincott Williams & Wilkins, Philadelphia.
20. **Boutin, H., F. Dauphin, E.T. MacKenzie, and Ph. Jauzac.** 1996. Cortical and subcortical alterations of the opioidergic system in response to cerebral ischemia, p. 601-617. *In* J. Krieglstein, H. Oberpichler-Schwenk, and Wissenschaftliche Verlagsgesellschaft (ed.), Pharmacology of cerebral ischemia. Medpharm Scientific Publishers, Stuttgart, Germany.
21. **Browning, J. L., M. L. Heizer, M. A. Widmayer, and D. S. Baskin.** 1997. Effects of halothane, alpha-chloralose, and pCO_2 on injury volume and CSF beta-endorphin in focal cerebral ischemia. Mol. Chem. Neuropathol. **31:**29-42.
22. **Buccafusco, J. J. (ed.).** 2001. Methods of behavior analysis in neuroscience. CRC Press, New York.
23. **Buchan A. M., D. Xue, and A. Slivka.** 1992. A new model of temporary focal neocortical ischemia in the rat. Stroke **23:**273-279.
24. **Burne-Taney M. J., J. Kofler, N. Yokota, M. Weisfeldt, R. J. Traystman, and H. L. Rabb.** 2003. Acute renal failure after whole body ischemia is characterized by inflammation and T cell-mediated injury. Am. J. Physiol. Renal Physiol. **285:**F87-F94.
25. **Busto, R., W. D. Dietrich, M. Y. Globus, I. Valdes, P. Scheinberg, and M. D. Ginsberg.** 1987. Small differences in intraischemic brain temperature critically determine the extent of intraischaemic neuronal injury. J. Cereb. Blood Flow Metab. **7:**729-738.
26. **Cai, H., H. Yao, S. Ibayashi, H. Uchimura, and M. Fujishima.** 1998. Photothrombotic middle cerebral artery occlusion in spontaneously hypertensive rats: influence of substrain, gender, and distal middle cerebral artery patterns on infarct size. Stroke **29:**1982-1986.
27. **Caplan, L. and P. Gorelick.** 1983. "Salt and pepper on the face" pain in acute brainstem ischemia. Ann. Neurol. **13:**344-345.
28. **Carr, F. J., M. W. McBride, H. V. Carswell, D. Graham, P. Strahorn, J. S. Clark, F. J. Charchar, and A. F. Dominiczak.** 2002. Genetic aspects of stroke: human and experimental studies. J. Cereb. Blood Flow Metab. **22:**767-773.
29. **Carswell, H. V., N. H. Anderson, J. S. Clark, D. Graham, B. Jeffs, A. F. Dominiczak, and I. M. Macrae.** 1999. Genetic and gender influences on sensitivity to focal cerebral ischemia in the stroke-prone spontaneously hypertensive rat. Hypertension **33:**681-685.
30. **Chileuitt, L., K. Leber, T. McCalden, and P. R. Weinstein.** 1996. Induced hypertension during ischemia reduces infarct area after temporary middle cerebral artery occlusion in rats. Surg. Neurol. **46:**229-234.
31. **Code of Federal Regulations.** Title 9–Animals and Animal Products, Chapter 1–Animal and Plant Health Inspection Service, Department of Agriculture, Subchapter A–Animal Welfare, U.S. Government 1-1-01 Edition.
32. **Cramer, S. C.** 2003. Clinical issues in animal models of stroke and rehabilitation. ILAR J. **44:**83-84.
33. **Crockard, A., F. Iannotti, A. T. Hunstock, R. D. Smith, R.J. Harris, and L. Symon.** 1980. Cerebral blood flow and edema following carotid occlusion in the gerbil. Stroke **11:**494-498.
34. **Crowell, R. M., Y. Olsson, I. Klatzo, and A. Ommaya.** 1970. Temporary occlusion of the middle cerebral artery in the monkey: clinical and pathological observations. Stroke **1:**439-448.
35. **Crowell, R. M., F. W. Marcoux, and U. DeGirolami.** 1981. Variability and reversibility of focal cerebral ischemia in monkeys. Neurology **31:**1295-1302.
36. **Curry, B. B., III.** 2001. Animal models used in identifying gender-related differences. Int. J. Toxicol. **20:**153-160.
37. **Daffertshofer, M., Z. Huang, M. Fatar, M. Popolo, H. Schroeck, W. Kuschinsky, M. A. Moskowitz, and M. G. Hennerici.** 2004. Efficacy of sonothrombolysis in a rat model of embolic ischemic stroke. Neurosci. Lett. **361:**115-119.
38. **Dalkara, T., I. J. Namer, R. Onur, and T. Zileli.** 1989. Intravenously and iontophoretically administered naloxone reverses ischemic changes in rat hippocampus. Stroke **20:**1059-1064.
39. **Dahlqvist, P., A. Ronnback, S. A. Bergstrom, I. Soderstrom, and T. Olsson.** 2004. Environmental enrichment reverses learning impairment in the Morris water maze after focal cerebral ischemia in rats. Eur. J. Neurosci. **19:**2288-2298.
40. **Dahlqvist, P., A. Ronnback, A. Risdal, R. Nergardh, I. M. Johansson, J. R. Seckl, B. B. Johansson, and T. Olsson.** 2003. Effects of postischemic environment on transcription factor and serotonin receptor expression after permanent focal cortical ischemia in rats. Neuroscience **119:** 643-652.
41. **Dahlqvist, P., L. Zhao, I. M. Johansson, B. Mattsson, B. B. Johansson, J. R. Seckl, and T. Olsson.** 1999. Environmental enrichment alters nerve growth factor-induced gene A and glucocorticoid receptor messenger RNA expression after middle cerebral artery occlusion in rats. Neuroscience **93:**527-535.
42. **DeGirolami, U., R. M. Crowell, and F. W. Marcoux.** 1984. Selective necrosis and total necrosis in focal cerebral ischemia. Neuropathological observations on experimental middle cerebral artery occlusion in the macaque monkey. J. Neuropathol. Exp. Neurol. **43:**57-71.
43. **Déjerine, J., and G. Roussy.** 1906. Le syndrome thalamique. Rev. Neurol. (Paris) **14:**521-532.
44. **De Lecinana, M. A., E. Diez-Tejedor, F. Carceller, and J. M. Roda.** 2001. Cerebral ischemia: from animal studies to clinical practice. Should the methods be reviewed? Cerebrovasc. Dis. **11(Suppl. 1):**20-30.
45. **Del Zoppo, G. J., K. J. Becker, and J. M. Hallenbeck.** 2001. Inflammation after stroke: is it harmful? Arch. Neurol. **58:**669-672.
46. **Del Zoppo, G. J., B. R. Copeland, L. A. Harker, T. A. Waltz, J. Zyroff, S. R. Hanson, and E. Battenberg.** 1986. Experimental acute thrombotic stroke in baboons. Stroke **17:**1254-1265.
47. **DeVries, A. C., R. J. Nelson, R. J. Traystman, and P. D. Hurn.** 2001. Cognitive and behavioral assessment in experimental stroke research: will it prove useful? Neurosci. Biobehav. Rev. **25:**325-342.
48. **Eklof, B. and B. K. Siesjo.** 1972. The effect of bilateral carotid artery ligation upon acid-base parameters and substrate levels in the rat brain. Acta Physiol. Scand. **86:**528-538.

49. **Eklof, B. and B. K. Siesjo.** 1972. The effect of bilateral carotid artery ligation upon the blood flow and the energy state of the rat brain. Acta Physiol. Scand. **86:**155-165.
50. **Farrell, R., S. Evans, and D. Corbett.** 2001. Environmental enrichment enhances recovery of function but exacerbates ischemic cell death. Neuroscience **107:**585-592.
51. **Fukuda, S. and G. J. del Zoppo.** 2003. Models of focal cerebral ischemia in the nonhuman primate. ILAR J. **44:**96-104.
52. **Garcia, J. H.** 1984. Experimental ischemic stroke: a review. Stroke **15:**5-14.
53. **Garcia, J. H. and Y. Kamijyo.** 1974. Cerebral infarction. Evolution of histopathological changes after occlusion of a middle cerebral artery in primates. J. Neuropathol. Exp. Neurol. **33:**408-421.
54. **Gartshore, G., J. Patterson, and I. M. Macrae.** 1997. Influence of ischemia and reperfusion on the course of brain tissue swelling and blood-brain barrier permeability in a rodent model of transient focal cerebral ischemia. Exp. Neurol. **147:**353-360.
55. **Ginsberg, M. and R. Busto.** 1989. Rodent models of cerebral ischemia. Stroke **20:**1627-1642.
56. **Gorson, K. C., M. S. Pessin, L. D. DeWitt, and L. R. Caplan.** 1996. Stroke with sensory symptoms mimicking myocardial ischemia. Neurology **46:**548-551.
57. **Hansson, P.** 2004. Post-stroke pain case study: clinical characteristics, therapeutic options and long-term follow-up. Eur. J. Neurol. **11(Suppl. 1):**22-30.
58. **Hata R., G. Mies, C. Wiessner, K. Fritze, D. Hesselbarth, G. Brinker, and K. A. Hossman.** 1998. A reproducible model of middle cerebral artery occlusion in mice: hemodynamic, biochemical, and magnetic resonance imaging. J. Cereb. Blood Flow Metab. **18:**367-375.
59. **Hattori, K., H. Lee, P. D. Hurn, B. J. Crain, R. J. Traystman, and A. C. DeVries.** 2000. Cognitive deficits after focal cerebral ischemia in mice. Stroke **31:**1939-1944.
60. **Hildebrandt-Erikson, E. S., T. Christensen, and N. H. Diemer.** 2002. Mild focal cerebral ischemia in the rat. The effect of local temperature on infarct size. Neurol. Res. **24:**781-788.
61. **Hossman, K. A.** 1991. Animal models of cerebral ischemia. I. Review of the literature. Cerebrovasc. Dis. **1:**2-15.
62. **Hou, S. T. and J. P. MacManus.** 2002. Molecular mechanisms of cerebral ischemia-induced neuronal death. Int. Rev. Cytol. **221:**93-148.
63. **Iadecola, C. and M. Alexander.** 2001. Cerebral ischemia and inflammation. Curr. Opin. Neurol. **14:**89-94.
64. **Institute of Laboratory Animal Resources, Commission on Life Sciences, and National Research Council.** 1996. Guide for the care and use of laboratory animals. National Academy Press, Washington, D.C.
65. **Internet Stroke Center at Washington University in St. Louis.** 2004. Stroke Scales & Clinical Assessment Tools. [Online] Available at http://www.strokecenter.org/trials/scales. Accessed 10/04/04.
66. **Johansson, B. B.** 1996. Functional outcome in rats transferred to an enriched environment 15 days after focal brain ischemia. Stroke **27:**324-326.
67. **Johansson, B. B. and P. V. Belichenko.** 2002. Neuronal plasticity and dendritic spines: effect of environmental enrichment on intact and postischemic rat brain. J. Cereb. Blood Flow Metab. **22:**89-96.
68. **Johansson, B. B. and M. Grabwoski.** 1994. Functional recovery after brain infarction: plasticity and neural transplantation. Brain Pathol. **4:**85-95.
69. **Kagstrom, E., M. L. Smith, and B. K. Siesjo.** 1983. Recirculation in the rat brain following incomplete ischemia. J. Cereb. Blood Flow Metab. **3:**183-192.
70. **Kaplan, J. R. (ed.).** 2004. Nonhuman primate and other animal models of women's health. ILAR J. **45:**83-219.
71. **Kaplan, H. and C. Miezejeski.** 1972. Development of seizures in the Mongolian gerbil (*Meriones unguiculatus*). J. Comp. Physiol. Psychol. **81:**267-273.
72. **Kawai, N., R. F. Keep, and A. L. Betz.** 1997. Hyperglycemia and the vascular effects of cerebral ischemia. Acta. Neurochir. Suppl. (Wien) **70:**27-29.
73. **Kawai, N., R. F. Keep, A. L. Betz, and S. Nagao.** 1998. Hyperglycemia induces progressive changes in the cerebral microvasculature and blood-brain barrier transport during focal cerebral ischemia. Acta. Neurochir. Suppl. (Wien) **71:**219-221.
74. **Kimura, T., K. Sako, K. Tanaka, M. Kusakabe, T. Tanaka, and T. Nakada.** 2002. Effect of mild hyperthermia on energy state recovery following transient forebrain ischemia in the gerbil. Exp. Brain Res. **145:**83-90.
75. **Kirino, T.** 1982. Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res. **239:**57-69.
76. **Kirsch, J. H., J. A. Klaus, K. K. Blizzard, P. D. Hurn, and S. J. Murphy.** 2002. Pain evaluation and responses to buprenorphine in rats subjected to sham middle cerebral artery occlusion. Contemp. Top. Lab. Anim. Sci. **41(6):**9-14.
77. **Kirsch, J. R., R. J. Traystman, and P. D. Hurn.** 1996. Anesthetics and cerebroprotection: experimental aspects. Int. Anesthesiol. Clin. **34:**73-93.
78. **Kiyota, Y., M. Miyamoto, A. Nagaoka, and Y. Nagawa.** 1986. Cerebral embolization leads to memory impairment of several learning tasks in rats. Pharmacol. Biochem. Behav. **24:**687-692.
79. **Klein, H. J. and R. J Nelson (ed.).** 2002. Advanced physiological monitoring in rodents. ILAR J. **43:**121-182.
80. **Kofke, W.A., R. H. Garman, R. Garman, and M. E. Rose.** 1999. Opioid neurotoxicity: fentanyl-induced exacerbation of cerebral ischemia in rats. Brain Res. **818:**326-334.
81. **Kofler, J., K. Hattori, M. Sawada, A. C. DeVries, L. J. Martin, P. D. Hurn, and R. J. Traystman.** 2004. Histopathological and behavioral characterization of a novel model of cardiac arrest and cardiopulmonary resuscitation in mice. J. Neurosci. Methods **136:**33-44.
82. **Koizumi, J., Y. Yoshida, T. Nakazawa, and G. Ooneda.** 1986. Experimental studies of ischemic brain edema. I. A new experimental model of cerebral embolism in rats in which recirculation can be introduced in the ischemic area. Jpn. J. Stroke **8:**1-8.
83. **Komitova, M., E. Perfilieva, B. Mattsson, P. S. Eriksson, and B. B. Johansson.** 2002. Effects of cortical ischemia and postischemic environmental enrichment on hippocampal cell genesis and differentiation in adult rat. J. Cereb. Blood Flow Metab. **22:**852-860.
84. **Kramer, K., L. Kinter, B. P. Brockway, H. P. Voss, R. Remie, and B. L. Van Zutphen.** 2001. The use of radiotelemetry in small laboratory animals: recent advances. Contemp. Top. Lab. Anim. Sci. **40(1):**8-16.
85. **Landi, M. S. (ed.).** 2001. Impact of noninvasive technology on animal research. ILAR J. **42:**187-262.
86. **Legos, J. J., A. A. Mangoni, S. J. Read, C. A. Campbell, E. A. Irving, J. Roberts, F. C. Barone, and A. A. Parsons.** 2002. Programmable microchip monitoring of post-stroke pyrexia: effects of aspirin and paracetamol on temperature and infarct size in the rat. J. Neurosci. Methods **113:**159-166.
87. **Levine, S.** 1960. Anoxic-ischemic encephalopathy in rats. Am. J. Pathol. **36:**1-17.
88. **Levine, S. and D. Sohn.** 1969. Cerebral ischemia in infarct and adult gerbils. Relation to incomplete Circle of Willis. Arch. Pathol. **87:**315-317.
89. **Li, X., K. K. Blizzard, Z. Zheng, A. C. DeVries, P. D. Hurn, and L. D. McCullough.** 2004. Chronic behavioral testing after focal ischemia in the mouse: functional recovery and the effects of gender. Exp. Neurol. **187:**94-104.
90. **Lin, C. L., T. Calisaneller, N. Ukita, A. S. Dumont, N. F. Kassell, and K. S. Lee.** 2003. A murine model of subarachnoid hemorrhage-induced cerebral vasospasm. J. Neurosci. Methods **123:**89-97.
91. **Longa, E. Z., P. R. Weinstein, S. Carlson, and R. Cummins.** 1989. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke **20:**84-91.
92. **Loskota, W. J., P. Lomax, and S. T. Rich.** 1974. The gerbil as a model for the study of epilepsies. Seizure patterns and ontogenesis. Epilepsia **15:**109-119.
93. **MacLellan, C. L., J. Girgis, and F. Colbourne.** 2004. Delayed onset of prolonged hypothermia improves outcome after intracerebral hemorrhage in rats. J. Cereb. Blood Flow Metab. **24:**432-440.
94. **MacLellan, C., A. Shuaib, and F. Colbourne.** 2002. Failure of delayed and prolonged hypothermia to favorably affect hemorrhagic stroke in rats. Brain Res. **958:**192-200.
95. **Macrae, I. M.** 1992. New models of focal cerebral ischaemia. Br. J. Clin. Pharmacol. **34:**302-308.
96. **Macrae, I. M., M. J. Robinson, D. I. Graham, J. L. Reid, and J. McCulloch.** 1993. Endothelin-1 induced reductions in cerebral blood flow: dose dependency, time course, and neuropathological consequences. J. Cereb. Blood Flow Metab. **13:**276-284.

97. **Marcoux, F. W., R. B. Morawetz, R. M. Crowell, U. DeGirolami, and J. H. Halsey.** 1982. Differential regional vulnerability in transient focal cerebral ischemia. Stroke **13:**339-346.
98. **Marshall, J. W., R. M. Cummings, L. J. Bowes, R. M. Ridley, and A. R. Green.** 2003. Functional and histological evidence for the protective effect of NXY-059 in a primate model of stroke when given 4 hours after occlusion. Stroke **34:**2228-2233.
99. **Marshall, J. W. and R. M. Ridley.** 2003. Assessment of cognitive and motor deficits in a marmoset model of stroke. ILAR J. **44:**153-160.
100. **Marston, H. M., E. S. Faber, J. H. Crawford, S. P. Butcher, and J. Sharkey.** 1995. Behavioral assessment of endothelin-1 induced middle cerebral artery occlusion in the rat. Neuroreport **6:**1067-1071.
101. **McAuley, M. A.** 1995. Rodent models of focal ischemia. Cerebrovasc. Brain Metab. Rev. **7:**153-180.
102. **McBean, D. E., and P. A. Kelly.** 1998. Rodent models of global cerebral ischemia: a comparison of two-vessel occlusion and four-vessel occlusion. Gen. Pharmacol. **30:**431-434.
103. **McBean, D. E., V. Winters, A. D. Wilson, C. B. Oswald, B. J. Alps, and M. J. Armstrong.** 1995. Neuroprotective efficacy of lifarizine (RS-87476) in a simplified rat survival model of 2 vessel occlusion. Br. J. Pharmacol. **116:**3093-3098.
104. **McCullough, L. D., N. J. Alkayed, R. J. Traystman, M. J. Williams, and P. D. Hurn.** 2001. Postischemic estrogen reduces hypoperfusion and secondary ischemia after experimental stroke. Stroke **32:**796-802.
105. **McCullough, L. D. and P. D. Hurn.** 2003. Estrogen and ischemic neuroprotection: an integrated view. Trends Endocrinol. Metab. **14:**228-235.
106. **McPherson, R. W., J. R. Kirsch, S. K. Salzman, and R. J. Traystman.** 1994. Anesthetic management, p. 328-339. *In* S. K. Salzman and A. I. Faden (ed.), The neurobiology of central nervous system trauma. Oxford University Press, New York.
107. **Megyesi, J. F., B. Vollrath, D. A. Cook, and J. M. Findlay.** 2000. In vivo animal models of cerebral vasospasm: a review. Neurosurgery **46:**448-460.
108. **Miyazawa, T., A. Tamura, S. Fukui, and K. A. Hossmann.** 2003. Effect of mild hypothermia on focal cerebral ischemia. Review of experimental studies. Neurol. Res. **25:**457-464.
109. **Morton, D. B. and P. M. H. Griffiths.** 1985. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Vet. Rec. **116:**431-436.
110. **Murakami, K., T. Kondo, M. Kawase, and P. H. Chan.** 1998. The development of a new mouse model of global ischemia: focus on the relationships between ischemia duration, anesthesia, cerebral vasculature and neuronal injury following global ischemia in mice. Brain Res. **780:**304-310.
111. **Murphy, S. J., M. T. Littleton-Kearney, and P. D. Hurn**. 2002. Progesterone administration during reperfusion, but not preischemia alone, reduces injury in ovariectomized rats. J. Cereb. Blood Flow Metab. **22:**1181-1188.
112. **Murphy, S. J., L. D. McCullough, and J. M. Smith.** 2004. Stroke in the female: role of biological sex and estrogen. ILAR J. **45:**147-159.
113. **Murphy, S. J., P. Smith, A. B. Shaivitz, M. I. Rossberg, and P. D. Hurn.** 2001. The effect of brief halothane anesthesia during daily gavage on complications and body weight in rats. Contemp. Top. Lab. Anim. Sci. **40(2):**9-12.
114. **Murphy, S. J., R. J. Traystman, and P. D. Hurn.** 2000. Progesterone exacerbates striatal stroke injury in progesterone-deficient female animals. Stroke **31:**1173-1178.
115. **Naritomi, H.** 1991. Experimental basis of multi-infarct dementia: memory impairments in rodent models of ischemia. Alzheimer Dis. Assoc. Disord. **5:**103-111.
116. **Nasreddine, Z. S. and J. L. Saver.** 1997. Pain after thalamic stroke: right diencephalic predominance and clinical features in 180 patients. Neurology **48:**1196-1199.
117. **Nedergaard, M. and N. H. Diemer.** 1987. Focal ischemia of the rat brain, with special references to the influence of plasma glucose concentration. Acta Neuropathol. (Berlin) **73:**131-137.
118. **Neigh, G. N., J. Kofler, J. L. Meyers, V. Bergdall, K. M. La Perle, R. J. Traystman, and A. C. DeVries.** 2004. Cardiac arrest/cardiopulmonary resuscitation increases anxiety-like behavior and decreases social interaction. J. Cereb. Blood Flow Metab. **24:**372-382.
119. **Nelson, R. J. and R. J. Nudo (ed.).** 2003. Animal models of stroke and rehabilitation. ILAR J. **44:**81-174.
120. **Nicholson, B. D.** 2004. Evaluation and treatment of central pain syndromes. Neurology **62(5 Suppl 2):**S30-S60.
121. **Nordstrom, C. H., S. Rehncrona S., and B. K. Siesjo.** 1987. Effects of phenobarbital in cerebral ischemia. Part II: Restitution of cerebral energy state, as well as of glycolytic metabolites, citric acid cycle intermediates and associated amino acids after pronounced incomplete ischemia. Stroke **9:**335-343.
122. **Office of Laboratory Animal Welfare.** 2002. Public Health Service Policy on Humane Care and Use of Laboratory Animals. National Institutes of Health, Bethesda, Md.
123. **Okamoto, K., Y. Yamori, and A. Nagaoka.** 1974. Establishment of the stroke-prone spontaneously hypertensive rats (SHR). Circ. Res. **34-35 (Suppl. 1):**I143-I153.
124. **Parrent, A. G., A. M. Lozano, J. O. Dostrovsky, and R. R. Tasker.** 1992. Central pain in the absence of functional sensory thalamus. Stereotact. Funct. Neurosurg. **59:**9-14.
125. **Patel, P. M., J. C. Drummond, and D. J. Cole.** 1991. Induced hypertension during restoration of flow after temporary middle cerebral artery occlusion in the rat: effect on neuronal injury and edema. Surg. Neurol. **36:**195-201.
126. **Pulsinelli, W. A. and J. B. Brierley.** 1979. A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke **10:**267-272.
127. **Pulsinelli, W. A. and A. M. Buchan.** 1988. The four-vessel occlusion rat model: method for complete occlusion of vertebral arteries and control of collateral circulation. Stroke **19:**913-914.
128. **Pulsinelli, W. A. and T. E. Duffy.** 1983. Regional energy balance in rat brain after transient forebrain ischemia. J. Neurochem. **40:**1500-1503.
129. **Pulsinelli, W. A., D. E. Levy, and T. E. Duffy.** 1982. Regional cerebral blood flow and glucose metabolism following transient forebrain ischemia. Ann. Neurol. **11:**499-502.
130. **Ren, Y., M. Hashimoto, W. A. Pulsinelli, and T. S. Nowak, Jr.** 2004. Hypothermic protection in rat focal ischemia models: strain differences and relevance to "reperfusion injury." J. Cereb. Blood Flow Metab. **24:**42-53.
131. **Report of the Laboratory Animal Science Association (LASA) Working Party.** 1990. The assessment and control of the severity of scientific procedures on laboratory animals. Lab. Anim. **24:**97-130.
132. **Rice, J. E., R. C. Vannucci, and J. B. Brierley.** 1981. The influence of immaturity on hypoxic-ischemic brain damage in the rat. Ann. Neurol. **9:**131-141.
133. **Risedal, A., B. Mattsson, P. Dahlqvist, C. Nordborg, T. Olsson, and B. B. Johansson.** 2002. Environmental influences on functional outcome after a cortical infarct in the rat. Brain Res. Bull. **58:**315-321.
134. **Rossetti, A. O., J. A. Ghika, F. Vingerhoets, J. Novy, and J. Bogousslavsky.** 2003. Neurogenic pain and abnormal movements contralateral to an anterior parietal artery stroke. Arch. Neurol. **60:**1004-1006.
135. **Roughan, J. V. and P. A. Flecknell.** 2000. Effects of surgery and analgesic administration on spontaneous behavior in singly housed rats. Res. Vet. Sci. **69:**283-288.
136. **Roughan, J. V. and P. A. Flecknell.** 2001. Behavioral effects of laparotomy and analgesic effects of ketoprofen and carprofen in rats. Pain **90:**65-74.
137. **Rusa, R., N. J. Alkayed, B. J. Crain, R. J. Traystman, A. S. Kimes, E. D. London, J. A. Klaus, and P. D. Hurn.** 1999. 17β-Estradiol reduces stroke injury in estrogen-deficient female animals. Stroke **30:**1665-1670.
138. **Russell, W. M. S. and R. L. Burch.** 1959. The principles of humane experimental technique. Methuen, London.
139. **Sadoshima, S., Y. Nakatomi, K. Fujii, H. Ooboshi, T. Ishitsuka, J. Ogata, and M. Fujishima.** 1988. Mortality and histological findings of the brain during and after cerebral ischemia in male and female spontaneously hypertensive rats. Brain Res. **454:**238-243.
140. **Sakurada, O., C. Kennedy, J. Jehle, J. D. Brown, G. L. Carbin, and L. Sokoloff.** 1978. Measurement of local cerebral flow with iodo[^{14}C]antipyrine. Am. J. Physiol. **234:**H59-H66.

141. **Salford, L. G., F. Plum, and B. K. Siesjo.** 1973. Graded hypoxia-oligemia in rat brain. I. Biochemical alterations and their implications. Arch. Neurol. **29:**227-233.
142. **Salford, L. G., F. Plum, and B. K. Siesjo.** 1973. Graded hypoxia-oligemia in rat brain. II. Neuropathological alterations and their implications. Arch. Neurol. **29:**234-238.
143. **Schaller, B. and R. Graf.** 2004. Cerebral ischemia and reperfusion: the pathophysiologic concept as a basis for clinical therapy. J. Cereb. Blood Flow Metab. **24:**351-371.
144. **Schallert, T., M. T. Woodlee, and S. M. Fleming.** 2003. Experimental focal ischemic injury: behavior-brain interactions and issues of animal handling and housing. ILAR J. **44:**130-143.
145. **Schwarz, D. A., G. Barry, K. B. Mackay, F. Manu, G. S. Naeve, A. M. Vana, G. Verge, P. J. Conlon, A. C. Foster, and R. A. Maki.** 2002. Identification of differentially expressed genes induced by transient ischemic stroke. Brain Res. Mol. Brain Res. **101:**12-22.
146. **Seisjo, B. K., K. Katsura, and T. Kristian.** 1996. Acidosis-related damage. Adv. Neurol. **71:**209-233.
147. **Sharkey, J. and S. P. Butcher.** 1995. Characterization of an experimental model of stroke produced by intracerebral microinjection of endothelin-1 adjacent to the rat middle cerebral artery. J. Neurosci. Methods **60:**125-131.
148. **Smith, M. L., R. N. Auer, and B. K. Siesjo.** 1984. The density and distribution of ischemic brain injury in the rat following 2-10 min of forebrain ischemia. Acta Neuropathol. (Berlin) **64:**319-332.
149. **Smith, M. L., G. Bendek, N. Dahlgreen, I. Rosen, T. Wielock, and B. K. Siesjo.** 1984. Models for studying long-term recovery following forebrain ischemia in the rat. A 2-vessel occlusion model. Acta Neurol. Scand. **69:**385-401.
150. **Smrcka, M., C. S. Ogilvy, R. J. Crow, K. I. Maynard, T. Kawamata, and A. Ames, III.** 1998. Induced hypertension improves regional blood flow and protects against infarction during focal ischemia: time course of changes in blood flow measured by laser Doppler imaging. Neurosurgery **42:**617-624.
151. **Soonthon-Brant, V., P. M. Patel, J. C. Drummond, D. J. Cole, P. J. Kelly, and M. Watson.** 1999. Fentanyl does not increase brain injury after focal cerebral ischemia in rats. Anesth. Analg. **88:**49-55.
152. **Spetzler, R. F., W. R. Selman, P. Weinstein, J. Townsend, M. Mehdorn, D. Telles, R. C. Crumrine, and R. Macko.** 1980. Chronic reversible cerebral ischemia: evaluation of a new baboon model. Neurosurgery **7:**257-261.
153. **Stefanovich, V. (ed.).** 1983. Stroke: animal models. Pergamon Press, Oxford.
154. **Stroke Therapy Academic Industry Roundtable (STAIR).** 1999. Recommendations for standards regarding preclinical neuroprotective and restorative drug development. Stroke **30:**2752-2758.
155. **Sundt, T. M., W. C. Grant, and J. H. Garcia.** 1969. Restoration of middle cerebral artery flow in experimental infarction. J. Neurosurg. **31:**311-322.
156. **Tabuchi, M., K. Umegaki, T. Ito, M. Suzuki, M. Ikeda, and T. Tomita.** 2001. Disturbance of circadian rhythm in heart rate, blood pressure and locomotive activity at the stroke-onset in malignant stroke-prone spontaneously hypertensive rats. Jpn. J. Pharmacol. **85:**197-202.
157. **Takagi, K., W. Zhao, R. Busto, and M. D. Ginsberg.** 1995. Local hemodynamic changes during transient middle cerebral artery occlusion and recirculation in the rat: a [^{14}C]iodoantipyrine autoradiographic study. Brain Res. **691:**160-168.
158. **Takahashi, H., R.J. Traystman, K. Hashimoto, E. D. London, and J. R. Kirsch.** 1997. Postischemic brain injury is affected stereospecifically by pentazocine in rats. Anesth. Analg. **85:**353-357.
159. **Takizawa, R. and A. M. Hakim.** 1991. Animal models of cerebral ischemia. II. Rat models. Cerebrovasc. Dis. **1:**16-21.
160. **Tamura, A., D. I. Graham, J. McCulloch, and G. M. Teasdale.** 1981. Focal cerebral ischemia in the rat. I. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J. Cereb. Blood Flow Metab. **1:**53-60.
161. **Teasell, R. W., J. W. Jutai, S. K. Bhogal, and N. C. Foley.** 2003. Research gaps in stroke rehabilitation. Topics Stroke Rehabil. **10:**59-70.
162. **Thiessen, D. D., G. Lindzey, and H. C. Friend.** 1968. Spontaneous seizures in the Mongolian gerbil, *Meriones unguiculatus*. Psychon. Sci. **11:**227-228.
163. **Toung, T. K., P. D. Hurn, R. J. Traystman, and F. E. Sieber.** 2000. Estrogen decreases infarct size after temporary focal ischemia in a genetic model of type I diabetes mellitus. Stroke **31:**2701-2706.
164. **Traystman, R. J.** 2003. Animal models of focal and global cerebral ischemia. ILAR J. **44:**85-95.
165. **Tsuchiya, D., S. Hong, T. Kayama, S. S. Panter, and P. R. Weinstein.** 2003. Effect of suture size and carotid clip application upon blood flow and infarct volume after permanent and temporary middle cerebral artery occlusion in mice. Brain Res. **970:**131-139.
166. **Tyson, G. W., G. M. Teasdale, D. I. Graham, and J. McCulloch.** 1984. Focal cerebral ischemia in the rat: topography of hemodynamic and histopathological changes. Ann. Neurol. **15:**559-567.
167. **Vannucci, R. C., J. R. Connor, D. T. Mauger, C. Palmer, M. B. Smith, J. Towfighi, and S. J. Vannucci.** 1999. Rat model of perinatal hypoxic-ischemic brain damage. J. Neurosci. Res. **55:**158-163.
168. **Vannucci, S. J., L. B. Willing, S. Goto, N. J. Alkayed, R. M. Brucklacher, T. L. Wood, J. Towfighi, P. D. Hurn, and I. A. Simpson.** 2001. Experimental stroke in the female diabetic, *db*/*db*, mouse. J. Cereb. Blood Flow Metab. **21:**52-60.
169. **Virley, D., S. J. Hadingham, J. C. Roberts, B. Farnfield, H. Elliot, G. Whelan, J. Golder, C. David, A. A. Parsons, and A. J. Hunter.** 2004. A new primate model of focal stroke: endothelin-1-induced middle cerebral artery occlusion and reperfusion in the common marmoset. J. Cereb. Blood Flow Metab. **24:**24-41.
170. **Walker, W. C.** 2004. Pain pathoetiology after TBI: neural and nonneural mechanisms. J. Head Trauma Rehabil. **19:**72-81.
171. **Wass, C. T. and W. L. Lanier.** 1996. Glucose modulation of ischemia brain injury: review and clinical recommendations. Mayo Clin. Proc. **71:**801-812.
172. **Watson, B. D.** 1997. Animal models of photochemically induced brain ischemia and stroke, p. 52-73. *In* M. D. Ginsberg, and J. Bogousslavsky (ed.), Cerebrovascular disease: pathophysiology, diagnosis, and management. Blackwell Science, Oxford, UK.
173. **Weinstein, P. R., G. G. Anderson, and D. A. Telles.** 1986. Neurological deficit and cerebral infarction after temporary middle cerebral artery occlusion in unanesthetized cats. Stroke **17:**318-324.
174. **Working Party of the Association of Veterinary Teachers and Research Workers (AVTRW).** 1986. Guidelines for the recognition of pain in animals. Vet. Rec. **118:**334-338.
175. **Xerri, C., Y. Zennou-Azougui, and J. O. Coq.** 2003. Neuroprotective effects on somatotopic maps resulting from piracetam treatment and environmental enrichment after focal cortical injury. ILAR J. **44:**110-124.
176. **Yamori, Y., R. Horie, H. Handa, M. Sato, and M. Fukase.** 1976. Pathogenetic similarity of strokes in stroke-prone spontaneously hypertensive rats and humans. Stroke **7:**46-53.
177. **Young, A. R., O. Touzani, J. M. Derlon, G. Sette, E. T. MacKensie, and J. C. Baron.** 1997. Early reperfusion in the anesthetized baboon reduces brain damage following middle cerebral artery occlusion: a quantitative analysis of infarction volume. Stroke **28:**632-638.
178. **Zauner, A., R. Bullock, X. Di, and H. F. Young.** 1995. Brain oxygen, CO_2, pH, and temperature monitoring: evaluation in the feline brain. Neurosurgery **37:**1168-1176.
179. **Zhang, R. L., M. Chopp, Z. G. Zhang, Q. Jiang, and J. R. Ewing.** 1997. A rat model of focal embolic cerebral ischemia. Brain Res. **766:**83-92.
180. **Zhang, Z., M. Chopp, R. L. Zhang, and A. Goussev.** 1997. A mouse model of embolic focal cerebral ischemia. J. Cereb. Blood Flow Metab. **17:**1081-1088.
181. **Zhang, Z., R. L. Zhang, Q, Jiang, S. B. Raman, L. Cantwell, and M. Chopp.** 1997. A new rat model of thrombotic focal cerebral ischemia. J. Cereb. Blood Flow Metab. **17:**123-135.