5: Preparing an in-vivo experiment

Rodents were used for the DOT temporal response measurements discussed in this dissertation for the reasons explained in the following chapter. Since surgical preparation of the animals was required, anesthesia was essential. In order to control the anesthetic dosage and maintain a stable physiologic state for these experiments over a period of 8 to 10 hours, custom biomonitoring equipment was required. The delivery of an adjustable electrical stimulus to the forepaw with current stability of better than 5% over 8 hours or more was also required.

Chapter 5 discusses many of the issues involved in the preparation of an in-vivo animal experiment. The topic of anesthesia is discussed in detail, followed by the development and optimization of the custom anesthesia equipment used for these rodent experiments. The importance of biomonitoring during in-vivo experiments is explained, and the design and development of the rodent biomonitoring instrumentation is covered in detail as well. The chapter then concludes with a discussion of the design and construction of the electrical forepaw stimulator.

5.1 Anesthesia

Modern neurophysiological experimentation often requires that the subject be anesthetized in order to both reduce discomfort and eliminate motion artifact. Some diffuse imaging experiments require imaging of intracraneal structures. In order to access the appropriate regions, preparatory surgery (tracheotomy, skull thinning, catheterization, etc.) is required. Stable anesthesia, ventilatory, and thermal support must be provided during the measurements – hence the need for a simple, portable anesthesia "workstation".

Due to the huge amount of literature that exists in this field (and for the sake of brevity), we address issues primarily relating to inhalation anesthesia – specifically involving the use of halocarbon anesthetics. Although injectable anesthetics are very popular and offer certain advantages over inhaled agents, the equipment and administration techniques involved are relatively straightforward and will only be discussed briefly.

5.1.1 Background

A (very) brief history of anesthesia

1100s	Opium, cannabis, mandrake and henbane, carotid artery compression
1540	Paracelsus notes that diethyl ether anesthetizes chickens
1800	Sir Humphrey Davy suggests the use of N_2O for anesthesia
1842	Diethyl ether first used for dental extractions by W. Clarke, but . . .
1846	Morton demonstrates diethyl ether on Oct. 16^{th} at MGH(!) and gets the credit
1847	Chloroform (trichloromethane) used on animals and humans
1862	N_2O gains acceptance for light anesthesia
1864	Trichlorethylene
1875	Chloral hydrate
1878	Cocaine suggested for local anesthesia (already in use for trephination)
1928	Cyclopropane
1930s	Barbiturates
1950	Phenothiazines used as tranquilizers

1956 Halothane
1959 Methoxyflurane
1972 Enflurane
1981 Isoflurane
1993 Desflurane
1995 Sevoflurane

Injectable anesthetics

Injectables comprise the largest class of anesthetics. In fact, most analgesics are also available in injectable form. Many of these drugs are also available in an enteral (ingestible) form, although any drugs administered enterally will suffer the "first pass" effect, as the blood supply from the splanchnic circulation passes through the liver prior to entering the systemic circulation. This gives the liver an opportunity to metabolize the drug on its first pass through that organ – hence the origin of the term. The pharmacodynamics and pharmacokinetics of most injectable anesthetics are well understood.

Barbiturates are effective, safe, and reliable as anesthetics. They provide good anesthesia, but poor analgesia. Their pharmacokinetics can range widely, with durations of action spanning a few minutes (for thiobarbiturates) to hours for long-acting varieties. All barbiturates create a dose-dependent reduction in blood pressure (BP), intracraneal pressure (ICP), CBF, and $CMRO_2$. [The ICP and $CMRO_2$ reducing effects of barbiturates are often used to protect closed-head injury and hemorrhagic stroke victims from the damaging effects of increased ICP and cerebral edema by placing them in a barbiturate coma until the problem can be surgically corrected.]

Opioids provide good analgesia, but are dangerous if used as general anesthetics, since dosages high enough to cause unconsciousness can also cause respiratory arrest. They are most often administered as a premedication prior to surgery, to reduce intraoperative and postoperative pain, and to alleviate anxiety.

Dissociatives do not produce true unconsciousness, but rather create a dissociative, or trancelike, state. They are excellent analgesics, with residual analgesia lasting for days after the initial dose. Dissociatives increase BP, CBF, ICP, and $CMRO_2$. For this reason they are excellent for use with hypovolemic patients, however they should be avoided if cerebral injury is suspected [51].

The **haloalcohols** (chloral hydrate, α-chloralose, and tribromoethanol) are hypnotics which provide moderate analgesia but only minimally effect BP, CBF, and $CMRO_2$. The preservation of cerebral hemodynamics is a feature for DOT and fMRI measurements of cortical function.

Urethane, the common name for ethyl carbamate, provides excellent analgesia and sedation. Like the haloalcohols, it only minimally affects BP, CBF, and $CMRO_2$), however it is a known animal carcinogen and a cancer suspect agent in humans.

Parenteral administration

There are a number of ways in which injectable drugs can be administered. Choosing the proper route of administration is important, since it directly affects the pharmacokinetics.

Intravenous (IV) injection requires direct access to a large collecting vein. It provides the fastest onset of action, but requires the most skill to administer, since vascular access is required. Since pharmacologic effects are often very rapid through the IV route, respiratory arrest can occur without warning, and resuscitation equipment should always be available whenever IV anesthetics are used.

Some drugs - barbiturates, for example - can only be administered intravenously, since they are very corrosive to tissue. Inadvertent extravasation can cause painful ulceration at the injection site. If frequent doses are anticipated, an indwelling catheter can be placed. This reduces the risk of tissue damage from both extravasation and multiple venipunctures, and provides immediate vascular access, should it be required.

Intramuscular (IM) injection provides a slower onset of action and is far easier to administer. IM preparations often contain a digestible oil, such as sesame or soybean oil, to slow absorption. For this reason, IM preparations should never be administered intravenously unless the label specifically states that IV use is acceptable. Vaccines are often given IM, since this route elicits the greatest immune response.

Subcutaneous (SC) injection provides variable absorption, which depends on the degree of perfusion of the local tissues at the injection site. SC injections are easy to administer and are less uncomfortable (for the patient) than IM injections. But due to the uncertainty in absorption, SC is best suited for very long acting drugs, such as urethane.

Intraperitoneal (IP) injection combines relatively rapid and uniform absorption with ease of administration, and is therefore quite popular in small animal surgery. There is always a risk of infection whenever any substance is introduced into the peritoneal cavity, so sterility must be assured [51].

In an ideal world . . .

The ideal general anesthetic would provide strong analgesia to minimize patient discomfort. It would relax all of the skeletal muscle tissue (except that surrounding the glottis to avoid airway obstruction), thus obviating the need for neuromuscular blocking agents like succinylcholine or pancuronium. It would not interfere with respiration or cardiac function, nor would it interact with any other pharmacologic agents used during surgery. If an inhalant, it should be mild and nonirritating to mucous membranes to prevent excessive bronchial secretions and to ease induction. It should either be a gas or a liquid with a boiling point somewhere between 30'C and 50'C to simplify delivery using standard variable-bypass vaporizer technology. Other features would include high potency, rapid induction and recovery, low toxicity, minimal biotransformation or nontoxic metabolites, elimination of post-recovery nausea and emesis, and low cost [51].

Inhalant anesthetics

The inhalant anesthetics evolved from the hydrocarbons (diethyl ether, ethylene, cyclopropane) to the safer and more effective halocarbon agents (halothane, isoflurane, methoxyflurane, enflurane, desflurane, sevoflurane, etc.). Other gases, used as anesthetics or anesthetic adjuncts, include nitrous oxide and xenon. Both are relatively weak agents, but they provide rapid induction and recovery with minimal metabolic degradation. Nitrous oxide, unlike most other inhaled agents, provides analgesia at sub-MAC doses. The pharmacokinetics of inhaled agents are understood, but their pharmacodynamics are still a mystery.

How we think inhalation anesthesia works

The underlying physiological mechanism of inhalation anesthesia is still not well understood, but interestingly the same physical properties which make a good inhalation anesthetic also make a good propellant for canned whipped cream: high solubility in both water and lipids. The current thinking is that a substance which has good fat and water solubility can pass through lipid membranes (i.e. many lipid/aqueous interfaces) and can then interact with the nervous system in some mysterious as-yet-unexplained manner. Note that substances which are soluble in fat alone (*n*-hexane, for example) may have some euphoric effects at low concentrations, but make poor inhalation anesthetics. Diethyl ether, which has high water and lipid solubility, provides only mild euphoria but excellent anesthesia.

Some current hypotheses are [51]:

1) The "lipid" hypothesis, which states that potency is directly related to lipid solubility. Indeed a direct correlation exists between anesthetic potency and O/W coefficient. [Note, however, that it is difficult to isolate this correlation to just the O/W coefficient, since the O/W and B/G coefficients tend to scale together with most agents. Some agents, such as trichlorethylene, exert their effects not

through solubility alone, but also through pharmacologically active metabolites (in this case trichloroethanol) as well.]

2) The "cell permeability" hypothesis, which states that normal ionic fluxes are inhibited through destabilization of cell membranes.

3) The "biochemical/metabolic" hypothesis, in which anesthesia is mediated by the inhibition of oxygen consumption in brain tissue (most relevant to barbiturates and opioids).

4) The "Neurophysiological" hypotheses, advocating inhibition of the reticular activating system through decreased synaptic transmission.

5) The "Physical" hypotheses, which include clathrate formation (a kind of chelation-mediated co-precipitation effect) in the brain. This interferes with neuronal excitability. Thus, anesthetic potency should be a function of the magnitude of Van Der Waals forces.

6) The "Physico-chemical" hypotheses, which postulate some membrane effect. A variant of this is the thought that anesthetics diffuse into the lipid portion of the membranes, causing them to swell, thus compressing ionic channels.

7) The "protein receptor" hypotheses, which favors a specific protein receptor, the nature of which remains to be established.

What seems to be mutually agreed upon is that the depth of anesthesia is a function of the partial pressure of the agent within the brain. Empirical measurements seem to show that the depth of anesthesia is directly proportional to the partial pressure of anesthetic agent dissolved in the brain tissue. This means that an anesthetic agent which has both a high blood/gas partition coefficient (high water solubility) and a high oil/water partition coefficient (high lipid solubility) will lead to a high partial pressure within the lipid-rich neural tissue in the brain. Unfortunately this potency comes at a price. Since there are large fat stores in the form of lipid-rich organs and adipose tissue throughout the bodies of most creatures (and most of us too), potent anesthetics also diffuse into these lipid-rich regions as well. This lipid uptake occurs mostly during induction, at the same time that the agent is also crossing the blood-brain barrier, so a temporary dilution ("lipid-steal") occurs, and the partial pressure of the anesthetic in the brain rises slowly with time, leading to slower induction.

Recovery is also delayed, since once the anesthetic concentration is lowered, the adipose tissue begins to release its sizable stores of anesthetic in the same fashion. Some agents, such as nitrous oxide, can exit the lungs in such high concentrations that they actually dilute the alveolar pO_2, leading to a potential disorder called **diffusion hypoxia**. The simplest way to avoid diffusion hypoxia upon recovery is to keep the patient on pure oxygen for a few minutes, until most of the nitrous oxide has been purged from the body. The **second gas effect** is a complementary process seen during induction, when the presence of a soluble gas such as N_2O can actually enhance the uptake rate of a volatile agent by diffusing into the blood quickly. This concentrates the volatile agent remaining within the alveoli, increasing its partial pressure and thus speeding its uptake.

Blood circulation has a somewhat counterintuitive effect on the induction rate. If the cardiac output is high (say, in an anxious patient) then the anesthetic ***concentration*** in the blood remains low (because the volume of blood passing through the alveoli per unit time is large, but the gas influx rate through respiration is relatively constant). So the partial pressure of the agent within the arterial blood entering the systemic circulation remains low, and thus the blood/brain concentration gradient remains low as well, delaying induction (per Fick's Law of Diffusion). This means that it is beneficial to premedicate a frightened patient (Propofol, Pentothal, Valium, etc.) before attempting induction with slower agents like ether or Methoxyflurane. Likewise, patients with compromised circulation (those in shock or with significant blood loss) will go deep quickly, and anesthetic overdose with cessation of respiration is possible.

In cases involving short or simple medical procedures, the advantages offered by faster induction and recovery offset the disadvantage of reduced potency and higher cost, and so a number of less potent anesthetics have been developed. Isoflurane and Sevoflurane are good examples. The MAC for Isoflurane is around 1.6% in O_2 (as compared to Halothane at 0.8%), but it provides rapid induction and recovery with minimal biotransformation. This is a result of its chemical configuration. The Isoflurane molecule consists of a methyl-ethyl ether backbone to with halogens are substituted in the appropriate locations to strike a good balance between solubility, vapor pressure, bioinertness, and (with the presence of two easily cleaved hydrogen atoms) ozone-friendliness. The highly polar oxygen atom in the center of the molecule along with the highly electrophilic fluorine atoms at both ends provides a sterically broad polar influence. This reduces lipid solubility and serves to keep the blood/air and oil/water partition coefficients low. Sevoflurane is similar to Isoflurane, but it is far less irritating during induction and so is better for use with anxious humans and children, who would likely choke if induced with Isoflurane alone.

Compare this to Halothane, which consists of a simple ethane backbone to which bromine, chlorine, and fluorine have been added. Note the distinct polar and nonpolar ends to the molecule (small as it is). Since no oxygen is present and the chloro-, bromo-, and hydro- moities are more lipophilic (in that order) than the fluoro-, Halothane has much larger B/G and O/W partition coefficients. Also, without the convenience of the larger mass of the bromine and chlorine atoms, the vapor pressure would be too high to use in standard variable-bypass vaporizers. Desflurane, another less-potent anesthetic agent, has a boiling point just barely above room temperature, and so requires the use of a unique (and expensive) flow, pressure, and temperature-controlled vaporizer.

Important properties of volatile anesthetic agents [51]

COMPOUND	MAC	Blood/Gas Partition Coefficient at 37'C	Oil/Water Partition Coefficient at 37'C	Boiling Point at 760mmHg	Vapor Pressure @20'C	Equilibrium Concentration in Air @20'C
Halothane	0.8%	2.5	330	49-51'C	243mmHg	32%
Isoflurane	1.3-1.6	1.43	170	48.5	239	31
Methoxyflurane	0.23	13	400	104.7	24	3.5
Enflurane	2.06	1.91	134	56.5	180	24
Desflurane	7.2	0.42	19	23.5	644	87
Sevoflurane	2.4	0.68	130	59	160	21
Nitrous Oxide	200(!)	0.46	3.3	-89.5	750psi	100%+

Volatile agents used in veterinary medicine

Halothane, Isoflurane and Nitrous Oxide are the most common agents used in veterinary inhalation anesthesia today.

Halothane is 2-bromo-2-chloro-1,1,1-trifluoroethane. It is a pleasant smelling agent and does not induce bronchospasm or stimulate excessive bronchial secretions. Analgesia, though weak, is better than with Isoflurane, although muscular relaxation is poorer. It is somewhat photosensitive, and is preserved with 100ppm of thymol, which contributes a mild but distinctive "phenolic" scent. It does not decompose in contact with warm soda-lime. There is a dose-dependent depression of the cardiopulmonary system. Blood pressure is lowered through both direct myocardial depression and through vasodilation. Halothane is a direct cerebral vasodilator and depresses the autoregulation of cerebral blood flow (which creates a significant confound with any hemodynamic measurements). Cerebral blood flow increases in direct proportion to increasing dose. This leads to an increase in intracraneal pressure as well. It also sensitizes the myocardium to catecholamines, so induction of frightened animals or people should be avoided without premedication. About 20% to 30% of the Halothane administered is metabolized in the liver, and metabolites include trifluoroacetic acid and

bromide ions, but few free fluoride ions, so renal toxicity is low (unlike Methoxyflurane). Halothane can cause a rare, acute, necrotic liver disorder in about 1 in every 30,000 adults, which has been nicknamed "Halothane hepatitis." The etiology is not well understood, although it is thought to be the result of an autoimmune reaction. Halothane is also considered to be the most potent trigger of malignant hyperthermia.

Isoflurane is 1-chloro-2,2,2-trifluoroethyl difluoromethyl ether. The ether linkage introduces a polarity to the molecule, rendering it more hydrophilic than Halothane (this can be seen from the lower B/G and O/W values). This raises the MAC by a factor of two, but it also shortens induction and recovery times. It is stable and inert to soda-lime. Isoflurane has a rather pungent odor, which can stimulate bronchial secretions and make induction somewhat difficult in conscious patients. It causes less cardiac sensitization than Halothane and provides better muscle relaxation, but poorer analgesia. Cardiovascular depression is dose-dependent, and results from a decrease in total peripheral resistance rather than by direct myocardial depression as with Halothane, although it has less of an inhibiting effect on cerebral autoregulation than Halothane. It is a stronger respiratory depressant, producing more profound hypoventilation and hypercapnia, so mechanical ventilation is recommended. The terminal CF_3 group confers great stability to the molecule, so only 0.2% is metabolized in the liver.

Nitrous oxide is a colorless, relatively insoluble, sweet-smelling gas. It is stored as a liquid (below its critical temperature) under a pressure of about 750 psi. The MAC in humans is around 100% and for animals it is closer to 200%, which means that it is never used as the sole anesthetic agent, but rather as an adjunct, to potentiate the effects of other agents and to provide analgesia. It speeds induction through the "second gas" effect, but this can also lead to diffusion hypoxia upon recovery, so patients should be ventilated with pure oxygen for a few minutes until most of the N_2O has left the body. It should always be used with a <u>minimum</u> of 30% oxygen to prevent hypoxia. As a result of its rapid mobility, N_2O should not be used in rebreathing systems, since a slight equilibrium shift can result in the equivalent of diffusion hypoxia within the breathing circuit, leading to the formation of a hypoxic gas mix.

N_2O has minimal cardiopulmonary effects, with a mild increase in sympathetic tone and no muscle relaxation. N_2O will diffuse into closed gas spaces within the body, which can sometimes present a problem during abdominal surgery (gas pockets within the gut swell up, making it difficult to operate). N_2O should never be used on patients with pneumothorax or abdominal obstruction for this reason [51].

Note that the halocarbons are very similar to other halogenated solvents such as chloroform and Freon TF in that they are powerful defatting and degreasing agents. They will rapidly soften or "fog" most non-crosslinked plastics. High vapor concentrations will leach the plasticizer from vinyl tubing, leaving it stiff and brittle. [Thus, when defeating the key-fill feature on your vaporizer while attempting to refill it, be sure to use PE, TFE, or PTFE (Teflon) tubing.] This is important because the phthalate plasticizers in vinyl have a very low vapor pressure and will accumulate in the vaporizer wick. The affinity of the anesthetic for these lipid-like compounds will reduce its vapor pressure (as seen from the O/W coefficient), gradually reducing the delivered concentration below that indicated by the dial setting on the vaporizer.

Pathophysiological Consequences of Anesthesia

Besides providing analgesia, muscle relaxation, and unconsciousness, most inhalation anesthetics suppress a number of normal homeostatic processes. These are things which your body does automatically, such as maintaining a stable body temperature (thermoregulation), controlling breathing rate and blood flow (respiratory and cardiac regulation), controlling the blood pressure throughout the body by controlling local vasomotor tone (hemodynamic autoregulation), etc. Note that many of these mechanisms are interrelated. For example, once vasomotor tone is altered, the blood flow to various

organs, including the brain, may increase or decrease. In conscious mammals, perfusion of both somatic and lung tissue is controlled locally through a vasoactive process called hemodynamic autoregulation. An excess of CO_2 or hydrogen ions in the extracellular fluid reduces its pH, stimulating the release of vasoactive substances such as nitric oxide, which cause the smooth muscles in the walls of the local arterioles to relax. This leads to vasodilation in somatic tissue, with a subsequent increase in blood flow to flush out the excess of CO_2 and return the local pH to normal. In the lungs, a complementary process – pulmonary hypoxic vasoconstriction – limits blood flow to poorly ventilated regions of the lung. This serves to keep the pO_2 as high as possible, since perfusing the unventilated alveoli would dilute the oxygen-rich blood from the rest of the lung, reducing oxygen delivery per Fick's Law.

In anesthetized mammals, this closed-loop vasoactive control is strongly suppressed. This interferes with hemodynamic autoregulation, vasoconstrictive thermoregulation, and both respiratory and metabolic pH regulation. As a consequence, tissue perfusion becomes blood pressure-dependent, so the V-Q mismatch grows and the body temperature falls (due to peripheral vasodilation combined with evaporative heat loss through the lungs – at least in nonrebreathing systems) without a compensatory increase in metabolic activity, since that too is inhibited under general anesthesia (except during a rare pathologic condition known as "malignant hyperthermia", which can be fatal if not treated quickly). Since pCO_2 sensitivity is inhibited, spontaneous breathing will be insufficient to maintain normocarbia, and mild to severe hypercarbia and hypoxia can develop. Persistent hypercarbia will eventually exceed the capacity of the bicarbonate and phosphate buffer systems, resulting in both respiratory and metabolic acidosis. An example of the effects of various anesthetics on CBF and $CMRO_2$ is shown in Figure 5.1.

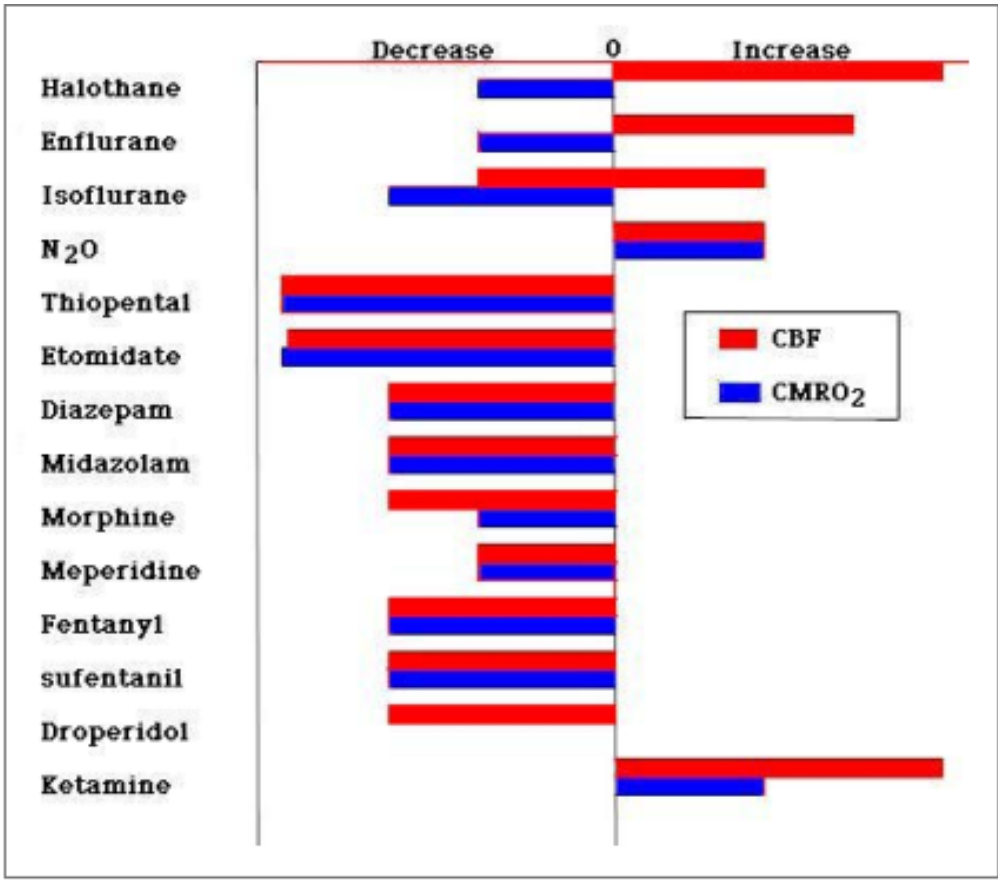

Figure 5.1. The effects of various anesthetic agents on cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen consumption ($CMRO_2$). Most common anesthetics significantly affect cerebral hemodynamics, which is why hypnotics such as α-chloralose or toxic anesthetics like urethane are preferred for DOT and fMRI measurements of brain function.

Overdosage of most inhalation anesthetics (except N_2O, which acts as a simple asphyxiant) causes respiratory arrest at a concentration of about 2.4 MAC. This means that the "therapeutic window" (the dynamic range, for drugs) is around 3:1. Most inhalation anesthesia is carried out at between 1.4 and 2.0 MAC with vapor alone. The addition of injectable agents (ketamine, fentanyl, propofol) can provide enough analgesia to extend this range down to 0.5 MAC or below [51].

The need for physiologic feedback during anesthesia

The two major physiologic systems which influence the delivery of volatile anesthetics to the brain tissue are the respiratory system and the cardiovascular system. Since these two systems are series-connected, it is important that the functioning of each system be closely monitored during all stages of anesthesia. In order to provide feedback about the status of both systems, a number of physiological parameters should be monitored throughout the course of anesthesia. Some of these are discussed in detail below.

Respiration parameters: This is very important with free-breathing anesthesia since all volatile agents depress natural respiration. The more potent the agent, the greater the risk of apnea and respiratory arrest. If the gas mix contains a high concentration of oxygen, the vascular oxygen reserve may permit short periods of apnea without harm. If air alone is used, some degree of hypoxia is already present (due to both respiratory depression combined with a significant V-Q mismatch), so even short periods of apnea or impaired ventilation from increased tracheal or bronchial resistance can lead to brain damage, cardiac dysrhythmia, and death. Therefore, respiratory monitoring during free-breathing anesthesia is vital.

Respiration monitoring devices range from simple (inexpensive) optical or strain gauge-based chest wall expansion sensing to complex (costly) end-tidal oximeter/capnometer systems which monitor the final portion of the exhaled breath (which spends the longest time in the alveoli and so best represents the alveolar gases. It contains the most CO_2 and the least O_2). For rodent monitoring, respiration rate sensing is easier to arrange, since their small (few cc) tidal volumes make accurate end-tidal gas measurement difficult.

Blood gas measurement: Since end-tidal gas measurement can be difficult to perform on rodents with accuracy, a useful substitute is arterial blood gas measurement. This requires the placement of an indwelling arterial catheter to allow periodic blood samples to be withdrawn. The disadvantages of arterial blood gas sensing are the limited rate of measurement, bounded on the high end by the need to prevent hypovolemia and hemodilution through repeated withdrawal of blood, and on the low end by the need for an update frequency sufficient to allow the anesthetist to react to physiological changes in a reasonable time.

The important parameters in a blood gas measurement are:

Plasma pH, which indicates the degree of metabolic acidosis (during anesthesia with spontaneous ventilation, acidosis is the norm, however with mechanical ventilation alkalosis can also occur). It also serves as a rough measure of how long uncorrected respiratory acidosis has existed. Normal arterial pH should be around 7.40. A pH below 7.35 or above 7.45 would suggest acidosis or alkalosis respectively, and the ventilation minute rate (total breath volume per minute) should be increased or decreased as needed. Bicarbonate replacement is rarely needed during short surgical procedures.

Oxygen tension (pO_2) indicates whether sufficient oxygen is present in the blood to prevent global hypoxia. Note that regions of tissue with compromised perfusion may become hypoxic, despite an adequate pO_2. An oxygen tension of between 90mm and 100mmHg indicates normoxia. Values well above 100mm generally do no harm for short (few hour) periods, and are considered beneficial. High oxygen tensions (hyperoxia) provide a temporary "oxygen surplus", which can hold a patient over during small ventilatory or circulatory mishaps (removal of tracheal tube, hose disconnection, empty O_2 tank, cardiac arrhythmia, etc.). A pO_2 value below 90mm indicates some degree of hypoxia, which should be corrected quickly.

CO_2 tension (pCO_2) directly indicates the degree of respiratory acidosis (through hypercapnia) or alkalosis (hypocapnia) present. During free-breathing inhalation anesthesia, some hypercapnia and respiratory acidosis is expected. ***Hypo***capnia in a free-breathing patient is a result of hyperventilation, and indicates either a serious problem (inadequate anesthesia, metabolic acidosis) or an inaccurate blood gas measurement. With mechanical ventilation, changes in minute rate or anesthetic dosage can lead to either acidosis or alkalosis, and the simple fix is to vary either the breathing rate or the tidal volume.

Cardiovascular parameters: The simplest way to monitor heartrate is with either a pulse oximeter or an ECG system. Blood pressure can be measured either through sphygmomanometric devices (tail cuffs) or more accurately through a pressure transducer connected to an indwelling arterial catheter.

Pulse oximetry provides both pulse rate and blood oxygen saturation level. Normal arterial HbO_2 saturation (oxy sat) levels are between 95% and 100%. An oxy sat level below 80% indicates a problem which should be dealt with, and a subsequent arterial blood gas measurement should be made to confirm normoxia.

ECG systems are simple to use, and they provide vital information about the health and functioning of the myocardium as well as a general measure of perfusion. ECG can only measure the electrical activity of the myocardium, not cardiac output, so the presence of a normal sinus rhythm does not imply proper ventricular function.

Arterial pressure sensing provides real-time measurement of heartrate and arterial blood pressure, from which cardiac output can be inferred. It provides the fastest and most accurate measure of cardiovascular performance. Proper operation involves periodic catheter flushing and zeroing to maintain accuracy.

Core temperature: Thermal maintenance is critical for any anesthesia lasting more than about 30 minutes. Rodents have a large surface-to-volume ratio, and the combination of evaporative, radiative and convective cooling can lead to a rapid drop in core temperature. If the core temperature drops below 35'C, the risk of cardiac dysrhythmia and arrest increases significantly. Core temperatures above 38'C cause hyperthermia, with an increased metabolic demand for oxygen and the possibility of sudden cardiac arrest.

The best approach to thermal management involves rectal insertion of a temperature probe connected to a heating blanket driven by an electronic controller. The temperature setpoint (typically between 35'C and 37.5C) is maintained through a combination of internal metabolic processes and external heating. Since the heat balance for most anesthetized animals is usually (but not always) negative, some degree of external heating is required to maintain core body temperature. In some cases, small animals exposed to high-power surgical illuminators can actually gain enough heat through optical absorption alone to become hyperthermic! An "overtemp" alarm is strongly recommended.

In summary, what this all means is that during general anesthesia, the anesthetist (you!) must monitor the respiration, heart rate, MAP, body temp, pH, and blood gases – and take whatever steps

are necessary to keep all of them within their normal physiologic ranges. In some cases, though, this control can be used to advantage (i.e. the induction of hypothermia to reduce tissue oxygen demand during certain surgical procedures, or hypocapnia to facilitate mechanical ventilation, etc.)

Medical concerns with volatile agents

Medical issues to be aware of include the potential for:

- Cardiac hypersensitivity to catecholamines (epinephrine and norepinephrine – both exogenous and endogenous!).
- Allergic hepatotoxicity ("Halothane hepatitis"), potentiation of certain drugs, including nondepolarizing muscle relaxants (Pancuronium, Rocuronium, Vecuronium, Tubocurarine, etc.)
- Malignant hyperthermia.

Although some agents (specifically Halothane) pose more of a hazard in this regard than others, it is wise to become familiar with the appropriate emergency procedures, in case they occur.

Other issues with volatile agents

- Flammability (ether, cyclopropane, ethylene)
- Drug potentiation (many halocarbons: with catecholamines, atropine, nondepolarizing paralytics)
- Chronic and acute toxicity (TCE, chloroform, Methoxyflurane, N_2O, Halothane)
- Cost and availability (Desflurane, xenon)
- Bronchial irritation (ether, Isoflurane)
- CO formation w/ desiccated soda-lime (many of the halocarbons)
- Malignant hyperthermia (primarily the halocarbons)
- Nausea and vomiting upon recovery (most anesthetics)
- Diminished mental capacity for 24-48h post-recovery (most anesthetics)
- Depressed homeostatic mechanisms (respiration, thermoregulation, acid-base equilibrium, etc.)

PROBLEM	EMERGENCY PROCEDURE
Cardiac Hypersensitivity (common)	Avoid induction of frightened rodents without premedication. Use epinephrine sparingly, if at all.
Allergic Hepatotoxicity (rare)	Halothane "tolerance testing". Use other anesthetics in patients showing signs of hepatotoxicity following past procedures
Drug Potentiation (common)	Reduce dosages of muscle relaxants accordingly.
Malignant Hyperthermia (rare)	Immediate administration of Dantrolene, aggressive attempts to reduce body temperature, respiratory and circulatory support (as needed), and maintenance of normal electrolytes and proper pH balance.
Respiratory arrest (due to overdosage)	Oxygen flush, ventilate mechanically until spontaneous respiration returns.

Some halocarbon anesthetics can react with the strongly alkaline material in the CO_2 absorber canisters if they become desiccated (which is usually rare, since they are formulated to be hygroscopic) to form small amounts of carbon monoxide. Although the patient's COHb concentration rarely reaches 30%, it further reduces the oxygen carrying capacity of the blood when perfusion may already be compromised, and tissue damage can occur through carboxemic hypoxia [51].

5.1.2 Hardware design and development

The anesthesia equipment sold by vendors such as Harvard Apparatus, Vetequip, and Kent Scientific is designed primarily for conventional veterinary use (i.e. for surgery on cats and dogs), and is therefore not optimized for small animals and rodents. They distribute full-size anesthetic vaporizers and rodent ventilator systems which were never designed to interface with each other. DOT measurements require a more stable and precise anesthetic and ventilatory environment than is required for surgery, since the goal is to provide as normal and stable a hemodynamic state as possible. Commercial rodent ventilators are not designed to provide this level of performance.

Since no commercial vendor offers a veterinary anesthesia system suitable for both surgery and experimentation, an integrated ventilator/anesthesia system was developed to meet this need. This section discusses the design and development of a self-contained anesthetic delivery system for use with rats or other small mammals weighing less than 500g. It contains a miniaturized vaporizer equipped with a real-time anesthetic concentration monitor and a complete time/demand-triggered pressure-cycled ventilator. It can accept oxygen or O_2/N_2O gas mix from any regulated source, or it can function on room air, if necessary.

Design of the anesthetic vaporizer

A number of different techniques were evaluated, mostly in concept, prior to selecting the saturated vapor dilution approach. A discussion of these alternate techniques is included in the Appendix.

Saturated vapor dilution involves splitting up the incoming gas stream into two paths and recombining them downstream of the vaporizer: The main gas path continues uninterrupted, while the "vapor" path passes through an ambient temperature vaporizer and throttle valve. The throttle valve varies the flow resistance of the vapor path, and so determines the relative blend ratio between the pure gas and the saturated vapor/gas mixture.

Advantages: Simple to design and build, can be constructed with common parts, vapor concentration is gas flow-independent, electronic concentration control possible (with a duty-cycle modulated solenoid throttle valve), predictable and linear vapor concentration vs. gas blend ratio (solenoid duty cycle), near-zero power dissipation (need to power solenoid valve).

Disadvantages: anesthetic concentration is room temperature-dependent, not fail-safe (solenoid latchup could generate a lethal anesthetic concentration).

The saturated vapor dilution approach offered many advantages and was easy to construct. The prototype proved to be very practical and effective. It consisted of a small Nalgene (LDPE) solvent delivery squeeze bottle container fitted with a hermetic screwtop cap. The existing spout was removed and the remaining hole was threaded and chamfered to accommodate a #10-32 tubulated fitting to serve as the exhaust port. Another hole was drilled and tapped in the cap to accommodate the gas entrance port.

Inside the container, a short length of (de)plasticized vinyl tubing was secured to the internal tubulation on the intake port and was bent in an attempt to create a cyclonic gas flow. This was done to boost the evaporation efficiency over that of a noncyclonic "straight-through" gas flow design. [I am using the term "evaporation efficiency" to mean the percent ratio of the actual delivered agent vapor concentration to its theoretical saturated vapor concentration in the gas mix at the same exit

temperature]. Although the walls of the container are vertical, the exhausted air is forced out from the center of the container. Because angular momentum must be conserved, any kinetic energy not expended through frictional or thermal (evaporative cooling) losses will manifest itself through cyclonic gas flow near the exhaust tube.

Since this gas circulates directly above the agent sump, the evaporation efficiency is expected to be quite high. A piece of white unscented toilet paper (Scott Paper Products) was used as the wick, and was cut to provide a small exposed portion of the container wall for use as an agent level view port. The molded plastic diptube was shortened by 1cm and two small "safety" holes were drilled about 1cm above this point to prevent a hydraulic plug from developing if too much agent was inadvertently introduced into the container. Although these holes were feared to reduce evaporation efficiency, testing did not show this. No change in agent concentration was noticed at the same duty cycle after these holes were made.

The only obvious disadvantage noted was the transient venting of some saturated vapor into the gas stream when the supply pressure was reduced (This "pumping" effect is also seen with commercial variable-bypass vaporizers as well). Since the solenoid throttle valve was placed ahead of the vaporizer (to keep the agent vapor from swelling the rubber valve disk and O-Ring), a reduction in feedline pressure allowed some saturated vapor to escape from the vaporizer housing and enter the gas flow. The simplest fix for this would be to place the solenoid valve at the output of the vaporizer and to introduce a length of tubing at the fresh gas input to act as an accumulator to accommodate the small outflow of saturated vapor. Since this was a transitory effect and it only occurred during changes in supply pressure, it was judged not to be serious enough to fix.

Controlling the saturated vapor flow

The vapor/gas blending was performed in different ways in both instruments. Mechanical valves are compact, simple, and rugged, and a mechanical needle valve was used in the initial prototype version. Unfortunately it was discovered that mechanical valves are imprecise at low flow rates and at low differential pressures since they become plugged with accreted debris or wetted with plasticizer eluted from the vinyl tubing, nor can mechanical valves be electronically controlled.

Solenoid valves can be electronically controlled, and some valves can toggle flow very quickly (<10ms FWHM). Solenoid valves are generally "digital" in nature because the armature motion creates a variable reluctance, which generates magnetic hysteresis. The hysteresis acts to force the valve into either the "on" or "off" states. So flow control must be achieved through duty cycle modulation.

A Clippard EV-2 solenoid valve (Clippard, Inc., Cincinnati, OH) was chosen due to its high throttle speed and its availability. Since the flow aperture was too small (~800um), it acted like a constant-flow choke at nominal (~100ml/min) flow rates, so it was drilled out to double its bore to solve this problem. Luckily there was enough compliance in the neoprene seal to cover the machining scars and a large enough throw to correct for the additional ~100um of distance needed to reach the "new" valve seat location. The spacer shim was left in place to preserve as much on-state clearance as possible. Luckily this did not significantly increase the pull-in voltage, which still remained around 8V. The following gas flow and vapor mix ratios were used to size the gas handling components:

- Typical average flow rate for a 400g rat: 60BPM, 5cc/breath = 300cc/min.
- Worst case high: 80BPM, 10cc/breath = 800cc/min
- Worst case low: 20BPM, 3cc/breath = 60cc/min
- Agent vapor concentration range: 0 to 5% in air, O_2, O_2/N_2O

Design of the ventilator

Modern positive-pressure ventilator designs are either of the pressure-controlled or volume-controlled variety:

Pressure-controlled ventilator: Use solenoid valves to gate the flow of pressurized anesthetic gas mix from a reservoir into the patient's airway until the proximal airway pressure reaches the inspiration setpoint. Then vent the airway to the exhaust port until the airway pressure reaches the expiration (or optional PEEP) setpoint. All setpoints are controlled electronically.

GOOD POINTS: Easy to design and build using common parts, relatively compact and lightweight, low risk of barotrauma, allows special features like PEEP and CPAP, periodic sigh or sigh on command, demand (patient-initiated inspiration) mode, variable (pressure-triggered) or fixed (time-triggered) inspiration/expiration, direct electronic control of respiration, sigh, insp. and PEEP setpoints, etc.

BAD POINTS: Gas flow to lungs is more abrupt than volume-controlled design and can contain pressure fluctuations introduced by the reciprocating pump (if used).

Volume-controlled ventilator: Basically a variable-displacement pump with mechanical valving to control the direction of gas flow (think of a bicycle pump – but one designed to supply fresh air to the patient during each downstroke and then vent the patients lungs to ambient during each upstroke). Force a preset volume of gas mix into the patient's lungs, then vent to exhaust port. Inspiration/expiration ratio adjustable manually (on standard designs, although this could be variable in a stepper motor-driven system).

GOOD POINTS: Rugged and generally very reliable, mechanically simpler design (no compressor needed), smoother gas flow to lungs,

BAD POINTS: Risk of barotrauma if exhaust port becomes plugged for any reason, no sigh or PEEP feature, hard to build – requires custom-designed mechanical components, wear issues with piston seal.

Basically there was no clear advantage to attempting a volume-controlled design, and the option to include useful features like sigh, PEEP, pressure or time-triggering, etc. seemed appealing in light of the direct hemodynamic modulation from positive-pressure ventilation. This way we can control and/or record the inspiration rate and max. and min. tracheal pressures, arrest breathing at any point in the ventilation cycle, apply and maintain a fixed lung pressure, etc.

Since there were resource constraints for the prototype version, I had to use a large displacement reciprocating air pump (model UN05 AVI, KNF Neuberger, Princeton, NJ). This meant that the output would be pulsatile – much like a rectified AC power supply. In an effort to attenuate these pressure fluctuations, I included a two-stage pneumatic filter. The first stage consisted of a relatively stiff-walled polyethylene squeeze bottle as the high-pressure accumulator (the pneumatic capacitor) followed by a manually adjustable needle valve (the resistor). The goal here was to operate the pump at a relatively high pressure (so that the gas contained in each stroke comprised only a small fraction of the total mass of gas contained within the accumulator) and throttle the needle valve down (to increase the series resistance). This both increases the pneumatic time constant, and it forces the first stage to appear more like a current source: a high voltage [pressure] followed by a large resistance [small valve opening], so that slight pressure variations downstream will have little effect on the actual gas flow rate - and thus on the "minute ventilation" - through the patient's lungs.

The second filter stage would be directly connected to the patient's lungs during inspiration, and so had to store a moderate volume of gas at a safe low pressure – on the order of 50cm H_2O column. This was achieved by using a very flexible accumulator (a large red unvulcanized latex party balloon, later changed to green when the red one developed a leak). One of the unique features of elastomers is the extremely low and nonlinear stress/strain (force/displacement) coefficient. What this means is that it is initially quite easy to inflate a balloon (very low pressure). Once the loose balloon form is filled and elastic deformation begins to occur, it quickly becomes more difficult to inflate (the pressure

begins to rise). As the radius increases (and the balloon is thin enough) , a peak pressure point is crossed (a local maximum, an inflection point, on the pressure/volume curve) and the balloon then becomes progressively easier to inflate (reducing pressure) until an abrupt endpoint is reached at which the polymer chains in the latex are maximally extended. Further inflation causes the pressure to rise rapidly until rupture occurs. [This inflection point is due to an interesting consequence of LaPlace's Law, which states that the pressure within a bubble, balloon, alveolus, etc., is directly proportional to the surface tension and inversely proportional to the radius of curvature. Thus the highest pressure occurs at the smallest radius. This is why collapsed lungs do not readily reinflate and why thin-walled balloons are easier to inflate as they grow larger. Thick-walled bladders may not show this inflection point, since the stress/strain coefficient may be too large and the maximal extension point will be reached too early.]

My goal was to operate close to (but safely below) the inflection point on the pressure/volume curve. This would provide a nearly perfect "infinite" reservoir – the volume could change at a nearly constant pressure. A simple manometer measurement performed on a similar red balloon yielded an inflection pressure of around 75cm H_2O, which was perfect. One hazard with unvulcanized latex is an annoying feature of plastic deformation called elastic hysteresis: once inflated, the pressure/volume curve is permanently shifted to the left (which is why a balloon is always easier to inflate the second time).

So as long as the supply and outflow rates to this balloon accumulator were carefully controlled, this would work fine – but that never happens in real life! As a protective measure, an "inflection protection" switch (actually the "1" key from an old computer keyboard) was mounted above the balloon so that overinflation would close the keyswitch, reducing the drive current to the compressor – and thus the supply pressure – to a safe "idling" level. The actual ventilation pressure would then be controlled by a combination of compressor speed and throttle valve setting. This provides the flexibility of trading gas throughput for pressure stability. So during chamber and mask induction, where a high flow rate is required, the throttle valve can be opened fully to provide flow rates exceeding 3lpm. Mechanically ventilated anesthesia through a tracheal tube would only require around 0.3lpm, so the throttle valve could be partially closed to provide a more stable gas flow.

The inspiration and expiration control was performed with two Clippard EV-2 solenoid valves in combination with the throttle valve. Since a pressure sensor was not available at this point in the design, simple duration controls were used – one knob controlled the "on" time of each solenoid. Thus the inspiration time and expiration time were completely independent, allowing for separate control over tidal volume (through adjustment of the inspiration time) and respiration rate (through adjustment of the expiration time). The inspiration rate could be controlled by varying the balloon pressure with the throttle valve, with due diligence exercised at high rates to guard against barotrauma. The option of "frequency" and "duty cycle" controls was also considered, but was judged to be impractical, since varying the respiration rate with the "frequency" control would have also varied the tidal volume as well, requiring compensatory adjustment of the "duty cycle" knob to reestablish the tidal volume.

Unfortunately the prototype system had no means of monitoring peak airway pressure, and one rodent was lost, likely due to tension pneumothorax secondary to barotrauma. When a pressure sensor later became available, the option of true pressure-controlled ventilation became possible, and was integrated into the next version. In the second version, the "inspiration" knob controlled the peak tracheal pressure, the "expiration" knob controlled the minimum (or PEEP) pressure, and the inspiration rate controlled the throttle valve. Since the peak pressure was actively controlled, the risk of barotrauma was substantially reduced, and no additional cases of barotrauma-related injury occurred while using the second version.

Design of the anesthetic concentration monitor

Since the anesthetic vapor concentration produced by saturated vapor dilution is a function of both the gas blending ratio and the vapor pressure of the anesthetic, some direct means of monitoring the relative (or better yet, absolute) vapor concentration is required. Here are the requirements for a suitable agent concentration monitor:

1) **Vapor Concentration Range:** Since most halocarbon anesthesia is performed at or below a vapor concentration of 5% by volume, a measurement range 0-5% is desired.

2) **Linearity, Resolution, Accuracy, Stability, Hysteresis, Response Time:** For anesthesia monitoring, what matters most is the ability to track ***relative*** changes in anesthetic concentration during the course of a single surgical procedure. Since pharmacologic sensitivity can vary widely between patients (or even with the same patient under different physiological conditions), linearity, resolution, and absolute accuracy of +/-10% FS is probably adequate, although the short-term (<4 hour) stability and hysteresis should all fall within +/-5%, if possible, since these could affect the measured concentration value during a procedure. The output should settle to +/-10% of the final value within 20 seconds or less, and the droop should be less than 10% FS over a period of at least an hour.

3) **Agent Selectivity:** Since the gas composition will be well-known, selectivity (the ability to measure the concentration of one agent while ignoring another) can be nonexistent, so long as the sensor can accurately distinguish the halocarbon vapor from the carrier gas (0_2) or gas mix (air). Actually, a 100% cross-sensitivity to both Halothane and Isoflurane would be very helpful indeed, since each may be used for different reasons.

4) **Agent selection:** The two agents most commonly used in our lab are Halothane and Isoflurane. The sensor should respond to both of these agents in a stable and reproducible fashion. Since the agent in use will be known, a conversion factor (or a "HAL / ISO" toggle switch) can be used to select the appropriate sensor gain.

A number of different techniques were evaluated, mostly in concept, prior to selecting the acoustic velocity modulation approach. These alternate techniques are discussed in Chapter 9.

The acoustic velocity modulation sensor uses an ultrasonic transducer to pass a CW acoustic wave through an anechoic gas cell and detect the acoustic signal with another transducer. The increase in transit time is measured as a phase shift between the generated and detected waveforms. Since the time delay/phase shift increase should be linearly proportional to the halocarbon concentration-pathlength product, the acoustic frequency, pathlength, and amplitude should determine the sensitivity (through zero-crossing uncertainty) and the frequency and pathlength should determine the maximum unambiguous concentration (360' = 0'). Since most piezo transducers are designed to be operated as high-Q resonators for maximum efficiency, any oscillator drift or instability may translate into an apparent concentration drift thorough the electrical/acoustic phase shift within the transducers. Acoustic cavity resonances should also be well damped for similar reasons: as the concentration changes, the cavity resonances will change as well, leading to unpredictable phase fluctuations. These sharp resonances then translate into localized "kinks" in the concentration/output curve (this was noted during development). Since cavity length should directly affect sensitivity, a value should be chosen to provide a maximum unambiguous concentration range (similar to the maximum unambiguous range with pulsed radar) above 6% or so at the acoustic frequency of operation. increasing the length beyond this would increase sensitivity further, but at the cost of slower response time (since the cavity will create a "volume-averaging" effect due to dilution by the finite gas flow. This means that the volume-limited response time would be gas flow-dependent.) Since the sound velocity through a gas is proportional to temperature, and is expected to be very nonlinear close to the boiling point (due to nonideal gas behavior), thermal stability is expected to be poor. Features of the acoustic velocity sensing approach are:

- Flow rate invariant (except for temporal latency)

- Linear concentration response (below maximum unambiguous concentration)
- Large dead volume
- Good temporal response
- High sensitivity
- Temporal stability unknown
- Thermal stability expected to be poor
- No hysteresis expected
- Low cost

This method was developed, or rather discovered, during the evaluation of the mass-balance sensor design. Since it offered some definite advantages over the mass-balance approach, a prototype was constructed. It consisted of a short cylindrical tube, fashioned from an empty polystyrene wire spool, with two identical 40kHz ultrasonic transducers sealed into either end and facing each other. A wad of nonspun bonded polyester, fashioned from a bouffant hair cover (Kimberly-Clark Corp.), was placed within the cylinder to serve as the resonance dampener (without this, the linearity was quite poor near each cavity resonance peak. The dampener reduced the sensitivity to cavity resonances substantially and had very little effect on the temporal response). Two ¼" diameter copper tubes were inserted into holes drilled in the wall of the cylinder and glued in place. The final dimensions of the sensor head were approximately 2.5" tall and 1" in diameter. The electronics consisted of:

- A (relatively stable) 40kHz oscillator which both drove the source transducer and supplied the phase reference for the digital phase detector
- The AC-coupled preamplifier
- The phase detector
- Amplitude scaling circuitry for calibration adjustment

The oscillator was based on a 7555 CMOS timer chip operated as an astable, with a resistor attenuator between pin 3 and the source transducer. The resistor served as both an attenuator to protect the piezoceramic from being overdriven, and combined with the parasitic capacitance of the piezo, as a lowpass filter to prevent exciting any of the (annoyingly numerous) overtone modes. It also helped damp the Q a bit to reduce the resonant peakiness, and thus the sensitivity to oscillator frequency drift.

The preamplifier provided a reasonably low (~1kΩ) load impedance for the receiver transducer to reduce its Q (as with the source) without attenuating too much of the signal. Since phase detection is an edge-sensitive process, the transducer signal was treated as an FM or PM signal: It was first amplified through a few AC-coupled high-gain stages and then limited (clipped) to produce a good likeness of a squarewave. This limiting feature provided a good measure of amplitude drift tolerance, much like the IF stage of an FM receiver. The phase detector consisted of a D-type flip-flop, configured to produce a digital pulse train whose duty cycle is a direct function of the phase difference between the detected signal and the reference signal. This digital signal was then fed directly to a single-stage lowpass RC filter to recover the "baseband" concentration signal. The last stage contained offset and gain adjustments for calibration against known anesthetic concentrations. A voltage output with a gain of 1V/% of agent and a meter with a 0 to 5% agent concentration scale were included.

Since this sensing technique proved to be so successful (and a prototype was already built and working), it was installed into the system as-is. The calibration process consisted of using a calibrated

vaporizer to expose the sensor to a known range of concentrations of both Halothane and Isoflurane in pure oxygen and in 30%O_2/N_2O. The results are shown in Figure 5.2 below.

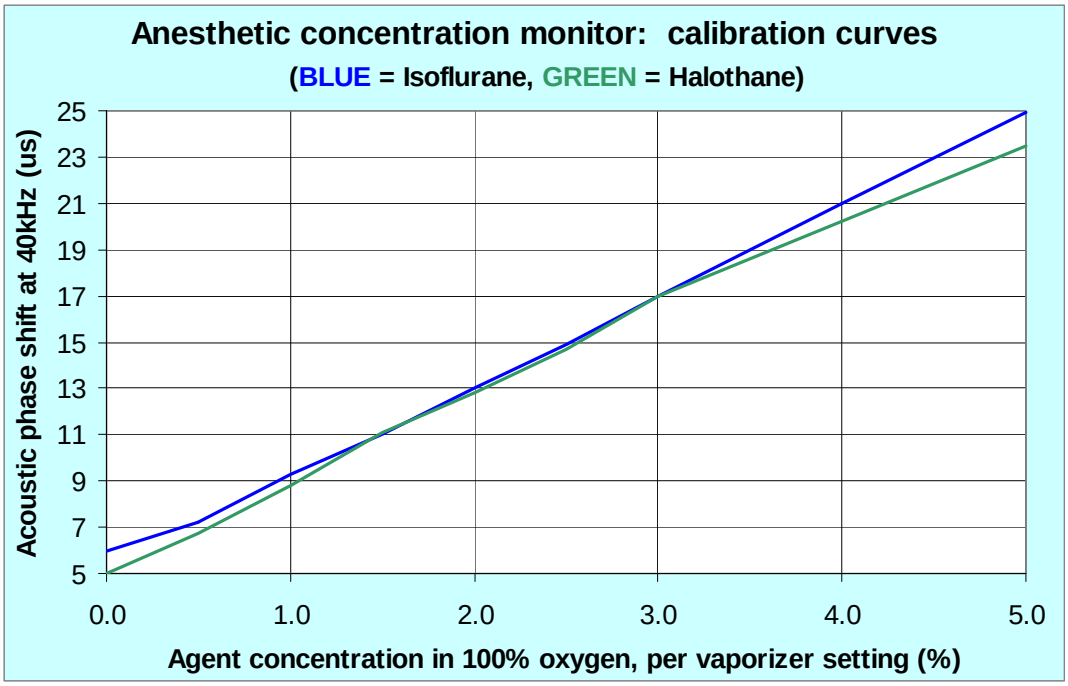

Figure 5.2. Calibration curves for the anesthetic concentration monitor based upon acoustic velocity detection. Standard Tec-series vaporizers were used to generate a range of agent concentrations and the acoustic phase shift was then plotted as a function of the dial setting on the vaporizers.

5.1.3 Anesthesia delivery systems

The prototype system: Sleeper 1

Sleeper I, shown in Figure 5.3, was designed to provide both anesthesia and ventilation. A block diagram of the system is shown in Figure 5.4. It employs a time-triggered, time-cycled positive-pressure ventilator design, because it was believed that absolute temporal control over ventilation would simplify the data analysis. This would place the pulmonary hemodynamic changes in discrete temporal sidebands, which could then be easily distinguished from the hemodynamic fluctuations of interest to DOT measurements.

Tidal volume can be controlled through a combination of inspiratory time and gas flow rate. The breathing rate can be controlled through the expiratory time adjustment. The inspiration/expiration (I/E) ratio would then be a function of both inspiratory time and expiratory time adjustments. Although titrating ventilation in this fashion may seem somewhat confusing in principle, it was actually relatively straightforward in practice, since once the basic ventilatory parameters were set, further adjustments often involved only small tweaks in the breathing rate to accommodate minor changes in metabolism.

These are the features of Sleeper I:

Anesthetic vaporizer

- External O_2/N_2O intake manifold with room air makeup
- Variable duty cycle modulated solenoid for vapor/gas mix ratio control
- Polyethylene reservoir with cyclonic flow
- Backflow prevention loop to minimize agent leakage through pressure-pumping

Anesthetic concentration monitor

- Acoustic time-of-flight sensor, 0% to 5%, Halothane or Isoflurane
- Switchable correction for air and pure oxygen (May work with O_2/N_2O, but not sure)

Ventilator portion

- Internal compressor with dual accumulators for better pressure stability
- High pressure flow control, motor speed control (trade pressure stability for flow rate during induction)
- Separate inspiration and expiration time adjustment knobs (will change to insp/exp. pressure trip points)
- Sigh feature
- Internal or external ventilation control
- Continuous-flow option for faster induction
- Inspiration pressure control
- Max. pressure safety limiter (not designed to prevent barotrauma, only to prevent bladder rupture)
- Airway pressure monitor with analog output (added later)

Figure 5.3. Front and side views of Sleeper I.

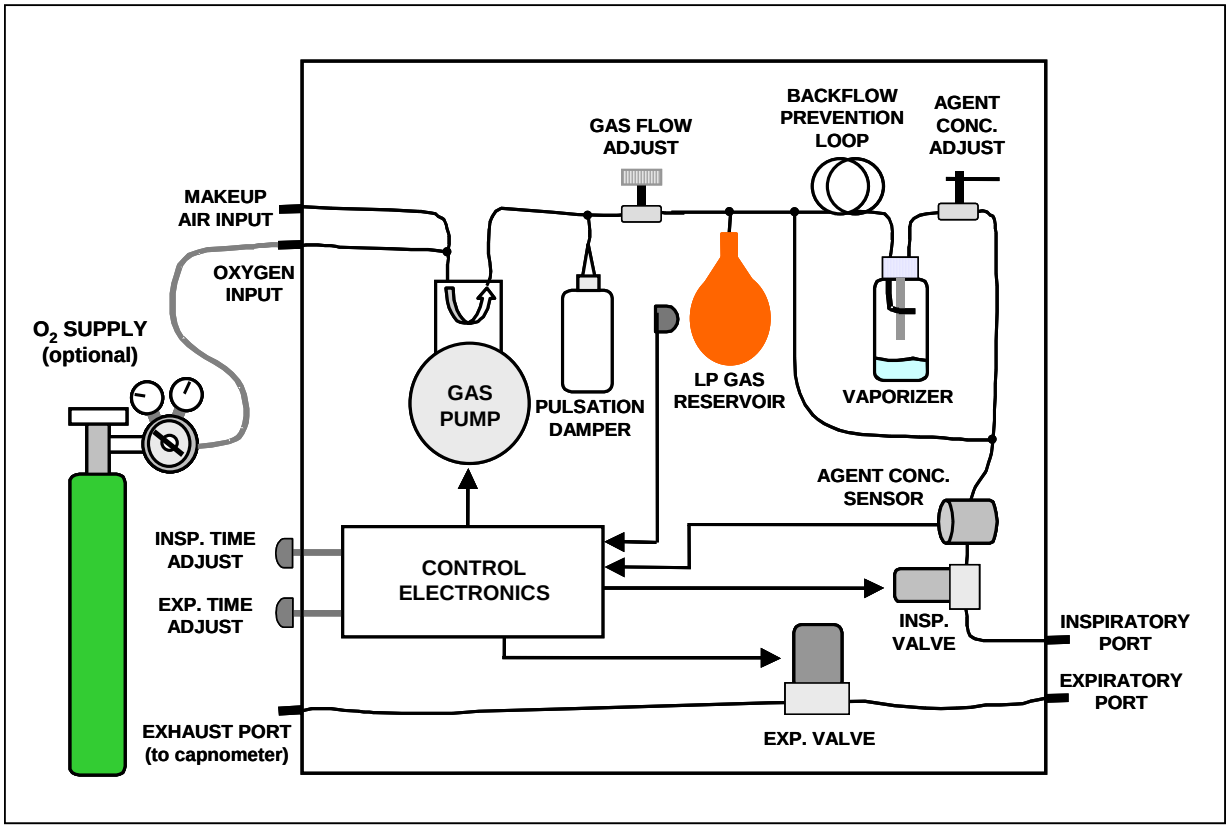

Figure 5.4. A block diagram of Sleeper I, showing the functional and pneumatic connections. The flow rate of an external regulated oxygen source was adjusted to provide an adequate inspiratory O_2 concentration.

The improved version: Sleeper 2

Sleeper II was designed as an improved version of Sleeper I, with more precise ventilatory control and better fault detection. The final decision to redesign Sleeper I was made after an incident which occurred as a series of DOT measurements was about to begin: While a rat was under anesthesia with Sleeper I, the $etCO_2$ began to drop inexplicably. A number of sigh breaths were attempted, and instead of improving ventilation, they appeared to actually impede gas exchange. Unfortunately the health of the rat deteriorated and it expired approximately 15 minutes later. A simple necropsy seemed to indicate that the lung may have ruptured at its base, near the diaphragm. It was later learned that these symptoms: progressively worsening ventilation and gas exchange made worse by increasing airway pressure, deteriorating vital signs, and death, are pathognomonic of a form of pulmonary barotrauma referred to as "tension pneumothorax." This sometimes occurs in patients with certain forms of obstructive pulmonary disease, and in patients undergoing positive-pressure ventilation in which the maximal lung volume has been exceeded and the alveolar pressure rises to the point of rupture. Gas then accumulates and becomes trapped in the pleural space (the sealed region between the outer lung surface and the inner thoracic wall), gradually reducing vital capacity. In humans, this problem can be treated in a number of ways, however in rats, treatment is difficult and would be impractical to perform during a DOT experiment.

In order to ensure that this problem never recurred, a completely new anesthesia/ventilator system was designed. This would be more flexible than Sleeper I in a number of ways. This ventilator would be pressure-cycled, providing an inherent level of safety to protect against barotrauma. Pressure-cycling also allowed the inclusion of an apnea sensor: if the slope of the airway pressure curve were too steep (due to either pulmonary obstruction from accumulated mucus or a tubing occlusion for any

reason), then an alarm would sound, alerting the researcher to the problem. [This feature was responsible for the detection of tracheal obstructions early enough to permit successful suctioning, thus salvaging at least three experiments.] The apnea sensor was also upgraded to include a leak-detection feature: If the breathing circuit was opened or became disconnected from the patient for any reason (say, if the Y-piece slipped out of the tracheal tube), then the apnea alarm would sound. Breath stacking (a problem common with fixed-volume ventilators when the trachea or the exhaust line becomes plugged, trapping gas in the lungs) cannot occur in pressure-cycled ventilators.

The inspiration phase was redesigned to incorporate demand-triggering with a time-triggered backup (also referred to as "assist/control" mode), such that the patient could trigger a new breath cycle simply by attempting to inhale. If respiratory drive were weakened by an excess of anesthesia, time-triggering would occur, providing a reduced, yet survivable, breathing rate until spontaneous respiration returned. This provided better respiratory management than fixed-rate breath control, since the patient's own homeostatic drive was observed to better accommodate for metabolic and thermal changes than an experimenter could using manual controls. It also freed up the experimenter to concentrate on the optical measurements at hand.

Sleeper II also contained an improved gas delivery system. The old KNF Neuberger pump was replaced with a compact diaphragm pump originally designed for continuous air sampling in a portable methane gas monitor. The pump weighed only about 100g and drew less than 250mA at 4.5V while providing 500ml/min. at 40mmHg pressure, an ideal match for the rodent ventilator. Another addition was the use of a true feedback control system to stabilize the bladder reservoir pressure. These would eliminate the need for two air chambers, and would provide far better long-term pressure stability. Front, side, and top views of Sleeper II are shown in Figure 5.6, Figure 5.7, and Figure 5.8, respectively. A simplified block diagram depicting gas flow through the unit is shown in Figure 5.9.

The single bladder reservoir performed two main functions. It acted as a large gas capacitor, filtering out the pulsatile pressure fluctuations from each rotation of the fixed-displacement diaphragm pump, thus stabilizing the gas flow. It also prevented the pump from operating in the slow, hysteretic speed region which could lead to limit-cycling – an annoying form of oscillation common to servo systems driving hysteretic loads. Although some servo systems such as room heaters and refrigerators are designed to limit-cycle for efficiency reasons, this mode of operation leads to significant variations in the stabilized output – an undesirable feature for this application.

An example of a typical motor speed vs. voltage plot is shown in Figure 5.5. When voltage is first applied, the motor is in the boundary lubrication regime, in which static friction dominates. Shaft rotation occurs only after the shaft torque exceeds the resistance posed by static friction. This is referred to as the "breakaway torque." Once the shaft begins to rotate, hydrodynamic lubrication rapidly reduces the friction coefficient to the dynamic value, and the shaft speed rapidly accelerates until an equilibrium between shaft torque and dynamic frictional drag is reached. Further increases in shaft torque result in a relatively linear increase in shaft speed. A reduction in torque results in a relatively linear reduction in speed, even below the initial breakaway torque point. At some point, hydrodynamic lubrication cannot be sustained, and the shaft speed drops to zero. This "stall speed" (not to be confused with stall torque – a different term) nearly always occurs at a torque value well below the breakaway torque, and this is what creates the hysteresis. The magnitude of this hysteresis is greatest in motors equipped with sleeve bearings, lower with ball and roller bearings, and can be essentially nonexistent with pressurized air or fluid bearings, since these are designed to always operate in the hydrodynamic lubrication regime.

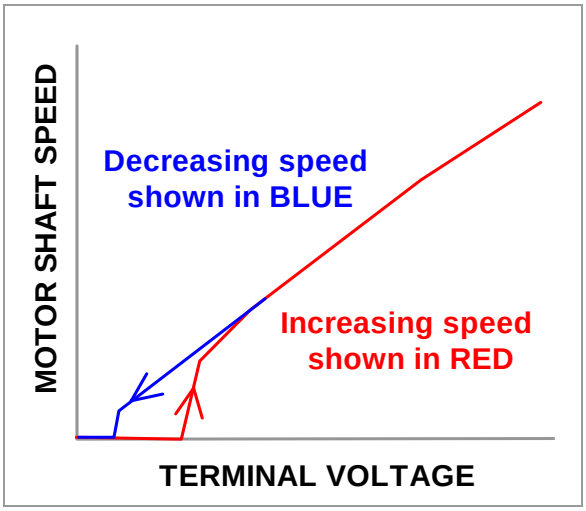

Figure 5.5. Plot of a typical motor speed-voltage curve, showing the difference in shaft speed with increasing and decreasing motor terminal voltage. This hysteretic behavior, common to mechanical machinery, results from "stiction," – the abrupt shift from the boundary (static friction) regime to the hydrodynamic lubrication (dynamic friction) regime. The simplest way to avoid stiction-induced instability is to operate the motor in the linear region above the breakpoint shown by the blue curve.

A large elastic gas reservoir allowed a type of feedback called dominant-pole compensation – where the bladder capacitance itself created the dominant pole. The motor could then be driven in proportional (gain-only) fashion, maintaining a relatively constant motor speed, simplifying the servo electronics, and providing excellent loop stability. Pump speed stability could then be traded in exchange for pressure stability, and both would benefit from increased bladder size and compliance. The bladder consisted of a thick walled latex balloon ("Punch Ball," National Latex company, Ashland, OH), and was initially set for an inflation pressure of 40mmHg. However this was later reduced to 30mmHg to minimize the risk of barotrauma with no noticeable change in performance.

The anesthetic vaporizer was completely redesigned to reduce its size, improve the evaporation efficiency, and provide easier fill access during use. The new vaporizer has a cylindrical wick and a more tortuous gas flow. The smaller internal volume significantly reduced pressure-pumping, so the backflow-prevention loop was shortened. Connection to the fill port was through standard Luer-lock fittings for easier access. The liquid agent level was readily visible through the clear glass wall. Although there was some concern over the possible photodegradation of Halothane, it was felt to not be an issue, since the vaporizer is usually freshly filled prior to each use, and waste agent can easily be removed and the vaporizer rinsed with Isoflurane to remove any impurities or residual thymol (Isoflurane is more chemically stable than Halothane, and does not require preservatives or special storage).

An anesthesia monitor was considered unnecessary for Animal II, since the Datex Capnomac now provided accurate anesthetic agent monitoring, and would be used during all future experiments.

Here is a summary of the features of Animal II:

Anesthetic vaporizer

- External O_2/N_2O intake manifold with adjustable gas mix control and room air makeup
- Variable duty cycle modulated solenoid for vapor/gas mix ratio control
- Compact glass vaporizer with tortuous gas flow path and Luer fitting for easier access

Ventilator portion

- Internal feedback-stabilized compressor with single high-volume accumulator
- Separate inspiration pressure, expiration time, and assist threshold adjustment knobs
- Sigh, inspiratory hold, expiratory hold, fresh gas purge, and pause features
- Internal/external ventilation control
- Automatic crossover from continuous-flow to triggered mode – simplifies mask-to-tracheal switch
- Inspiration pressure safety cutoff w/ alarm
- Apnea and airway obstruction alarm with adjustable apnea threshold

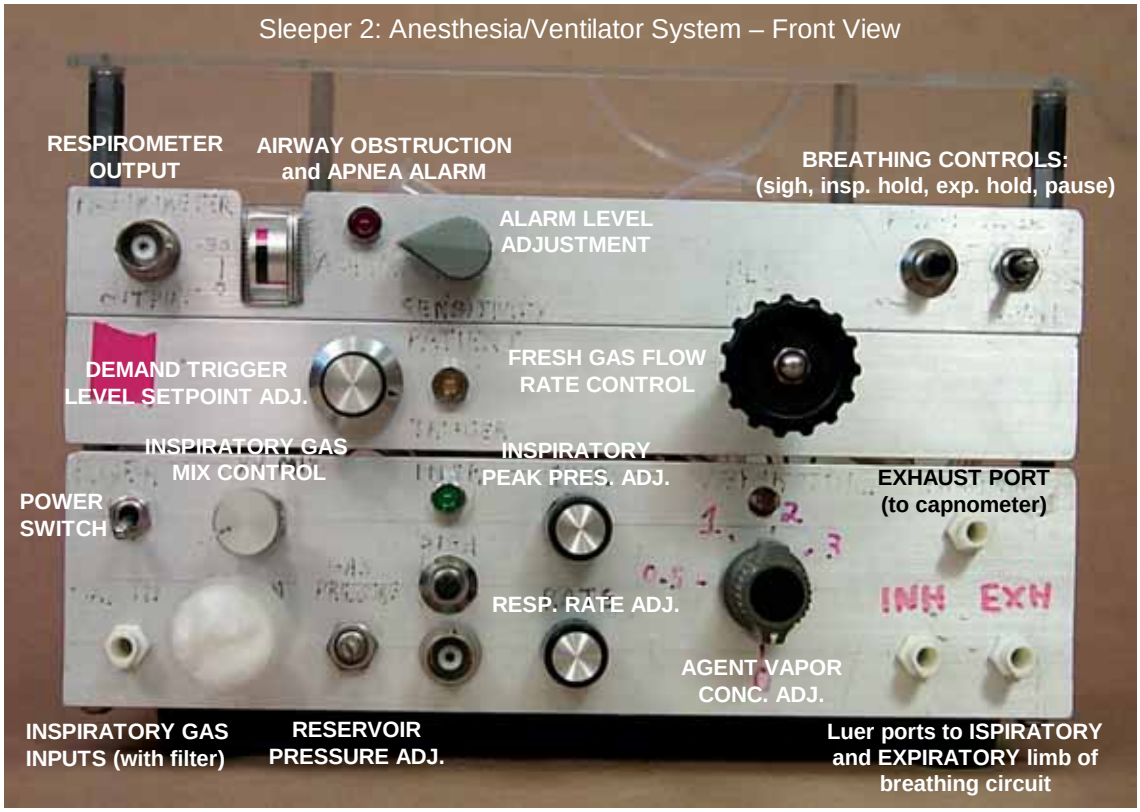

Figure 5.6. A front view of Sleeper II. Although more compact than Sleeper I, this version incorporates more features, and provides better control over gas flow and anesthetic depth.

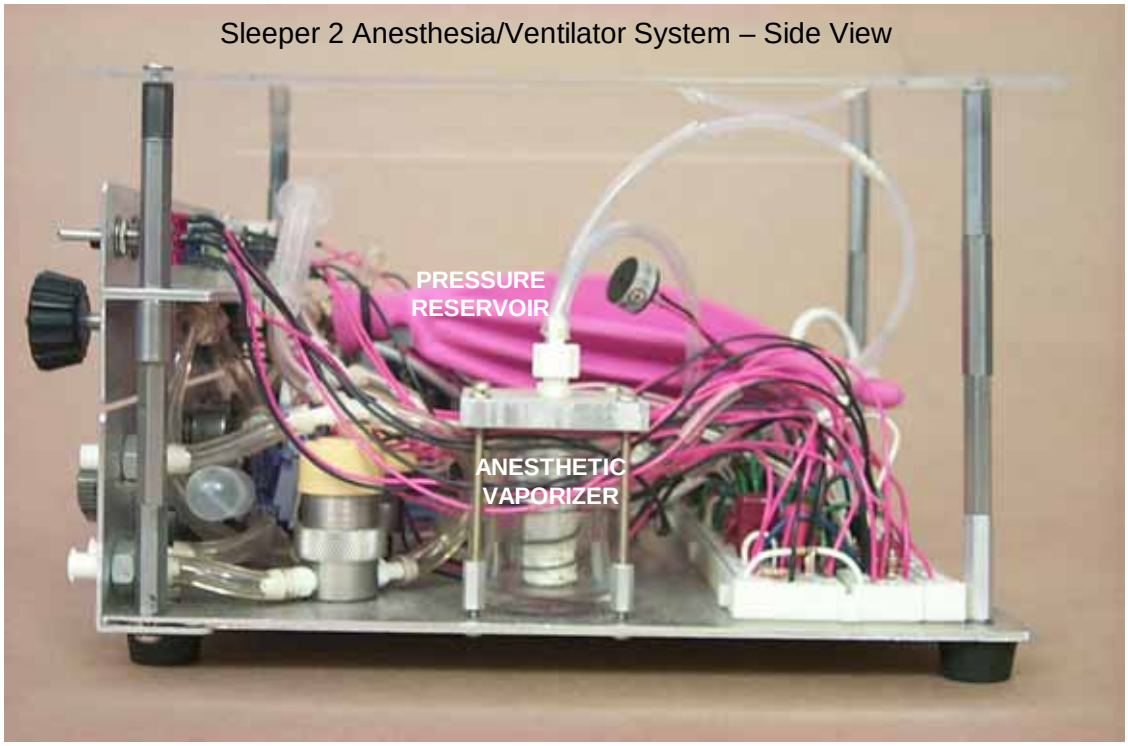

Figure 5.7. A side view of Sleeper II, showing the anesthetic vaporizer. The tubular wick can be seen, surrounded by a carbon steel spring which provided mechanical support. All of the circuitry fit onto one solderless breadboard. The circuitry was constructed on a solderless breadboard instead of the more conventional printed wiring board because it allowed troubleshooting and upgrades to be performed in real-time. Although some feared that reliability would suffer, no problems were observed despite hundreds of hours of use.

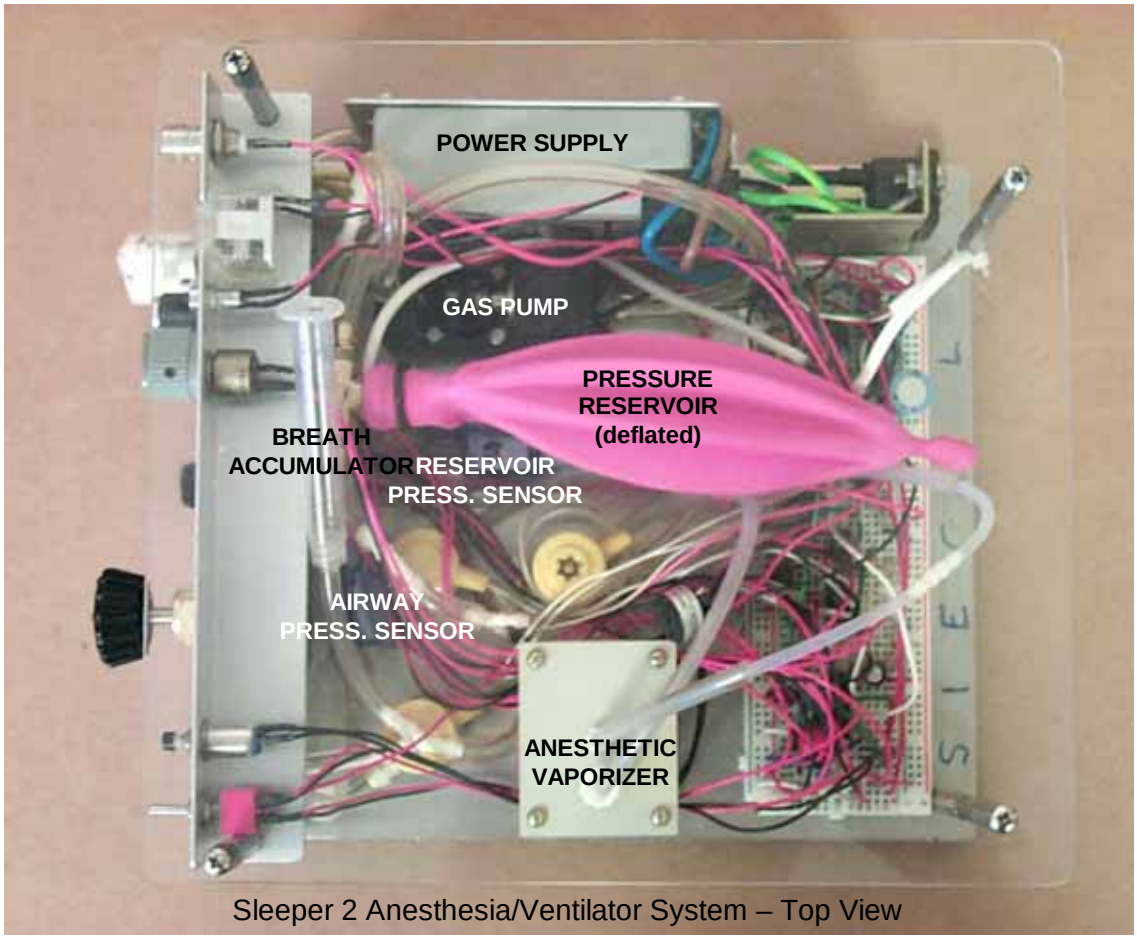

Figure 5.8. A top view of Sleeper II, showing the placement of all of the major components.

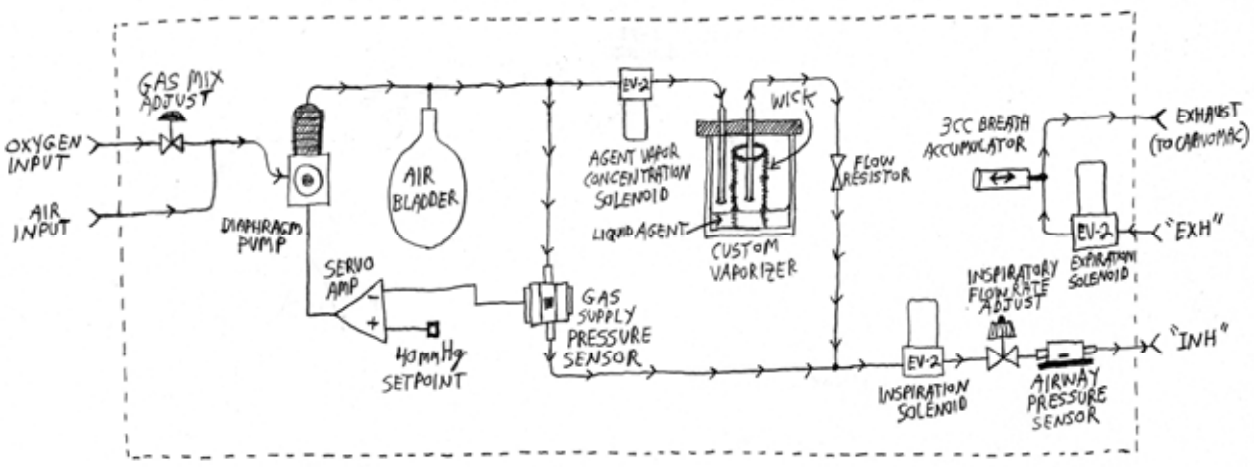

Figure 5.9. A simplified block diagram of Sleeper II, showing the direction of gas flow through the system.

Conclusion

Since no commercial vendor offered a veterinary anesthesia system suitable for both surgery and experimentation, an integrated ventilator/anesthesia system was designed and constructed. The first version, Sleeper I, functioned, but was inadequate in many respects. The second version, Sleeper II, addressed the inadequacies in Sleeper I, and offered a number of advanced features not available on any commercial rodent ventilator (Automatic crossover from continuous-flow to triggered mode, inspiration pressure safety cutoff, apnea and airway obstruction alarm with adjustable apnea threshold, etc.) These features proved their worth on many occasions.

5.1.4 Common anesthetic terms and definitions

Barotrauma: Damage to the alveoli resulting from overinflation of the lung with positive pressure. Since the alveoli are essentially gas exchangers, their walls are extremely thin (to minimize the concentration gradients developed during gas exchange within the respiratory cycle) and are very susceptible to rupture. Since our diaphragm operates in a negative-pressure, or vacuum mode, the alveoli are never subjected to *positive* pressure during normal respiration. The old "iron lung" style ventilators escaped this problem by replicating the diaphragmatic function, using vacuum to achieve ventilation. Modern ventilation equipment is of the positive pressure type, and applies a low pressure (or a fixed volume) to inflate the lungs. Most small animals should be ventilated to 10-15cm (H_2O column, or g/cm_2). Pressures above 40cm or so can lead to rupture of the walls of the alveoli and seepage of air into the thoracic cavity (pneumothorax) or even air entrained in the vascular system (air embolism), which can be fatal.

Blood Gas Measurement: Typically these include the partial pressure of oxygen, carbon dioxide, the pH of the blood, and sometimes the bicarbonate ion concentration as well.

The arterial partial pressure of oxygen (pO_2) in humans breathing air (21% oxygen) is normally between about 90 and 100 Torr (mmHg). Any value above this is acceptable, and breathing gas mixtures normally contain at least 30% oxygen to compensate for any ventilation-perfusion mismatch. A pO_2 below 90 Torr indicates some degree of hypoxia.

The CO_2 partial pressure (pCO_2)represents the respiratory component of the pH balance, and should fall between 35 and 45 Torr. A pCO_2 below 35 Torr indicates respiratory alkalosis and greater than 45 Torr indicates respiratory acidosis.

The pH and HCO_3^- concentration are a measure of the metabolic component of the pH balance. A pH below 7.35 indicates acidemia and above 7.45, alkalemia. Likewise a bicarbonate ion concentration below 24 mEq/l indicates metabolic acidosis and above 25 mEq/l, alkalosis. Note that pCO_2 and bicarbonate are linked by a reversible dissociation reaction: An acute drop in pCO_2 will reduce HCO_3^-, and vice-versa. Short-term hypercarbia will have a small effect on HCO_3^-, but chronic hypercarbia leads to a much larger compensatory shift in both HCO_3^- and pH, so the duration of hypercarbia should be noted when interpreting the results.

Dead Space: This is the volume of gas which is contained within the tracheal tube and the stem of the Y-piece, and does not get fully exchanged with every respiratory cycle. Normally the volume of air contained within our bronchial passages is effectively "dead", in that it does not contribute to the ventilation of the alveoli, and thus dilutes the inspired air, reducing the partial pressure of oxygen and increasing the partial pressure of CO_2 in the alveoli, hindering gas exchange. Adding to this "anatomical" dead space is the volume of any tubing in which gases pass in both directions during respiration. Dead space should be minimized wherever possible. Note that the effect is relative: 20ml of dead space with a large dog having a tidal volume in excess of one liter is insignificant, but 1ml of

dead space with a rodent whose tidal volume is only 3ml is significant. Also note that since dead space acts as a diluent to the tidal volume, simply increasing the tidal volume of the respirator to include the extra dead space will help somewhat, but will not solve the problem, and may even lead to barotrauma.

Hemoglobin Dissociation Curve: A sigmoidal curve showing hemoglobin saturation (or volume percent of oxygen) vs. oxygen partial pressure under a given set of conditions. It reveals that under standard conditions: 21%O_2, ambient pO_2=160mm, the hemoglobin in arterial blood (pO_2=100mm) is almost fully saturated with oxygen. When the blood has made its pass through the body tissues, the venous (pO_2=40mm) oxygen saturation is still 75%. This means that there is an "oxygen reserve" within the blood, which comes in handy in the short-term when breathing would be unwise or not possible (such as swimming underwater or driving through an exhaust-filled tunnel). If one were to hold their breath for an extended time, the drop in blood pH (from CO_2 buildup (respiratory acidosis) and anaerobic respiration (metabolic acidosis)) would help to liberate this oxygen reserve by "shifting" the Hb dissociation curve to the right, (i.e. freeing O_2 from the blood and increasing its delivery to the tissues). The tradeoff for this shift is a reduced affinity for fresh oxygen, and thus a drop in arterial blood oxygen saturation. Since a rightward shift in the Hb curve often occurs during periods of poor ventilation anyway, this is usually the best physiologic trade to make. When ventilation is eventually restored, the pH returns to the 7.40 range and the Hb curve returns to normal. If one were to hyperventilate, however, the rise in blood pH would shift the Hb curve to the left, retaining the excess oxygen and inhibiting its release.

Temperature also affects the Hb curve: An increase in body temperature shifts the curve to the right, releasing oxygen to the metabolically active tissue. Low temperature shifts the curve to the left, reducing the oxygen delivery to colder and less metabolically active tissues. Some drugs can also affect the Hb curve or can interfere with oxygen delivery to the tissues.

During anesthesia, a combination of hypercapnia and acidosis shift the Hb curve to the right. This shift, in combination with the unavoidable V-P mismatch, is what motivates the use of a higher than normal (>21%) oxygen concentration in the anesthetic gas mix.

MAC is the abbreviated term for Minimum Alveolar Concentration. It refers to the alveolar concentration of anesthetic at which 50% of people tested are unable to make purposeful movements in response to noxious stimuli. The lower the MAC, the more potent the anesthetic. Most anesthesia is performed between 1.5 and 2.0 x MAC. Note that MAC values often differ between species, and even within one species, being dependent on a number of variables such as circadian rhythm, hypercarbia (reduces MAC), age (older patients have smaller MAC values), and synergism with other pharmacologic agents such as N_2O. Thus, the actual MAC value should be viewed more as a guideline rather than to indicate a specific vaporizer setting.

Mechanical Ventilator: Two types of positive-pressure ventilators are in use today: volume-controlled systems and pressure-controlled systems. Both apply positive pressure to the lungs through the trachea, either through a plastic tube inserted through the mouth and sealed to the walls of the trachea with an inflatable cuff (endotracheal tube) or through a smaller tube surgically inserted into the trachea (tracheostomy).

During normal inspiration, the chest muscles and diaphragm act in concert to expand the thoracic cavity. The slight vacuum created by this expansion then "pulls" the lungs open, drawing air in through the trachea and bronchial passages. Note that since the chest muscles expand actively, they serve to both lift the ribcage and, in doing so, expand the lungs too. Thus the actual pressure exerted against the alveolar walls is quite low. During positive-pressure ventilation, however, the chest and

diaphragm are not active, and the ventilator must apply sufficient pressure to both overcome the airway resistance necessary to inflate the lungs (which is relatively small in most cases) and to expand the ribcage and distend the diaphragm too (not as small). Thus, during positive-pressure ventilation, the alveolar pressure varies significantly as compared to normal negative pressure respiration. In some cases, this can actually be helpful, since a positive pressure differential across the alveoli increases the partial pressure of oxygen and anesthetic vapor.

Too much positive pressure, however, can overinflate the lungs, tearing the very thin alveolar walls, creating a condition called "barotrauma". What this means from a hemodynamic standpoint, though, is that the blood pressure fluctuates by a value nearly equal to the pressure differential applied by the ventilator. Why – you ask – not use a vacuum, or a combination of pressure and vacuum to ventilate through the trachea? Because unlike diaphragmatic breathing, which relies on passive expiration, evacuating the trachea would create a *negative* pressure differential across the alveolar walls, which can lead to pulmonary edema and atelectasis (collapse of the alveoli).

Nonrebreathing Circuit: This is the most common breathing circuit used with small animals, since it is inexpensive and simple to use. Fresh gases passes through rotameters with individual metering valves (to set the gas mix ratio) and then to the vaporizer. The anesthetic gas mix is then fed either directly to the patient, or through a mechanical ventilator, which allows the gas to pass first into the lungs of the patient and then from the lungs directly to a vacuum vent or an anesthetic scavenger cartridge. Since the gases only pass through the lungs once and are never reused (rebreathed), nonrebreathing circuits consume lots of anesthetic, and so are best suited for short procedures or with small animals. A number of rebreathing circuits are currently in use, and can be characterized using the "Mapleson" classifications: Mapleson A: McGill and Lack circuits, Mapleson B and C: not used in veterinary practice, Mapleson D and E: Bain and Ayre's T-piece circuits.

Partition Coefficient is a unitless ratio which represents the relative distribution of a given quantity of anesthetic vapor in equilibrium with two different media. In general, the more an agent can actually cross the blood-brain barrier, and dissolve into the lipid-rich neural tissue in the brain, the more potent it is. Thus, the measure of a physical property (the partition coefficient) that aids in the transport of the agent from the lungs into the neural tissue will also represent its potency.

The two main interfaces that the agent must cross are the ***air/blood interface*** within the alveoli of the lungs and the ***extracellular fluid/lipid*** interface between the capillaries and the lipid components of the nervous tissue. The **Blood/Gas partition coefficient** represents the blood/air interface and the **Oil/Water partition coefficient** represents the lipid/aqueous interface. A large blood/gas partition coefficient means that most of the vapor will reside in solution in the blood rather than as a gas in the lungs. It also means that the agent will take a long time to diffuse back out of the blood, and is thus indicative of both the induction and recovery time of the anesthetic agent. The larger the B/G coefficient, the slower the induction and recovery.

A large oil/water partition coefficient has been empirically observed to scale with anesthetic potency, and thus inversely with MAC. Adipose tissue consists primarily of lipids, and acts as a huge anesthetic storage reservoir. Since adipose tissue is poorly perfused (it has a very low metabolic rate as compared to other tissue), the diffusion time constant is very long. Thus, a large O/W value (like the B/G value), also indicates a slower agent. [Although in principle POTENCY and SPEED OF ACTION are unrelated, this seems to conflict with simple logic. Clearly both the B/G and O/G coefficients should affect the induction and recovery times, since they both influence the rate of partial pressure rise and fall within the brain.

Classical thinking states that lipid solubility alone determines anesthetic potency. Potency must be a function of both water ***and*** fat solubility, or else any volatile hydrocarbon would be an anesthetic,

and this is clearly not the case. As evidence of my point, just compare the partition coefficient and MAC data for Methoxyflurane, Enflurane, and Desflurane.]

PEEP: Stands for Positive End Expiratory Pressure. This is the positive pressure remaining in the lungs of the patient at the end of expiration (of the breath – not the patient!). Since PEEP maintains a residual pressure differential in the lungs, it increases the partial pressures of all of the gases involved. It is often used to both prevent partial collapse of the lungs (which would normally remain inflated due to the muscle tone of the chest wall) during anesthesia or other major trauma, and to prevent pulmonary edema which can result from the reduced cardiac output during anesthesia. It also serves to improve the oxygen tension by increasing the average partial pressure of O_2 in the alveoli and by keeping as much of the lung ventilated as possible, thus reducing the ventilation-perfusion mismatch. Normal PEEP pressures fall within the 5cm to 20cmH_2O range.

Pin-Indexing: All medical "E" size gas tanks designed for use in anesthesia equipment are equipped with a standard medical valve and mating arrangement. Since the hermetic sealing components are all identical, the chosen method of distinguishing between tanks containing different gases (O_2 and N_2O, for example), is by placing protruding metal pins at key locations in the yoke fixture on the anesthesia machine. These pins act much like a key and lock, fitting into precisely located holes drilled into the valve wall of the appropriate gas tank. If one were to attempt to insert the incorrect tank into the yoke, the pins and holes would not line up properly and a proper seal would not be established. This was designed as a "foolproof" safety feature to prevent casualties due to accidental gas substitution during cylinder changes.

Unfortunately, sometimes the indexing pins are either sheared off (through accidental rotation of otherwise unrestrained tanks in the yoke fixtures) or fall out due to "deswaging" of the surrounding metal after years of use. As a consequence, some patients have died. Larger tanks generally use valves equipped with standardized connections originally established by the Compressed Gas Association. These "CGA" connectors are unique to each gas type and serve the same function as pin-indexing. [Moral: After changing tanks, always take a moment to check the gas. With some practice, oxygen, nitrous oxide, nitrogen, and CO_2 can all be easily distinguished by differences in their taste and smell.]

Rebreathing Circuit: The gas mix passes first through the rotameters and vaporizer (almost always in the VOC configuration) and then into the breathing circuit. A simple rebreathing circuit consists of an elastic rebreathing bag, a soda-lime or Baralime CO_2 absorber canister, a waste vent or "pop-off" valve, and a couple of unidirectional valves around the Y-piece (which connects to the patient). Although there are a variety of circuit configurations, the basic idea is that the anesthetic-laden gas mixture accumulates in the rebreathing bag during exhalation, passes through the patients lungs once, passes by the pop-off valve, then gets fed through the soda-lime canister, where it ends up back in the rebreathing bag. Since the patient consumes only about 3 to 8ml/kg/minute of oxygen and very little anesthetic (once equilibrium has been reached), the fresh gas flow rate can be reduced significantly as compared to a nonrebreathing circuit. When the patient exhales and the rebreathing bag fully inflates, any excess gas will be forced out through the pop-off valve and on to the scavenger. The initial cost and complexity of a rebreathing circuit is high, but it repays itself in direct relation to the breath volume of the patient and time used. Thus, rebreathing circuits are most suited for use with large patients and with extended surgical procedures. Due to the dangers of hypoxia through gas dilution, N_2O is rarely used in rebreathing systems.

Respiratory Assist: An operating mode for mechanical ventilators. If a patient is showing signs of spontaneous respiration, switching the ventilator to "respiratory assist" mode allows the patient to set its own respiration rate. When the patient attempts to inspire, the airway pressure drops below the preset PEEP level, initiating an inspiration cycle. This is often a necessary step to "wean" patients off of mechanical ventilators, since they must recalibrate themselves to a normocarbic and normoxic breathing pattern.

Saturated Vapor Dilution Vaporizer: An older vaporizer design, also referred to as "measured-flow", which split the $0_2/N_20$ gas mix into two distinct streams. One stream entered the vaporizer, exited with a saturated anesthetic vapor concentration, and then passed through a rotameter equipped with a flow metering valve. The other gas stream went through a separate rotameter and metering valve. By adjusting the two metering valves, the saturated vapor was diluted with a given quantity of pure carrier gas to set the final anesthetic vapor concentration. Since vapor pressure, and thus the saturated vapor concentration, is a strong function of temperature, a thermometer was mounted to the vaporizer to allow for thermal corrections to the dilution ratio. This is a rugged and inexpensive design, but it requires frequent monitoring and adjustment, and so presents a large risk of error. The "Copper Kettle" is one example of a saturated vapor dilution vaporizer.

Soda-Lime: A mixture of sodium hydroxide and calcium hydroxide which is used to absorb respiratory CO_2 by converting it to calcium carbonate. The standard formula for soda-lime absorbent contains about 5% sodium hydroxide and about 95% calcium hydroxide. A pH-sensitive indicator, ethyl violet, is used to indicate when the soda lime granules are "depleted" (i.e. converted into their respective carbonates) and must be replaced.

The small percentage of sodium hydroxide serves as an "activator" for the calcium hydroxide, and is not consumed until all of the calcium hydroxide has been irreversibly converted into calcium carbonate through the addition of one molecule of CO_2 and the release of one molecule of H_2O. The sodium hydroxide serves a couple of purposes. Since it is quite hygroscopic, it draws enough water into the mixture to allow some initial ionization to occur. This is important, since calcium hydroxide is a very weak base (i.e. it does not readily dissociate into calcium ions and hydroxide ions on its own), and it is only mildly hygroscopic. Without some moisture present, dissociation cannot occur. Sodium hydroxide is a strong base and dissociates readily in water, encouraging the gasborne CO_2 to enter the solution in the form of carbonic acid, a compound which will dissociate readily into hydrogen ions and carbonate ions under alkaline conditions. The hydroxide ions from the NaOH then rapidly react with the hydrogen ions from the carbonic acid to produce water. What is left are some sodium ions, some carbonate ions, and some sodium carbonate salt. Since this reaction is reversible (because sodium carbonate is water-soluble), there are some carbonate ions always milling about. When a carbonate ion reaches one of the calcium ions, they react exothermically, forming calcium carbonate and heat. Since calcium carbonate is insoluble in water, this reaction is essentially irreversible at ambient temperature and so the CO_2 remains permanently trapped in this chalky material. As the carbonate ions are consumed, more sodium carbonate dissociates to restore equilibrium. This regenerates the sodium hydroxide, which encourages fresh carbon dioxide to dissolve into the moist alkaline film, and so on. When all of the calcium hydroxide has been consumed, further carbon dioxide exposure converts the remaining sodium hydroxide into sodium carbonate, which is only a weak base, and the pH of the mixture gradually drops, causing the ethyl violet indicator to change color from clear to purple.

Another important function of the sodium hydroxide is to prevent desiccation (complete dehydration) of the soda-lime. Some halocarbons (Desflurane in particular) tend to react with desiccated soda-lime to form carbon monoxide. Although rarely sufficient to cause death on its own, it can significantly reduce the oxygen carrying capacity of the blood – a serious problem for an already

hypoxic patient. COHb levels as high as 28% have been recorded! NEVER use trichlorethylene (Trilene) in closed-circuit rebreathing systems. It decomposes under alkaline conditions to form small amounts of dichloroacetylene (a neurotoxin) and phosgene (a poison gas used in WWII).

Variable-Bypass Vaporizer: The most common vaporizer in use today. It consists of a large evaporation chamber and a smaller bypass chamber. A fibrous wick, similar to that in a kerosene lamp, is used to increase the surface area of the liquid anesthetic agent exposed to the O_2/N_2O carrier gas mixture. It also keeps this area relatively constant so long as there is enough liquid agent in the sump to reach the wick. A large dial on the vaporizer is used to vary the fraction of gas mixture which bypasses the evaporation chamber – hence the name. Since all of the gas ratioing occurs internally, features such as automatic temperature compensation (using a bimetallic strip to shift the bypass ratio) are easy to include. The variable-bypass design is similar in concept to the saturated vapor dilution design discussed below. It is more expensive, but is much easier to operate. (This may seem like a trivial convenience at first, but consider that, given all of the activity going on during a surgical procedure and the limited therapeutic range of inhalant anesthetics, a simple miscalculation in concentration can possibly cost a patient their life.)

Ventilation-Perfusion (V/Q) Mismatch: In a patient under anesthesia, the lower regions of the lungs are under compression due to gravity and are thus poorly ventilated. Although gas exchange in these regions is very poor, alveolar circulation is still present, and this creates the problem: Under normal conditions, hemodynamic regulation is usually achieved through two locally-acting autonomic processes called hypoxic vasoconstriction (for perfusion control) and bronchiolar vasoconstriction (for ventilation control). By simultaneously adjusting both vascular and airflow resistances, the "gas-exchange efficiency" of both lungs is kept quite high, and the blood gases within the pulmonary venous flow remain constant, regardless of whether the patient is exercising or at rest, whether standing, recumbent, or even upside down!

Inhalation anesthesia relaxes smooth muscle tissue throughout the entire body, resulting in vascular and bronchial vasodilation. One consequence of this is that the alveolar circulation is high in both properly ventilated ***and*** poorly ventilated lung tissue. The normally ventilated alveoli provide proper gas exchange, and the blood exiting is rich in oxygen and low in CO_2. This blood is then "diluted" upon mixing with the blood leaving the poorly ventilated alveoli, leading to an average reduction in oxygen tension and an average increase in CO_2 tension - and therein lies the rub! This "diluted" blood provides lower than normal pressure gradients in the tissue, resulting in poorer gas exchange (per Fick's Law). Although seemingly counterintuitive at first (an overall higher blood flow should always contain ***more*** oxygen rather than less, right? . . .), what really matters is not simply the mass transport of oxygen in the blood, but the ***concentration gradient*** which the blood must maintain across the capillary walls near the tissue in order for gas exchange to occur. [An electrical analogy would be trying to charge a small 12 Volt battery pack with ten alkaline "D" cells. If the cells are all connected in parallel, the charge capacity (oxygen mass flow) is there, but the voltage (oxygen tension) is too low. If the "D" cells were connected in series, however, the charge capacity (oxygen mass flow) would drop by a factor of ten, but the voltage (oxygen tension) would increase by a factor of ten, which is large enough for at least some charging (gas diffusion) to occur.]

V/Q mismatch is one of the mechanisms that leads to hypercapnia and hypoxia during anesthesia. Mechanical ventilation with PEEP, and periodic sigh breaths tend to counteract some of this mismatch.

VIC and **VOC** stand for Vaporizer-In-Circuit and Vaporizer-Outside-Circuit, and refer to the placement of the anesthetic vaporizer in relation to the closed-loop breathing circuit (usually a "circle" system). VIC systems offer little control over the actual anesthetic vapor composition, and are

sometimes as simple as a jar with a variable opening to the breathing circuit. Problems such as the lack of temperature compensation and water blanketing (a thin layer of water, condensed from the gas stream by the cooler evaporating anesthetic, which floats on top of the anesthetic liquid, thus blocking access of the agent to the flowing gas) make VIC systems less desirable. They are safest when used with less volatile agents such as Methoxyflurane, with its saturated vapor concentration of only 3.5%. Their main virtue is their simplicity and low cost. For these reasons, most modern anesthesia equipment is of VOC design.

5.1.5 Important gas transport laws

Dalton's Law of Partial Pressures states that ***each constituent***, whether vapor or gas, act independently of each other, and thus exert the same pressure that each would have exerted in the same volume if present alone. So the total pressure is simply the sum of the partial pressures of all of the constituents. The fractional part of the total pressure contributed by each constituent is called its Partial Pressure or tension. You can think of the partial pressure as the number of gas molecules colliding with a given surface area per unit time.

The partial pressure can be reduced by dilution with another gas (so long as the total pressure remained the same) as is done within most anesthetic vaporizers, or increased by eliminating diluents, hence the use of pure oxygen with hypoxic patients. It can also be altered by varying the gas pressure, which is common knowledge to both SCUBA divers and bartenders. Victims of carbon monoxide exposure are placed in a hyperbaric oxygen chamber in order to both hasten the dissociation of carboxyhemoglobin and to increase the oxygen partial pressure within the blood. Even if most of the hemoglobin is deactivated, enough oxygen may dissolve into the blood plasma to prevent (or at least minimize the extent of) brain damage. In the early space capsules, the atmosphere consisted of pure oxygen, but only at a pressure of about 3psi. This provided the same O_2 partial pressure as air with 21% O_2 at 14.7psi, but it greatly reduced the weight of the capsule (since it did not need to be designed to withstand as much pressure while in the vacuum of space), and it simplified the gas handling hardware, since no gas mixing or concentration measurement was required. (The astronauts had to be "denitrogenized" prior to flight, the same as patients prior to anesthesia using rebreathing equipment).

Grahams Law of Diffusion: The diffusion rate of a gas through certain membranes is proportional to the square root of its molecular weight. This may explain why agents with low molecular weight and low solubility (N_2O and Xenon, for example) act quickly and yet still provide significant analgesia.

LaPlace's Law: The pressure within a soap bubble (or any other spherical vessel) is directly proportional to the surface tension and inversely proportional to the radius. Thus, for a constant surface tension, the highest internal pressure occurs at the smallest radius. This governs many common processes such as boiling fluids, inflating balloons, and collapsing (or exploding) lungs. It is one of the reasons why barotrauma to overinflated lungs occurs at comparatively low pressures.

Fick's Law of Diffusion: The diffusion rate of a gas is always proportional to the concentration gradient - essentially Ohm's Law for diffusion. This explains why induction is sped up by increasing the vapor concentration – and also why recovery is a function of blood flow, patient size (okay, fat content), and breathing rate. It also provides an explanation for the paradoxical observation that patients with reduced cardiovascular output often induce faster and at lower vapor concentrations than those with normal perfusion. Lower blood flow means less blood passing through the alveolar capillaries per unit time – say, during one breath. Lower blood volume means less dilution, so that a higher partial pressure of agent develops in the blood. This increased partial pressure creates a greater

diffusion rate once the blood reaches the brain. (To be fair, other mechanisms which may account for this include blood-shunting to vital organs during periods of compromised circulation and reduced agent metabolism – both of which also play a part) [51].

5.2 Biomonitoring and life support

Life is a noisy and chaotic process. While phantoms can be designed to be controlled to arbitrarily high levels of precision and will remain perfectly stable during a measurement, living systems are dynamic, and are thus extraordinarily difficult to control for more than very short periods of time. The preparation must be kept alive during (and sometimes after) the experiment. This leads to the following general philosophy:

Strive to understand as many physiologic variables as possible

- Stabilize those that you can, such as respiration rate and depth, body temperature, and long-term blood pressure.
- Monitor those that you cannot stabilize, such as short-term changes in blood pressure, blood gases (pO_2, pCO_2, pH, $pHCO_3^-$), and metabolic rate.

5.2.1 Biomonitoring

The two main motivations for biomonitoring during DOT experiments are to improve the quality of the data and to monitor the anesthetic depth.

Monitoring to improve the quality of DOT data

Any parameters which can affect cerebral hemodynamics in real-time should be recorded concurrently, so that their confounding effects can later be removed from the data. Both cardiac function and ventilation directly affect the systemic circulation, and by extension, cerebral hemodynamics as well. The cardiac rhythm produces a large pulsatile modulation in both arterial pressure and flow, which varies as a function of arterial vascular compliance. Ventilation, whether spontaneous or mechanically assisted, also modulates blood pressure at the ventilation frequency both pneumatically and through neural feedback [50]. Although the ventilatory modulation depth is smaller, the repetition rate is closer to the temporal bandwidth for DOT hemodynamic measurements. Hypoventilation caused by anesthetic inhibition of the respiratory drive can produce respiratory acidosis, with subsequent vasodilatation of the cerebral vasculature [51]. This leads to the motivation to monitor end-tidal carbon dioxide concentration, a good indicator of adequate ventilation.

IV administration can sometimes introduce potentially serious emboli within the vascular system. Air bubbles or small particulates entrained in the IV flow can lodge in the pulmonary vasculature, causing regional ischemia and necrosis of the lung parenchyma. One indication of this is an immediate reduction in the basal metabolic rate, leading to a reduction in both thermal and respiratory output. This can be detected through a drop in body temperature and a reduction in end-tidal CO_2 output [51].

Monitoring to control the depth of anesthesia

Proper anesthetic management is critical to maintaining stable hemodynamics. The pharmacokinetics of α-chloralose make anesthetic management far more difficult than with most other agents. The delayed onset, combined with a short duration of action, make dosing titration difficult. As a result, the vital signs must be monitored closely. The best performance metric for α-chloralose is mean arterial pressure, hence the need for real-time monitoring of MAP.

In order to acquire and record all of this physiological information, a complete biomonitoring system was constructed. Although all of these could be monitored using individual pieces of commercially available equipment, it was less confusing, far less expensive, and more educational to design a single integrated rodent biomonitoring system.

5.2.2 Design and development of the biomonitor unit

The main parameters of interest are:

- Heart rate and rhythm
- Mean arterial blood pressure
- Respiration
- Body temperature / basal metabolic rate
- End-tidal CO_2

Oxygen saturation was not necessary, since the fiO_2 could be maintained at arbitrarily high levels to ensure an adequate saO_2. Although a peripheral nerve stimulator was also considered, it was also not considered necessary, since the level of analgesia could be readily assessed through a toe pinch, and anesthetic titration to an MAP of 100mmHg also provided adequate analgesia during the DOT measurements.

Heart rate and rhythm

The two methods considered for monitoring heart rate were myoelectrical (ECG) and chest wall displacement. Although a prototype chest wall displacement sensor was able to monitor cardiac function, the signal strength varied considerably with sensor placement and there were numerous body motion and ventilation artifacts. ECG was able to provide a stable and artifact-free output. Temporal features in the ECG waveform also provided information on myocardial function [51]. Thus, ECG monitoring was implemented.

Since the main function was heart rate monitoring, the cuton frequency was set around 3Hz to provide a clean and stable R-wave output at the expense of some Q- and T-wave features. The cutoff frequency was set to around 30Hz to capture the fifth harmonic of the ~6Hz heart rate while still minimizing 60Hz AC pickup. An audio monitor with an amplitude-sensitive output was included in order to provide aural feedback during the experiment. This enabled the immediate detection of premature ventricular complexes, along with any electrode impedance problems, should they occur.

The circuit, shown in Figure 5.13, employed a two-stage design. The first stage was a DC-coupled FET-input bridge amplifier with a differential gain of 10. This provided >1000 MΩ input impedance along with some gain up front for better CMRR, while still permitting about +/- 1VDC of common-mode range. This was important here, because the electromotive gradients from dissimilar metals across the electrode-tissue interface could generate hundreds of mV in both differential and common-mode potential.

The differential output of this first stage was then AC-coupled to a conventional differential amplifier stage with an initial voltage gain of 100, providing an overall gain of about 1V/mV. This gain was later increased to 2V/mV in order to provide a larger output swing for recording purposes. The output of this stage was itself AC-coupled to an output buffer to eliminate any drift in the output offset voltage, and for connection through a BNC connector to an external ADC card.

Many electrode configurations were considered, ranging from the ordinary (Ag/AgCl) to the unique (aqueous saline immersion electrodes for each forepaw). Disposable Ag/AgCl electrodes, while considered the standard bioelectrode interface, were judged to be too expensive and cumbersome for these rodent preparations. Aqueous forepaw immersion electrodes worked well, and were used for

many months. However they were difficult to use if access to the forepaw was required, they caused corrosion of the brass baseplate, and they needed frequent fluid replenishment. Stainless steel hypodermic needles were found to make the best and simplest electrodes. The surface lubricant, applied by the manufacturer to reduce the discomfort of insertion, was removed either mechanically or with isopropanol to reduce the electrode impedance. Connection to the amplifier was made through a 1 meter length of Teflon insulated shielded twisted-pair cable equipped with three small alligator clips. Although the active electrode area of the needles was small, the convenience, flexibility in placement, lack of trauma, duration of performance, and reusability made these electrodes ideal. Since the amplifier was designed to provide a very high input impedance, DC current flow through the electrodes was extremely small, so high electrode impedances and 1/f noise (created by current flow across the metal/tissue interface) did not present a problem.

Mean arterial blood pressure

Although a number of devices claim to measure arterial pressure noninvasively, their repeatability is poor and the uncertainty is too large for use in anesthetic management. For this reason arterial catheterization was always performed to provide real-time measurement of arterial pressure. The pressure transducer was a commercially available disposable component which used a laser-trimmed silicon piezoresistive sensor (Deltran series, Utah Medical). A complete datasheet for these transducers is included in the Appendix. Piezoresistive sensors are as stable as conventional strain gauge sensors, but they offer significantly greater strain coefficients and they can be manufactured inexpensively using standard silicon fabrication techniques. These transducers employ the standard four-wire Wheatstone bridge configuration, which reduces the effects of process variability and nearly eliminates extraneous EMI and RFI interference.

The most accurate means of measuring the output of a Wheatstone bridge sensor involves modulating the drive voltage and synchronously demodulating the amplified AC output [93]. This eliminates electrical offset errors and also allows for narrowband filtering of the demodulated output, significantly reducing the noise floor. Although this approach would have been ideal, there was concern that the modulated drive signal could couple into sensitive electrophysiology measurements nearby, so a simpler DC-coupled design was chosen instead. The transducer was driven by a 5VDC source and the output was connected to a high-gain differential amplifier with offset and gain adjustments, as shown in Figure 5.14. Calibration was performed using a static water column ($136cmH_2O = 100mmHg$) in lieu of mercury for both availability and safety reasons. The offset null adjustment was placed on the front panel for convenience and was trimmed periodically as needed. The gain at the BNC output was set to provide 1VDC at 100mmHg, and the temporal response was limited to 30Hz to minimize noise and interference. This design performed well, and the readings were quite stable, despite the use of a DC-coupled preamplifier.

Respiration

The objective for respiration monitoring was to obtain a recordable measure of the ventilation rate, in the event that respiratory perturbations interfered with the DOT measurements. A number of technologies exist for monitoring ventilation rate and depth [94]. Two of the simplest measures are airway thermal contrast and chest wall expansion.

Airway thermal contrast uses the temperature change of a thermistor located in the tracheal limb of the breathing circuit to infer the respiration rate. A combination of sensible and latent (evaporative/condensing) heat flow creates a large temperature change as dry, cool inhaled gas and saturated, warm exhaled gas alternately pass over the sensor. Although simple in concept, this would have involved the introduction of a thermistor into the already small tracheal limb. Since it was important to minimize the mass and torque coupled to the tracheal limb (to reduce any forces being applied to the trachea), this approach, although conceptually elegant, was not pursued.

Chest wall expansion can be measured directly through force or extension of a strain sensor, however the thoracic constriction from a sensing strap could have impeded ventilation in anesthetized rodents, so noncontacting approaches were explored. Acoustic proximity sensing would work, however the presence of ultrasonic energy would have likely annoyed any rodents nearby, so ultrasonics were undesirable in this setting. Electrostatic proximity sensing would have also worked well, however detecting small capacitance changes would have required some form of modulation, and concerns about modulated AC signals leaking into electrophysiology equipment made this impractical.

An optical proximity sensor, normally used to detect the presence of reflective objects, was tested in the CW mode and found to work well enough to implement. Since near-IR light from the sensor could potentially interfere with the frequency-encoded DOT measurements, it was intentionally not modulated for this reason. In practice, the static IR retroreflective sensor did not produce any detectable change in the DOT signal levels. The output range of the respiration sensor spanned from –10V to +10V, however the analog data acquisition system was configured for a +/-5VDC range, so an LED was used to indicate when the signal was within the proper voltage range. The circuit is shown in Figure 5.14. It provided a graded intensity over the 0V to 5V range, so the sensor could be aligned over the chest without the need for a voltmeter or an oscilloscope.

Body temperature

A commercial 100kΩ thermistor was used for monitoring body temperature. Since the resistance of a negative temperature coefficient (NTC) thermistor is nonlinear, a simple passive linearization circuit was constructed. This exploited the nonlinear relationship between terminal voltage and thermistor resistance when the thermistor is driven from a voltage supply with a finite source impedance.

The original objective was to provide a calibrated thermal error signal to an external temperature controller, so the amplifier gain was adjusted to provide a 0V output at 37.0’C, with a slope of 1V/’C. The thermistor leads were encapsulated in epoxy resin and then covered in heat-shrink tubing to approximate a hermetic seal and to provide good mechanical support, given the rather hostile environment it would likely be exposed to. The circuit, shown in Figure 5.13, employed resistive linearization to provide a ~10’C linear region centered at around 35’C. Calibration was performed against a commercial electronic fever thermometer with a specified accuracy of +/-0.02’C.

Although the temporal response of this sensor was less than one second, there was concern about the effect of loop compensation and stability on metabolic monitoring, since metabolic rate was to be judged through heat balance. Achieving optimal compensation in a feedback control system of this type is difficult because the thermal properties of a living creature create a complex load term, which contains multiple nonlinear resistances (thermal conductivity paths), reactances (heat capacities of the rat and the support platform), and heat sources (oxidative metabolism, surgical lamps, etc.). If, for example, the gain value in the control loop was set low (to maintain stability and improve settling), then there would be a significant temperature error at equilibrium, complicating the estimation of metabolic rate. If the gain were greater, then the transient response of the loop would suffer, leading to a confusing, time-varying metabolism estimate.

To alleviate these concerns, I chose the researcher-in-the-loop approach: The heat flow was manually adjusted the researcher as needed to maintain a stable temperature. To this end, yellow, green, and red LEDs indicated “LOW TEMP,” “OK,” and “HIGH TEMP” conditions. As long as the external heat flow was held constant, any power change would appear as a first-order term, with the temperature exhibiting a nice exponentially-damped temporal response. Once this change was noted, the heat flow (or other ventilation parameters) could be adjusted as needed.

Figure 5.10. A front panel view of the biomonitor unit, which is shown mounted above the forepaw stimulator. Both circuits share a common +/- 12V power supply module and utilize a common chassis ground. Colored tape indicates specific BNC cable connections to the analog data acquisition system.

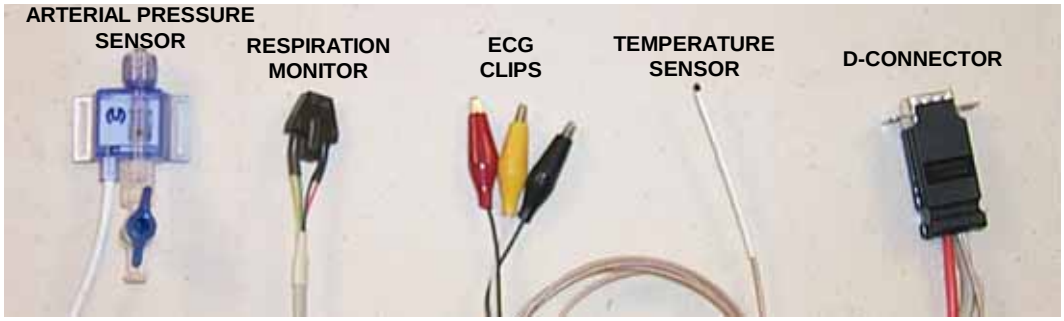

Figure 5.11. The biomonitor probe assembly. The four sensors connect to the front panel of the biomonitor unit through a 15 pin high-density D-connector.

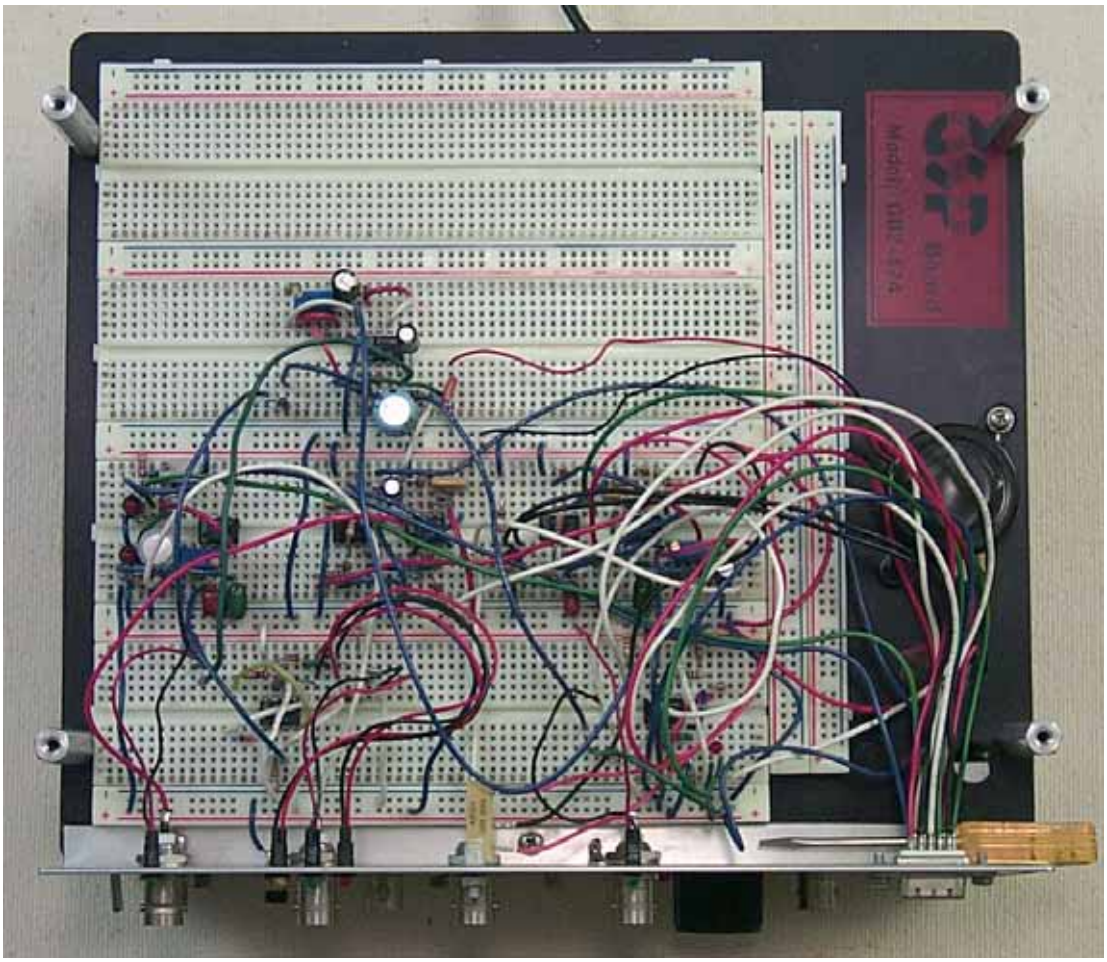

Figure 5.12. A top view of the biomonitor circuitry. All of the circuitry was constructed on a modified solderless breadboard, which provided the flexibility to perform in-situ prototyping and offered the option for future upgrades. Despite some initial concerns, no connectivity problems were experienced during three years of use.

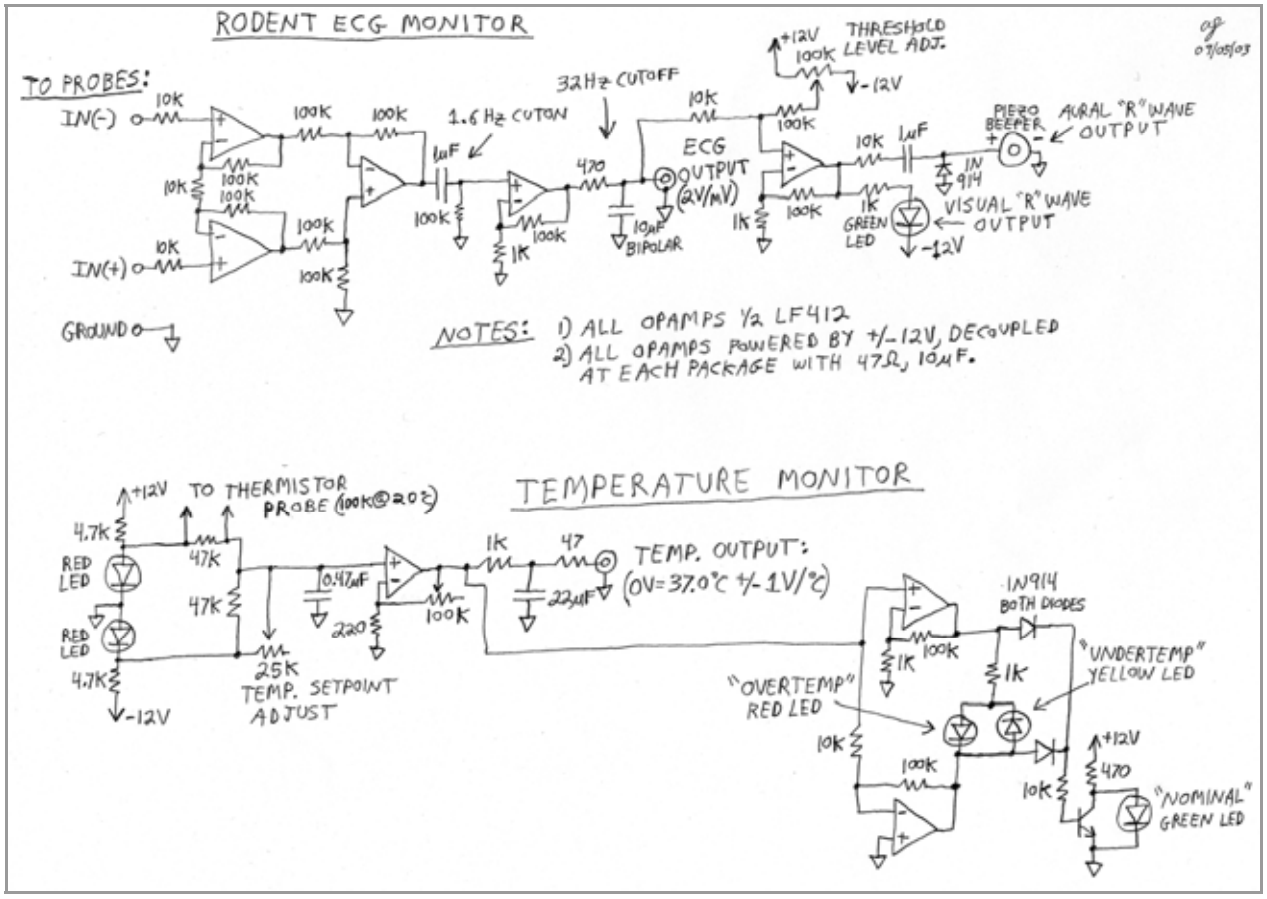

Figure 5.13. Schematic drawings of the ECG and body temperature monitor circuits.

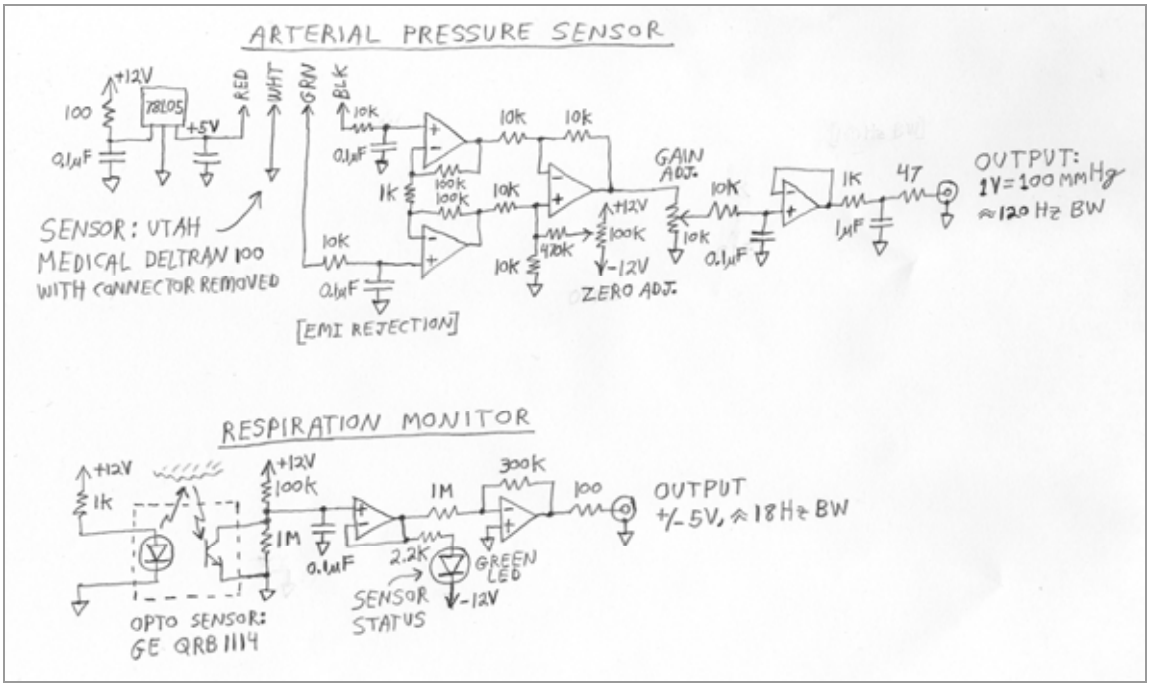

Figure 5.14. Schematic drawings of the arterial pressure sensor and respiration monitor circuits.

Capnometry and end-tidal CO_2 measurement

Through a combination of both luck and divine intervention, the surgical-grade anesthetic gas monitor shown in Figure 5.15 was acquired and repaired. It provided end-tidal CO_2, respiration rate, fiO_2, and halothane/isoflurane concentration. This obviated the need for some of the features of the biomonitor unit, however it was a welcome addition, and provided a valuable source of information. It was designed as a sidestream unit: a small volume of gas, about 75ml/minute, would be extracted from the breathing circuit to be analyzed, which could then be exhausted out the back of the unit or cycled back into the breathing circuit for low-flow anesthesia. Since the typical tidal volume for rodents is about 3cc and the sidestream sample flow rate is 75ml/minute, a small breath accumulation chamber (the barrel of a 3cc syringe) was used to capture enough of the exhaled gas to provide a measurable sample. So the connection between the exhaust port of the anesthesia/ventilator and the input port of the Capnomac was made as thin and short as practical to minimize dead space. This helped to reduce laminar dispersion (gas mixing within the tubing) to better preserve the temporal features of the capnogram. During use, it was discovered that the Clark-style amperometric oxygen sensor was quite old and only displayed only "19%" while sampling air, so the displayed value was scaled accordingly. $etCO_2$ measurements were calibrated using my exhaled breath as a reference, which always peaked between 38 and 40mmHg.

Figure 5.16 shows a rodent DOT experiment in progress. The anesthesia, biomonitoring, stimulus, and stereotactic equipment were all custom-built for these measurements. The exhaled output from the anesthesia/ventilator unit was fed directly into the Capnomac, which displayed $etCO_2$, respiration rate, fiO_2, and isoflurane concentration. Isoflurane was used for the initial surgery, during which a tracheotomy was performed and both arterial and venous lines were inserted to provide vascular access for monitoring arterial blood pressure and IV administration of α-chloralose. The probe of a commercial indoor/outdoor thermometer was used to monitor body temperature, and the black DVM atop the biomonitor/stimulator displayed the MAP. The power supply below the biomonitor/stimulator powered the Watlow heating pad (not visible) under the rat's abdomen. The patency of the arterial catheter was periodically assessed by checking for pulsatile fluctuations in the meter reading.

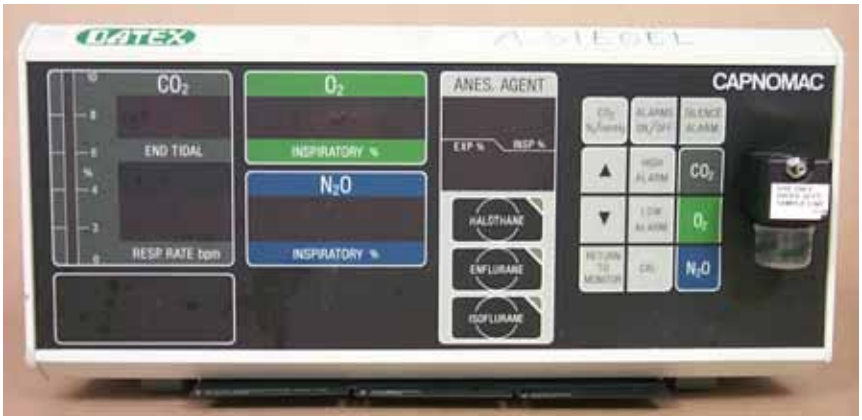

Figure 5.15. This Datex Capnomac anesthesia monitor, obtained at nominal cost from the MIT Flea Market with some mechanical damage, was repaired and used primarily for capnometry measurement.

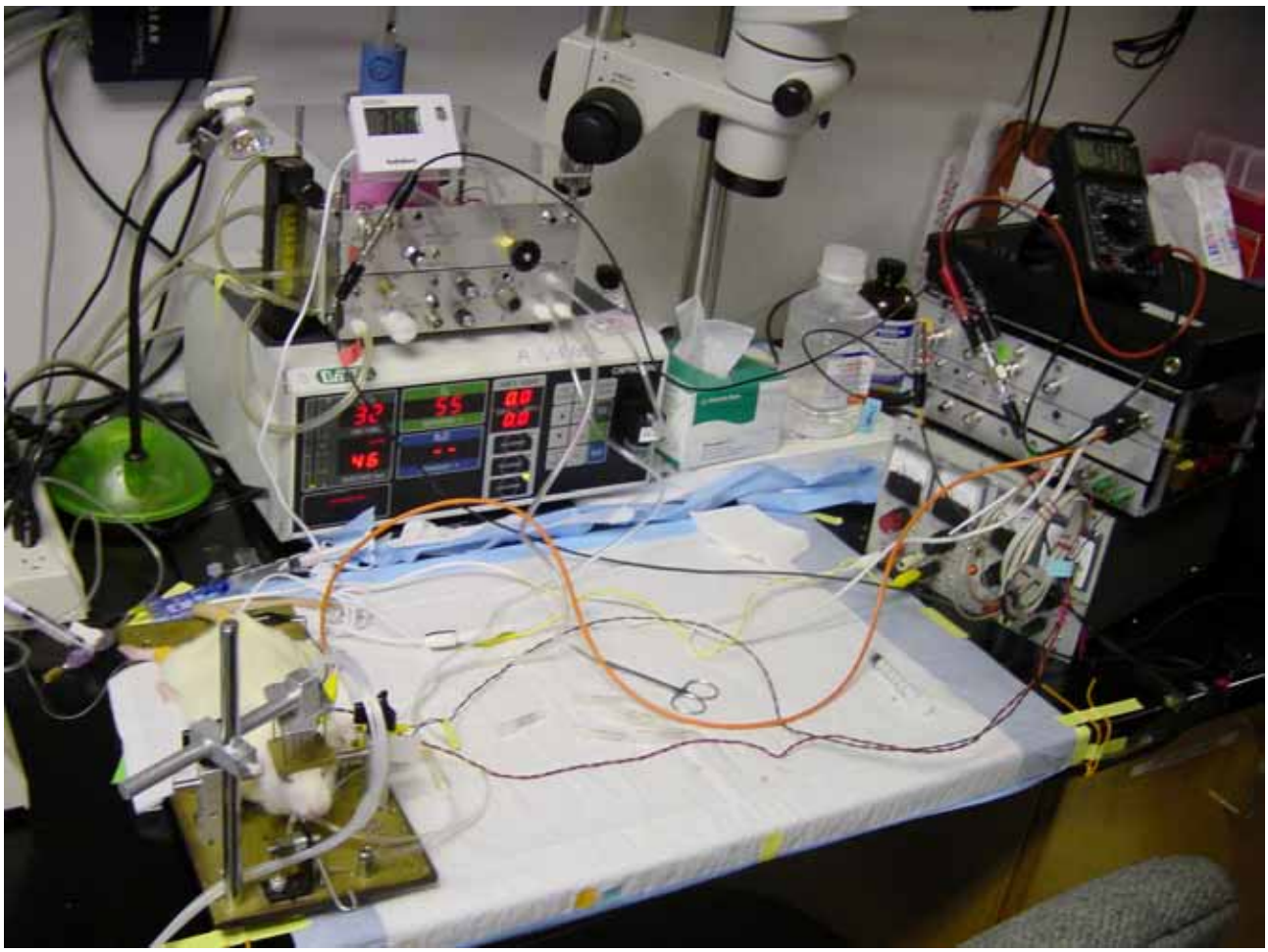

Figure 5.16. A DOT experiment in progress, showing the anesthesia, biomonitoring, and stimulus equipment in use.

5.3 Stimulation

Since many DOT experimental paradigms for rodents include electrical forepaw or hindpaw stimulation, a suitable stimulator was required. Although the absolute coupling of current flow to axonal activation is difficult to assess, it should remain relatively constant so long as the electrode placement does not change. Mechanical stability is easy to achieve, however DC current flow will eventually produce electrochemical changes at the electrode/tissue interface which could lead to localized tissue damage. The degree of axonal activation is a direct, albeit nonlinear, function of electrode current. Since the duration of some DOT experiments may exceed 8 hours, during which stimulation must continue to occur at predetermined intervals, it was important to both control and monitor the delivered current in real-time. This would both ensure that the correct current was being delivered, and allow any loss of continuity or changes in tissue conductivity over time to be observed and corrected. Unfortunately no commercial stimulator or stimulus isolation unit was equipped to provide real-time monitoring of stimulus delivery, therefore a custom stimulus delivery, isolation, and monitoring unit had to be constructed. The main design objectives were as follows:

- Galvanic isolation (to minimize interference and prevent stray electrode currents)
- Charge-conservative pulse delivery (to prevent electrochemical tissue damage)

- Pulse current monitor (to confirm proper current delivery)
- Tissue impedance (assumed): ~200Ω resistive
- Stimulus parameters:
    - Pulsewidth range: ~200+/-100us,
    - Stimulus frequency range: 1-10Hz,
    - Stimulus duration: 30sec to 180sec
    - Stimulus current: 0.1-10mA

The circuit is shown in Figure 5.17. An adjustable pulse generator (not shown) produces 200us wide digital pulses, derived from an internal free-running oscillator or from computer command via an external BNC input. These pulses drive a single stage current booster which applies a voltage pulse to the low voltage (secondary) winding of a commercial 24VAC stepdown transformer. The 120VAC (primary) winding is connected directly to the preparation through stainless steel stimulus electrodes. A 100Ω resistor in series with the transformer winding provides a means of monitoring current delivery and also protects the driver transistor from damage if the electrodes were inadvertently shorted. A low resistance trimpot on the driver output was adjusted to optimize the source impedance to provide clean rectangular current pulses to the tissue, as judged by the waveshape at the current monitor output. The combined input bias current of the LF412 opamps in the current monitor circuit was below 60pA, so electrode polarization was minimal.

On one occasion, the stimulus current was adjusted and the experiment had begun, but unbeknownst to us, one of the electrode leads had become intermittent. This was eventually detected, but not until most of the data had already been collected, resulting in the loss of an entire days' work. In response to this event, a "Delivery" indicator circuit was added. It provided a bright flash on the delivery of every pulse, and would not flash unless current was flowing through the tissue. No more wiring problems occurred once the delivery indicator was operational.

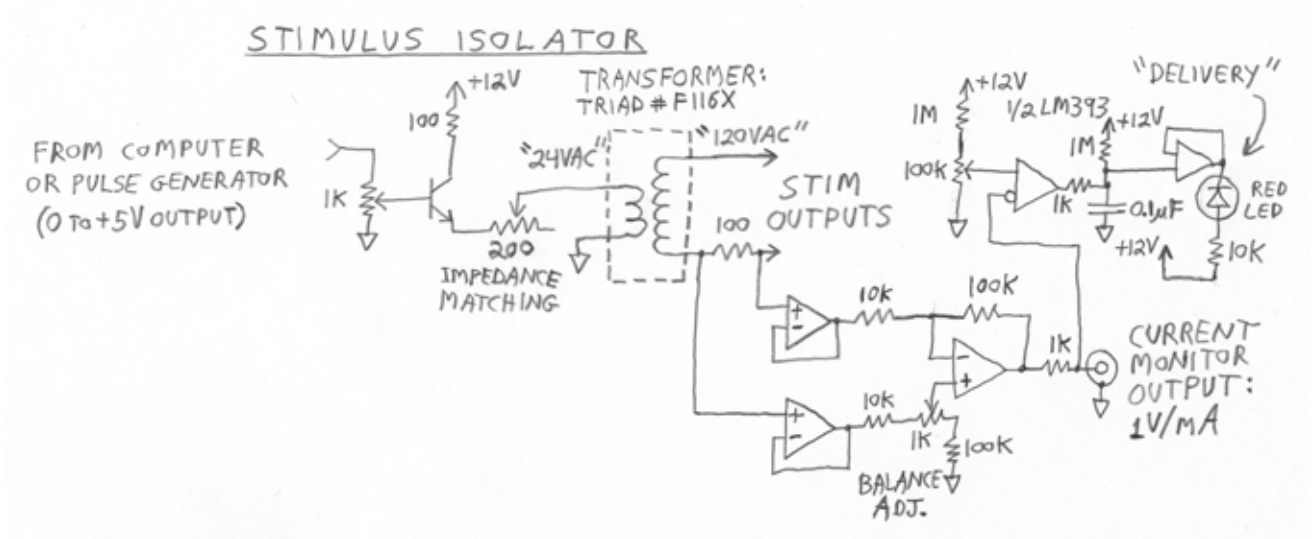

Figure 5.17. A schematic of the stimulus isolator circuit. All opamps are LF412. The current monitor output, when viewed on an oscilloscope, shows the current being delivered to the forepaw. The "delivery" LED provides a systemwide continuity check, since it only illuminates when pulses are being received and current is being delivered to the tissue.