ANESTHESIA FOR FMRI

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INTRODUCTION

The section is part of our standard operating procedures (SOP). It is written primarily for those collaborators in the laboratory who will be assisting in anesthetic management on a regular basis. Such people will be the laboratory technicians and those scientists who want to gain experience in laboratory animal anesthesia. Graduate students or postdoctoral fellows that do not wish to be involved in the induction and maintenance of anesthesia in monkeys are not obliged to read this section.

Details on the issues covered in this section may be found in any number of excellent text books of anesthesiology (Clinical Anesthesiology, by G. E. Morgan and M. S. Mikhail, Appleton & Lange; Principles & Practice of Veterinary Anesthesia, edited by Charles E. Short, Williams & Wilkins; Anesthesia, by Miller, Churchill Livingstone; Anaesthesiologie, by A. Doenicke, D. Kettler, W. F. List, J. Radke, and J. Tarnow, Springer Verlag). Details on our laboratory anesthesia machines can be found in those machines' manuals, and in various relevant textbooks (e.g. The Anesthesia Machine, by Clayton Petty, Churchill Livingstone Inc.).

General Anesthesia

Recent research shows that a meaningful description of general anesthesia is best given in terms of three basic components, *unconsciousness* (hypnosis), *muscle relaxation*, and *analgesia* (areflexia). However, it is still useful to remember the traditional definition of anesthesia-stages given by Güdel for *ether* anesthesia. These stages are easily distinguishable when barbiturates are used as sole anesthetics, and they can therefore help us understand the effects of a particular drug-dosage.

Stage I (Analgesia): It lasts from the onset of drowsiness to the loss of the eyelash reflex, and is associated with sedation, amnesia, and some analgesia. Characteristics: slow, regular breathing with the *diaphragm* and the *intercostal muscles*.

Stage II (Excitement): This stage is the result of inappropriate rate of drug administration, and can result in vomiting, laryngospasm, or arrhythmias. Characteristics: agitation, delirium, irregular respiration, salivation, dilated pupils, and divergent eyes.

Stage III (Surgical): It is further divided into 4 planes. *Plane 1* is characterized by slight somatic relaxation, regular periodic breathing, and active ocular muscles. In *Plane 2*, eyes become immobile, inhalation becomes briefer than exhalation, and a slight pause separates inhalation and exhalation. In *Plane 3*, the eyelid reflex is absent, the abdominal muscles are completely relaxed, and diaphragmatic breathing is very prominent. In *Plane 4*, pupils are dilated, the intercostal muscles are completely paralyzed, and paradoxical rib cage movement occurs.

Stage IV (Impending death): It lasts from the onset of apnea to failure of circulation. Muscles become flaccid, eyes are widely dilated, and respiratory and cardiovascular arrests are followed by cardiovascular collapse.

Anesthesia was initially done exclusively with single agents such as ether, chloroform, or nitrous oxide. Today the preferred type of anesthesia is usually called *balanced* or *cocktail* anesthesia. It involves multiple agents, each selected for a specific purpose, such as analgesia, unconsciousness and amnesia, muscle relaxation, or abolition of autonomic reflexes. Typically, hypnotics (e.g. inhalant agents, barbiturates) are combined with analgesics (e.g. opiates) and muscle relaxants. Balanced anesthesia is the type of anesthesia applied in this laboratory for most procedures. Single agent anesthesia (e.g. barbiturate anesthesia) may be used in case of brain injury, and opiates combined with muscle relaxants may be used for electrophysiological recordings. During the fMRI experiments anesthesia is maintained with *isoflurane (end-tidal 0.4 - 0.45% in air)* as hypnotic, *fentanyl (3 Ⓓg/kg/hr)* as analgesic, and *mivacurium (5mg/kg/h)* as muscle relaxant.

Anesthesia & Cerebral Physiology

Functional MRI is critically dependent on unperturbed, normal cerebral physiology. Those interested in the technique should therefore be aware of at least some very elementary concepts. The brain is restricted almost exclusively to glucose as the substrate for its energy. Extensive research has shown that the only incontrovertible and consistently positive arterio-venous differences demonstrated for the human brain under normal conditions have been for *glucose* and *oxygen*. Not surprisingly, negative arterio-venous differences significantly different from zero have been found consistently only for carbon dioxide, even though water, which has never been measured, is also produced.

Although comprising only 2 percent of the total body weight, the brain receives 12 to 15 percent of the cardiac output, consuming about 20% of the oxygen entering the body. The energy requirement, that is the cerebral metabolic rate (CMR) of the brain, is usually expressed in terms of oxygen consumption ($CMRO_2$) alone. This is so because about 90% of the glucose (5mg/kg/min) is aerobically metabolized, and therefore parallels oxygen consumption. $CMRO_2$ is proportional to neural activity and is 4 times greater in gray than white matter. At rest, the brain consumes oxygen at an average rate of approximately 3.5 ml of oxygen per 100 g of brain tissue per minute. Approximately 60 percent of the energy is used to support electrophysiological function, because a great deal of energy expenditure is required for the maintenance and restoration of ionic gradients and for the synthesis, transport, and reuptake of neurotransmitters. The remainder of the energy is used for cellular homeostatic activities, including the maintenance of the neuron's relatively large membrane mass.

The brain's substantial demand for substrate must be met by adequate delivery of oxygen and glucose, by means of the *cerebral blood flow* (CBF). This flow must be precisely controlled. It must be sufficient without being excessive, as the space constraints imposed by the noncompliant skull and meninges would immediately increase intracranial pressure. It's hardly surprising, therefore, that there are very elaborate mechanisms for the precise regulation of CBF and CBV (cerebral blood volume), which closely follows all CMR changes. CMR typically decreases during sleep and increases during sensory stimulation, mental tasks, or arousal of any kind. It increases dramatically during epileptic activity, and is substantially reduced in coma. This tight coupling of CBF to CMR is what makes most brain imaging techniques possible. It is thought to occur as a result of the opening and closing of sphincter-like vasomotor mechanisms in response to local alterations in metabolism. These mechanisms include chemical, myogenic, and neurogenic factors.

Normally CBF is "autoregulated." This *autoregulation* reflects the intrinsic capacity of the cerebral circulation to adjust its resistance in order to maintain CBF constant over a wide range of mean arterial pressure (MAP). The limits of autoregulation are approximately 50 and 150 mmHg. Above and below this range CBF is pressure-dependent (pressure-passive) and varies linearly with cerebral perfusion pressure (CPP). The latter is defined as the difference between mean arterial pressure (MAP) and intracranial pressure (ICP), which is approximately 90 mm Hg in the alert monkey. In addition, because ICP is usually less than 10 mm Hg, CPP is primarily a function of MAP. The precise mechanism by which autoregulation is accomplished is not known. It appears to be an intrinsic characteristic of cerebral vascular smooth muscle (i.e. myogenic), since it can be demonstrated in isolated vessels.

Anesthesia interferes with the brain's electrophysiological functions and its autoregulation. It therefore changes different aspects of cerebral physiology, including changes in CMR, CBF & CBV, CPP, and ICP. Anesthetics suppress CMR, ketamine being the notable exception. The component of cerebral metabolism on which they act is associated with electrophysiologic function rather than with the maintenance of cellular integrity (homeostasis). This means that an increase in the plasma concentrations of barbiturates, isoflurane, etomidate, etc. causes progressive suppression of EEG activity and a concomitant reduction in CMR, but increasing the plasma level beyond that required to first achieve isoelectric EEG does not result in any further depression of CMR. The $CMRO_2$ values observed when isoelectricity is established with different anesthetics are very similar. However, this does not mean that anesthetic-induced EEG isoelectricity represents a single physiologic state that is independent of the applied agent. Barbiturates, for instance, cause a uniform depression of CBF and CMR, while isoflurane suppresses the CMR and CBF of neocortex more than that of other portions of the cerebrum.

Anesthesia also interferes with other systemic physiological parameters, which in turn may strongly affect CMR, CBF and, as a result, fMRI in anesthetized monkeys. For example, CMR decreases by 6 to 7 percent per degree Celsius of temperature reduction. Just like certain anesthetics, hypothermia can also cause isoelectricity of the EEG (at about 20ºC). Note, however, that in contrast to anesthetics, temperature reduction beyond that at which isoelectricity first occurs does produce a further decrease in CMR. This is because hypothermia causes proportional decreases in the rate of energy utilization associated with both electrophysiologic function and the maintenance of cellular integrity. $CMRO_2$ at 18ºC is less than 10 percent of the normothermic control value, and this probably accounts for the brain's tolerance for moderate periods of circulatory arrest at these and lower temperatures. It is worth noting here that in our initial experiments hypothermia of a couple of degrees abolished the BOLD signal altogether. Hyperthermia has the opposite influence on cerebral physiology. Between 37 and 42ºC, CBF and CMR increase. However, above 42ºC a dramatic reduction in $CMRO_2$ occurs, indicating a threshold for the toxic effect of hyperthermia, which may result from enzyme degradation.

CMR and CBF (and consequently BOLD) are also influenced by the partial pressure of carbon dioxide (PaCO2) and oxygen (PaO2). In fact, CBF varies directly with PaCO2. The effect is greatest within the range of physiologic PaCO2 variation. CBF changes by 1 to 2ml/100g/min for each 1 mmHg change in PaCO2 around normal PaCO2 values. This response is attenuated below a PaCO2 of 25 mmHg. Under normal circumstances CBF sensitivity to changes in PaCO2 appears to be positively correlated with resting levels of CBF. Accordingly, anesthetics that alter resting CBF cause changes in the CO2 response of the cerebral circulation. However, CO2 responsiveness has been observed in normal brain during anesthesia with all the numerous anesthetics that have been studied. The changes in CBF caused by PaCO2 are apparently dependent upon pH alterations in the extracellular fluid of the brain. Note that in contrast to respiratory acidosis, acute systemic metabolic acidosis has little immediate effect on CBF because the blood-brain barrier excludes the hydrogen ion from the perivascular space. Changes in PaO2 from 60 to over 300 mmHg have little influence on CBF, but below a PaO2 of 60 mmHg, CBF increases rapidly. The mechanisms mediating cerebral vasodilation during hypoxia are not fully understood but may include neurogenic influences initiated by peripheral chemoreceptors and direct vascular hypoxic effects mediated by lactic acidosis. At high PaO2 values CBF decreases modestly.

In conclusion, correct maintenance of anesthesia and optimization of the animals' physiological state is absolutely critical, not only for ensuring the well-being of the animal, but also for running a meaningful fMRI experiment altogether.

ANESTHESIA PROCEDURE FOR MONKEYS

Scientists in the laboratory who intend to conduct experiments with anesthetized monkeys are strongly urged to read this section before they start familiarizing themselves with the practical aspects of our laboratory anesthesia. The section is brief and refers exclusively to the anesthesia regimes we use in

the lab. It concentrates on absolutely necessary background information rather than providing a general and thorough treatment of the relevant topics.

❑ **Arranging with the veterinarian the date and time of the procedure**
❑ **Notifying the animal care persons to ensure preoperative fasting**
❑ **Preparing all drugs, tools, and equipment**
❑ **Premedicating the monkey**
❑ **Inducing anesthesia**
❑ **Applying optical corrections**
❑ **Adjusting the visual stimulation system through the fundus camera**
❑ **Maintaining anesthesia during the experiment**
❑ **Guiding the emergence from anesthesia**
❑ **Returning the animal to its home cage**
❑ **Cleaning and organizing the room**

In general, the procedure for anesthetizing a monkey involves the following:

In our SOP there is a complete description of our preparative procedures. Please consult the appropriate sections or ask the laboratory technicians for relevant information. The following section will concentrate only on the premedication, induction, maintenance and emergence procedures. At the end of the chapter you will find some figures that illustrate the usage of various tools and devices that we typically use in our procedures.

Premedication

Premedication refers to the processes indicated for alleviation of anxiety, facilitation of the induction of anesthesia, reduction of salivary and bronchial secretions, and minimizing of anesthetic requirements. In monkeys anesthesia premedication is indispensable, as chemical restraint is always needed for intravenous injection of anesthetics or drugs in general. Preoperative fasting precedes the premedication of the monkeys (approximately 8 hours before a procedure) to decrease the risk of pulmonary aspiration. Water is given until a few hours before the procedure.

The most common preanesthetics used in monkey anesthesia are the anticholinergics, the benzodiazepines, and dissociative anesthetics such as phencyclidine and ketamine, the latter being the standard drug used in the laboratory. All preanesthetics are administered intramuscularly in the caudal thigh muscles. What follows gives a brief description of the preanesthetic drugs used in our laboratory.

Robinul (glycopyrrolate) i.m. We prefer glycopyrrolate over atropine because the highly polar quaternary ammonium group of glycopyrrolate limits its passage across lipid membranes such as the blood-brain barrier, in contrast to atropine sulfate and scopolamine hydrobromide, which are non-polar tertiary amines and penetrate lipid barriers easily.

The drug blocks the activity of parasympathetic nerves (vagal nerve). This has several advantages. First, during laryngoscopy the vagal nerve may be stimulated by mechanical force applied to the pharyngeal tissues. In response, the vagal nerve may cause bradycardia by slowing the sinus node of the heart. Secondly, ketamine, which is applied in the following step, causes salivation. If there is a lot of saliva present in the pharynx a few drops may reach the vocal cords or the entrance to the larynx. This may cause laryngeal spasm (laryngospasm), a reflex which can be life-threatening and often leads to hypoxia due to respiratory arrest. The vagal nerve is also responsible for control of salivation, so robinul protects against laryngospasm, too. Robinul begins to act immediately after uptake. However, to avoid problems with salivation is better to wait about 10 minutes after administration, because saliva that has already been secreted is obviously not influenced by the drug.

Ketamine i.m. This is a dissociation anesthetic that causes loss of consciousness and tolerance to minor surgical procedures. Ketamine is very different from all known anesthetics. Spontaneous respiration is preserved (and sufficient), and laryngeal reflexes such as coughing are maintained (although there are reports of aspiration in non-intubated human patients). Typically monkeys will make many unwanted movements that are unrelated to any stimulation, and show no anxiety whatsoever. The spontaneous tension (tone) of the muscles stays normal and the eyes open, so the animals (or humans) do not appear relaxed or sleeping. Each time a monkey moves during ketamine anesthesia one has to check carefully whether it was really correlated to some stimuli; generally it is not. Blood pressure and heart rate may increase during ketamine anesthesia, especially during induction. For i.m. administration the dosage is 2.5 times the dosage for intravenous sedation. Overdose of the drug does not pose any danger or highly undesired effect to the monkey despite an enhanced duration of anesthesia. Very low doses which do not cause loss of consciousness are good analgesics.

Induction

While the monkey is at the typical dissociation state induced by ketamine, noninvasive monitoring is applied, including a three-lead electrocardiographic (ECG) system, pulse oxymetry, noninvasive oscillometric blood pressure (NBP) measurement with the appropriate sized cuff, and temperature measurement with a rectal probe. SpO2 during the fMRI experiments works well when the Hewlett Packard probe is placed on the tongue of the animal and the Nonin probe around a finger. A 20-gauge catheter is subsequently introduced into the saphenous vein. When placing the catheter, remember to start as distal as possible, in case you fail to enter the vein with the first attempt. The catheter is used for the administration of various agents for the induction and maintenance of anesthesia, and the intravenous administration of fluids (lactated Ringer's). A drip rate of 10ml/kg/h is used. Anesthesia is then introduced with the following steps:

Preoxygenation

Preoxygenation is started without further drugs. It is meant to safeguard the animal against hypoxia for around 3-5 min should ventilation or spontaneous breathing fail for whatever reasons. During ketamine anesthesia you must make sure that the animal can be ventilated with a face mask. We use pediatric masks #0 and #1. You will need some help the first time you want to mask-respirate an animal. Mask respiration is done using pure oxygen to increase its concentration in the lung. In the normal lung breathing room air 80% of the volume is occupied by nitrogen. This nitrogen is washed out and to a large extent replaced by oxygen during the preoxygenation procedure.

Administration of Analgesics

Narcotics, such as fentanyl, morphine, meperidine, remifentanil, alfentanil, and sufentanil, are powerful painkillers which act on opioid receptors. They are used for pain relief and to suppress the strong stimulation of the autonomic nerves caused by the laryngoscopy and intubation of the trachea. They cause sedation but not loss of consciousness. Unfortunately, they may also cause vomiting and respiratory depression resulting in an impaired elimination of CO_2 that is easily recognized by the elevated end-tidal CO_2. In case of overdose use naloxone (remember to prepare it before the onset of the procedure), which has a short onset time. The analgesic we use is fentanyl. It may cause respiratory depression even in the doses used during induction (doses used for surgical anesthesia are certain to do this). Therefore – once again - it is important to preoxygenate. It takes up to 10 min for fentanyl to reach its peak effect. One has to wait for at least 3 min before proceeding with intubation, while maintaining ventilation by mask.

Administration of Hypnotics

We use thiopentone because it has the shortest time of action. Thiopentone and hypnotics, in general, cause loss of consciousness, relaxation of muscles, especially of the pharyngeal muscles, and short lasting respiratory depression. Unfortunately, they also causes pronounced cardiac depression, thus

lowering blood pressure. If additional hypnosis is needed because of complications during the intubation, half of the initial dose or less should be used, because while the hypnotic effects of the drug are reduced, the circulatory effects are very long lasting and add up immediately.

At very high doses thiopentone evokes a burst-suppression or even flat EEG. It is a free radical scavenger and depressant of immunologic function. You should therefore not anesthetize animals that may have infections etc. Spontaneous respiration is resumed within 1-2 min after thiopentone, despite the fact that hypnosis persists for a few more minutes. Thus, if you fail to maintain ventilation, the animal will resume breathing on its own in a short time. Hypoxia is an unlikely event with thiobarbiturates if the animals have been adequately preoxygenated.

Muscle Relaxation

Paralyzation (or muscle relaxation) can be achieved either by blocking the acetylcholine receptor at the postsynaptic membrane (competitive or non-depolarizing neuromuscular blocking agents) or by stimulating most receptors and thus exhausting the contractile response in a short time (depolarizing muscle relaxants). Competitive blockers (norcuronium bromide, pancuronium bromide, rocuronium bromide, atracurium, cis-atracurium) in general are long acting (more than 15 min) and have a long onset time (several minutes). Their side effects are few and not severe. Atracurium, which has the shortest time of action, is the only relaxant that causes a release of histamine that may lead to hypotension and bronchospasm.

Depolarizing blockers have a short onset time (1 min) and the effect is extremely short (around 3 min). They cause depolarization of the postsynaptic membrane and thus a lot of potassium leaks from muscles to the blood. High potassium levels in the blood (8 mmol/l) reaching the heart may cause arrhythmia or bradycardia but mostly for a short period. Cardiac arrest is possible, however, and can only be managed by external cardiac compression. Because a second dose of succinylcholine will liberate even more potassium from muscles, one has to wait for at least 5-8 minutes before administering a new dose. The drug should be applied as soon as consciousness is lost, i.e. shortly after thiopentone. But keep in mind that it is certain to cause respiratory arrest that leads to hypoxia in a short time. Apply it only if ventilation is successfully established. Do not try to facilitate ventilation by paralyzing the animal, in particular if you are using long lasting agents, e.g. norcuron, rocuronium, or atracurium.

Within 1 min after succinylcholine fasciculation of muscles will occur and intubation may be performed. Intubate as early as possible, if possible starting even before paralyzation is complete. The effect of succinylcholine is very short; there will be only time for 2-3 intubation attempts. Once again, do not reuse the drug within 5 min, as it can cause ventricular fibrillation (cardiac arrest). The action of succinylcholine will be short enough to avoid hypoxia if pre-oxygenation (ventilation by mask with pure oxygen, see above) was sufficient and intubation fails. Spontaneous breathing will restart in time.

Please note: If you cannot mask-ventilate the animal (for whatever reasons), and you still have to intubate (emergency surgery), do not paralyze the animal at all. The paralytic will strongly facilitate intubation, but intubation can be also performed without paralysis, following the administration of the analgesic, 5-10 minutes after spraying the vocal cords with a local anesthetic like xylocaine.

Immediately after intubation check the CO_2 waveform. If the CO_2 waveform starts with a small wave and increases within 5 respirations to above 25 mmHg it's a good sign, but if it starts around 25 mmHg, and it diminishes with successive expirations the tube is most likely not in the trachea. If no CO_2 waveform is detectable within 5 breaths check CO_2-monitor by measuring your own expiration. If the monitor is O.K. remove the tube and resume ventilation by mask with pure oxygen. Details about the laryngoscopy and intubation are given at the end of this section (Direct Laryngoscopy and Endotracheal Intubation).

Until the tube is secured hold it with one hand resting on the jaw of the animal. You can check the appropriate depth of the tube in the trachea by having another look at the larynx with the laryngoscope. Once you clearly see the intubation site pull the tube backwards very slowly until you notice that the vocal cords start slightly to expand. This indicates that the cuff of the tube is positioned directly behind the vocal cords. Push the tube 1cm back into the trachea to avoid unnecessary irritation

of the vocal cords. Tape the tube to the head and then release the tube. After the tube is secured check with a stethoscope whether the breath-sound is the same on both sides. If there is a difference in loudness pull out the tube very carefully while ventilating until the breath sounds are identical on both sides. If the endotracheal tube is inadvertently removed or intubation fails during induction resume mask ventilation immediately. Do nor try to re-intubate first. Keep in mind that the animal needs oxygen; the tube has secondary priority! The induction will keep the animal anesthetized for about 10 min. When the blood pressure starts to increase towards the initial value, start with maintenance of anesthesia.

Maintenance

In all fMRI experiments we use volatile anesthetics, more specifically isoflurane. The actions of volatile anesthetics are basically the same (but with different time courses). Vasodilation (reduction in vascular tone) occurs in arterial and venous vessels as well as reduction in cardiac performance (negative inotropic effect). To keep up performance under these conditions the heart needs a higher preload, i.e. a higher return of blood. The resulting circulatory change is a relative deficit in intravascular volume (blood volume), which must be replaced to keep blood flow within adequate limits. The replacement is done with artificial colloids not containing hemoglobin, resulting in hemodilution. In healthy animals hypovolemia can be detected by a decrease in arterial blood pressure (NIBP) that we restore by administering fluids or colloids. Another effect of volatile anesthetics is bronchodilation. Skeletal muscle tone is also reduced by volatile anesthetics and thus the demand for paralysis is also reduced. Isoflurane acts on the GABA receptors. Its cerebral effects are depression of neuronal activity (in monkeys 2.0% isoflurane usually depresses activity entirely). Despite the reduction in CMR following the depression of neural activity CBF is enhanced because of vasodilatation.

Isoflurane is started at 0.4% immediately after intubation. The isoflurane concentration is set to achieve the desired level end-tidally (end-tidal concentration resembles brain tissue concentration more closely than inspiratory concentration). In all our fMRI experiments, end-tidal isoflurane is about 0.4–0.45%. During the maintenance of anesthesia, vegetative stability and analgesia are achieved by administering fentanyl approximately every hour or by constantly infusing remifentanil (Ultiva) without using isoflurane. As mentioned above, the first dose of fentanyl is already administered during intubation, and may be supplemented 10 min before the fixation of the head and the placement of the contact lenses.

Muscle relaxation is accomplished by means of the short acting mivacurium chloride, which is started immediately after the monkey is correctly positioned, and after it has been established that the anesthetic depth is adequate. Remember that bolus injections of the drug cause severe drops in blood pressure. Avoid bolus injections whenever possible. Check the effectiveness of the paralysis by observing any possible movement in the animal's fovea through the fundus camera.

Careful examination of the respiratory and cardiovascular parameters is the core of successful anesthesia maintenance. At the outset, remember that isoflurane concentration cannot be changed much because of the experimental requirements. It can be arbitrarily increased only in emergency situations. When maintenance is running smoothly the actions you take are usually to increase blood volume, to adjust the amount of analgesics, to adjust respiratory parameters, and to control the perfusion of paralytic.

Changes in Blood Pressure and Heart Rate

High blood pressure and a fast heart rate should be thought of first as a sign of respiratory trouble, i.e. hypercapnia or hypoxemia, although such events have been rare in our procedures. If this can be ruled out by checking end-tidal CO2 and oxygen saturation by means of pulse oxymetry, then anesthesia may be too light, and another dose of the analgesic (fentanyl) should be given. Keep in mind that it will take 10 long minutes until the opiate takes effect.

Careful observation of the waveform of the pulse oxymeter, the so-called plethysmogram, is important for assessing the cardiovascular condition of the anesthetized monkey. The displayed signal reflects the absorption of infrared light by the tissue. The hemoglobin content, which is the main absorber of infrared light in tissue, changes with every heartbeat. During systole (contraction of the heart muscle) blood flows rapidly into the tissue and increases absorption. High amplitude on the plethysmogramm indicates good perfusion with hemoglobin. A diminishing amplitude indicates poor perfusion. Additionally, one can detect changes of the baseline and amplitude synchronous to the respiratory cycle.

A low-amplitude plethysmogramm may be the result of reduced cardiac output or missing hemoglobin, but also of significant vasoconstriction that is caused by light anesthesia or as a reflex to a low blood pressure (e.g. by posting the animal upright). Dilution of the blood (low hematocrit) to a great extent also reduces amplitude because the blood perfusing the tissue has a very low hemoglobin content. A flat plethysmogramm that cannot be accounted for by instrument failure or a misplaced probe is a very serious indication of heart failure. While this is very uncommon in healthy animals, it may still be caused by high positive end-expiratory pressure (PEEP).

Respiratory undulations in the plethysmogramm show that the output of the heart fluctuates greatly from inspiration to expiration. Because spontaneous breathing and - to an even greater extent - ventilation influences or impairs venous return to the heart these respiratory undulations indicate that the heart is not filled sufficiently during both respiratory phases, i.e. hypovolemia (see below) is starting to occur or may be present if fluctuations are pronounced.

Hypovolemia

Low blood pressure and tachycardia are signs of low intravascular volume and should be treated by means of volume expansion. If you administer a plasma expander quickly blood pressure will respond immediately. This is one way to check that hypotension was indeed caused by a volume deficit. If blood pressure cannot be restored by volume expansion and the pulse oxymetry signal is normal one should think about reducing anesthesia. As first step this should be done by delaying the next dose of fentanyl or by reducing isoflurane, especially if high concentrations are used.

Albumin and some other plasma protein solutions aid in maintaining osmotic pressure in the intravascular compartment. Maintenance of osmotic pressure has been identified as a principal factor in preserving intercompartmental water balance. Transcompartmental water balance may be affected by modifying one or more of the following factors: capillary membrane filtration rate, capillary perfusion pressure, interstitial tissue hydrostatic pressure, plasma oncotic pressure, and tissue oncotic pressure. If there is a lack of intravascular volume (low blood pressure, high heart rate, respiratory undulations in plethysmogramm) one should increase intravascular volume. This can be done with so-called crystalloids (saline, lactated Ringer, or Ringer) and colloids (HES). The first one (crystalloids) will stay intravascular for around 20 min, after which time 80% will have left circulation and caused edema in all organs. Of course water is needed to keep the body hydrated, but that's a different story.

Colloids stay intravascular for 2-4 hours and are eliminated by metabolic breakdown. These compounds keep themselves and water inside the vessels by oncotic means and are best for volume replacement. Every increase in intravascular volume is only possible without providing hemoglobin, i.e. hemoglobin content or concentration in the blood will decrease. Cardiac output must increase to keep oxygen supply up and will increase by physiologic mechanisms.

If volume expansion is performed it should be done rapidly. Around 10-20 ml of HES should be given within 1-2 min and plethysmogramm, blood pressure and heart rate must be monitored closely. One will see a transient increase in plethysmogramm amplitude and blood pressure and a decrease in heart rate. (In high dose opioid anesthesia the heart rate response will be blunt.) The presence of this pattern indicates that volume expansion was necessary. If the effect persists after the end of infusion the amount of volume expansion was sufficient and adequate intravascular volume is achieved for the next 30-60 min.

Intravascular volume is best done by monitoring urine excretion. As mentioned above, if cardiac output is not sufficient blood flow to the kidneys is reduced, resulting in reduced diuresis at an early

stage. A healthy animal or human being will be able to excrete huge amounts of fluids if one tries to overload them. Only in cases of heart failure does this not apply. Therefore a good or even more than normal diuresis indicates adequate cardiac output and intravascular volume because otherwise the body would have reduced diuresis. Maintenance of hydration is done with 80-100 ml/die/kg with saline or Ringer. Further addition of salts is not necessary within one day.

The total amount of liquids given during an experiment (Ringer, HES, Mivacron) should not exceed 10-15 ml/kg/h. In particular, remember to reduce the infusion rate of saline by 15-20 ml/h when you switch on the Mivacron infusion. Excessive use of HES or/and a too high infusion rate can result in a dangerously full bladder of the animal. You might not necessarily see any signs for that during the maintenance of anesthesia but run into a serious emergency situation without even noticing it.

Management of ventilation

Ventilation has three important functions: elimination of CO_2, uptake of oxygen (these both correspond only to gas volumes in the lung) and interface between circulation and lung tissue (gases and blood). Elimination of CO_2 is done by simple wash out via the respiratory gas, which never contains CO_2 in inspiration. Only minute volume is used to monitor CO_2. Increasing ventilation will decrease CO_2.

The arterial partial pressure of CO_2 is very important to the brain. Cerebral blood flow depends very closely on the arterial pCO_2. Unfortunately $paCO_2$ and end-tidal CO_2 are different. In general arterial pCO_2 is 4-8 mmHg above end-tidal CO_2. But this difference, the so-called delta CO_2, depends on lung function and circulation and may be influenced by measurement artifacts.

End-tidal CO_2 can only be a reliable indication of arterial CO_2 if at the end of expiration the plateau of the CO_2 waveform is horizontal or constant. If at the end of expiration the CO_2 waveform is still directed upwards this will not give a reliable reading of end-tidal CO_2. This can be overcome by applying a longer than normal expiration (expiratory hold) to see what the plateau really is. If there is any doubt about CO_2 one has to do an arterial/capillary blood gas analysis to check for pCO_2. But the difference between capillary and end-tidal CO_2 will only stay constant if no circulatory changes occur, for example in positioning or blood pressure.

If spontaneous ventilation occurs while the animal is still being ventilated, a serious problem has occurred. An animal which tries to breath on its own while anesthetized is being insufficiently ventilated. The endotracheal tube may have become dislodged inadvertently, the settings of the ventilator may be wrong, or the end-tidal CO_2 measurement may be false, e.g. because of sucking in additional air from the outside. This emergency can be detected by a camel-shaped CO_2 waveform or by inspiratory efforts of the animal visible in the airway pressure waveform (negative peak). You should thoroughly check the complete respiratory system, i.e. tube, connecting tubes and sampling tubes, and as a last resort interrupt the experiment to fix the problem. One should not try to continue if spontaneous respiratory efforts are present. This condition may lead to hypoxia and death.

Uptake of oxygen is not so much dependant on ventilation as on the gas content of the lungs or the inflation level of the lungs (The lungs are never completely empty. A residual volume of gas cannot be expired.) If one keeps ventilation constant but forces an additional amount of gas into the lungs oxygenation will improve. If oxygenation of blood is low as indicated by a low oxygen saturation (SpO2 below 94%) one has to increase the inflation level of the lung by increasing PEEP or increasing tidal volume. Increasing FiO2 will also increase oxygen saturation but will not improve the underlying condition and thus will only do the job for a limited time. There is no way to avoid increasing the inflation level if oxygenation is poor in the long run. This is done by increasing inspiratory pressure, which will in turn increase the tidal volume. The inspiratory pressure should be changed very carefully and slowly in steps of 0.5 cmH_2O. A tidal volume of 10 ml/kg should never be exceeded for longer periods to prevent damage to lung tissue; this is all the more true in longer-lasting anesthesia. In order to maintain a stable respiration and a good alveolic gas exchange it is recommended that the lung be inflated once every 3-4 hours with a PEEP of 16 cmH_2O for 3 breath strokes.

When increasing the inflation level of the lungs with the measures suggested one must keep in mind that an increased inflation level also means an increase in intrapulmonary and thus intrathoracic

pressure. Increased intrathoracic pressure works against venous return, i.e. the heart will become empty. Thus increasing the inflation level to improve oxygenation will cause a relative deficit in blood volume, causing a drop in blood pressure and cardiac output. If not done carefully the decrease in circulation can outweigh the improved oxygenation, resulting in no change at all or even deterioration of oxygen saturation. Therefore it may be wise sometimes if one feels pushed to use ever-increasing PEEP to reduce it to a great extent and see what happens.

Inspiratory oxygen concentration (FiO2) should be kept as low as possible (below 30%) because in the long run nitrogen keeps lung volume stabilized where no uptake is present. Furthermore, the animal can do quite well if awake and breathing 21% oxygen. If this is not sufficient during anesthesia one has to check the problems, mostly inflation level. Additionally because of the shape of the oxygen hemoglobin binding curve pulse oxymetry will only be a sensitive monitor if SpO2 is below 99%. Unnecessarily high FIO2 would damage lung tissue (oxygen is toxic above 400 mmHg), make pulse oxymetry ineffective as a monitor and remove an accurate feedback source for the management of ventilation.

Despite all this, during induction it is wise to use 100% oxygen for pre-oxygenation. This also applies to every other situation where one anticipates severe trouble with airways: Every time one feels uncertain about the position of the tube one should change to 100% oxygen immediately to use the few breaths which may be available (e.g. before the tube slips out entirely) to deliver as much oxygen as possible to the animal so as to have more time available to cope with the problems. Also during emergence with increasing CO2 pure oxygen should be used to build some reserve for the period following extubation of the trachea.

Emergence

Muscle paralysis

Atracurium has a half-life time of about 20 min. It takes about five times this half-life time for elimination; so you need more than one hour to establish safe, spontaneous respiration. Therefore this drug should be stopped first. One can try to monitor the decay of the drug effect by means of a neuromuscular monitor. In humans it is thought that if the T1/T4 ratio is above 25% one can use the reversal drug neostigmine combined with atropine. Mivacron, which has a 2-3 minute-long half-life, is a much better drug for our fMRI experiments, and this is what we commonly use. However, atracurium may be needed for animal showing no adequate response to mivacurium.

Anesthesia

Volatile anesthetics are eliminated quickly if ventilation is kept at normal levels (tidal volume of 10ml/kg, with a rate of 24 strokes/min). But this applies only if high concentrations of isoflurane were used. For the fMRI experiments isoflurane concentration is already 0.4-0.45%, and can be kept there until the paralysis is completely reversed to keep the animal sedated after extubation of the trachea. Sometimes it might be necessary to use much higher doses of isoflurane during emergence if the animal shows too much reflex activity.

During emergence spontaneous breathing has to restart. For this to occur an increase in CO2 to around 50 mmHg is generally required. This so-called weaning procedure is performed by reducing inspiratory pressure (and, as a result, tidal volume). This provides a sufficient stimulus for spontaneous breathing to start if respiratory depression by opioids has ceased. This can be seen in the CO2 waveform and at the thoracic and abdominal wall of the animal. This will also provide an indication of residual curarization (paralysis). In the respiration mode "Pressure Support" (Druckunterstützung), start with a trigger sensitivity below a PEEP of –2. The trigger sensitivity is used to set the value of negative pressure that the animal must produce in order to trigger a breath. The airway pressure, measured on the expiration side, is compared with the preset "Trig. Sensitivity + PEEP". If the airway pressure drops below that value a breath is triggered. Watch the CO_2 curve carefully and decrease the trigger sensitivity when the CO_2 curve indicates sufficient respiratory

activity from the animal. At a level of ~-10 cmO2 switch to CPAP (Continuous Positive Airway Pressure) and start again with a trigger sensitivity of –2. In this mode the animal breathes spontaneously through the ventilator at an elevated pressure level (PEEP). The trigger sensitivity is set at a position which allows the animal to trigger the ventilator. Upon triggering, the inspiration valve opens, and the animal can inspire through the ventilator and control the tidal volume and respiratory rate. You might have to switch back to pressure support if the respiratory activity of the animal is not sufficient.

The two remaining drug effects can be easily differentiated: If the movements look good and not weak but CO_2 remains high around 50 mmHg there is still a residual effect from the opioids. Respiratory rate will be low in this situation. If respiratory rate is high but the movements look a little bit like seizures and the animal apparently suffers stress this indicates an overhang from paralysis. Do not reduce ventilation further or try to provide more respiratory stimulus. Learn to wait; things will improve soon.

Extubation should be done when the animal can keep a constant end-tidal CO_2 within a range of 42-44 mmHg or better in CPAP mode with a trigger sensitivity of –12 to –16 cmH$_2$O. Shortly before extubation one person should lift the animal into a sitting position slightly leaned forwards to allow saliva to drain and prevent aspiration immediately after extubation. At the same time a second person has to secure the tube against the jaw of the animal to avoid movement of the tube in the trachea and, thus, irritation and a strong stimulus for swallowing and coughing. In some cases the animal will become quite agitated and alert before this happens because lack of breath is a very powerful stressor and wake up call. If spontaneous breathing does not look good or sufficient one should reinstate isoflurane for around 10 min.

OPTICAL CORRECTIONS

Following the restraint of the animal, add one drop of 1% ophthalmic solution of the anticholinergic cyclopentolate hydrochloride into each eye to achieve cycloplegia and mydriasis. Refractive errors must be measured after the induction of paralysis, approximately one hour after the application of cyclopentolate. Subsequently contact lenses (hard PMMA lenses, Firma Wöhlk, Kiel) with the appropriate dioptric power must be used to bring the animal's eye to a focus on the plane at which the stimuli are to be presented. The eyes of the monkeys are kept open with custom-made irrigating lid specula to prevent any drying of the tissues. The specula have been constructed so as to irrigate the eye at the medial and lateral canthus with a saline infusion at a rate of 0.05ml/min.

MANAGEMENT OF COMPLICATIONS

Respiratory Complications

Hypoxemia, with or without hypercapnia, is a consequence of all respiratory complications. Even a mild degree of hypoxemia has the potential to cause harm. Cyanosis is a late sign of impaired oxygenation; it is not obvious unless there is more than 5 g of deoxyhemoglobin in arterial blood. The color of mucous membranes - conjunctival, oral - are a more sensitive index of cyanosis than skin color. Poor lighting and increased pigmentation of the skin can make cyanosis even harder to detect. Observing the color of blood shed at the site of the operation instead of the color of the skin is a more sensitive method to detect arterial desaturation. In order to prevent hypoxemia, the inspired oxygen concentration should always be increased in the event that respiratory complications develop under anesthesia.

Coughing

Coughing is a protective reflex in response to an irritant in the airway. This is a complication seen with light anesthesia levels. Irritation may be caused by the vapor of a volatile agent, the endotracheal tube, saliva or mucus, or even gastric contents. Treatment should be directed at removal of the irritant. The concentration of volatile agents should be increased only gradually during induction; the oropharyngeal airway should be inserted only when the level of anesthesia is appropriate; and the fluid contents in the pharynx should be cleared by solution. If gastric fluid is suspected, tracheal intubation is indicated, and the patient should be examined for signs of aspiration.

Breath-holding

Breath holding is seen frequently during inhalation induction. It may also be seen in the animal breathing spontaneously during maintenance. During inhalation induction, breath-holding is a transient phenomenon. During maintenance, breath holding with or without laryngospasm can occur when a painful stimulus is applied at an inadequate level of anesthesia. Spontaneous respiration will return when the painful stimulus is withdrawn, but surgery should be not allowed to proceed until the depth of anesthesia is increased. If breath-holding persists, attempts should be made to ventilate the lungs manually. Manual ventilation is usually successful if laryngospasm is absent. If breath-holding occurs repeatedly, intubation with ventilation is suggested. Do not mistake breathholding for the agonal gasps seen in respiratory arrest.

Airway Obstruction

The most common cause of airway obstruction in an unconscious animal lying supine is the tongue falling backward to lie on the posterior pharyngeal wall. Snoring during inspiration and grunting during expiration are problems which can easily corrected by flexing the neck, extending the head, and supporting the angles of the jaw. In some cases, the placement of an oropharyngeal airway may be necessary. Kinking or compression of the endotracheal tube can cause airway obstruction in the intubated animal. The use of a reinforced tube will eliminate this problem.

Hypoventilation

There are many reasons for hypoventilation during anesthesia, including respiratory depression by anesthetic drugs, inadequate recovery from muscle relaxants, and airway obstruction. The consequence of hypoventilation is hypercapnia, i.e. excessive carbon dioxide levels. Hypercapnia can coexist with hypoxemia, but hypercapnia can also exist alone if a high inspired oxygen concentration is administered. An elevated $PaCO_2$ is the most reliable index of inadequate ventilation, but certain clinical signs are also helpful: tachycardia, hypertension and increased oozing in the surgical field. In addition, careful observation may reveal other clinical signs associated with the underlying cause of hypoventilation, for instance apnea following intravenous barbiturates, shallow breaths in the animal receiving a volatile agent, a slow respiratory rate in an animal receiving opiate, signs of partial paralysis, and signs of airway obstruction. Severe hypoventilation is a potentially fatal emergency. Animals found in hypoventilation should be given assistance immediately, and the inspired atmosphere should be enriched with oxygen.

Circulatory complications

Hypotension

It is not uncommon to see blood pressure fall by 10-15% following the induction of anesthesia in healthy animals. A fall in blood pressure greater than 20%, however, should be investigated. Severe hypotension calls for decisive actions. The inspired oxygen concentration should be increased immediately, and specific treatment should be directed at the elimination of the underlying cause. Decrease the depth of anesthesia if possible; rectify any mechanical interference with venous return or

cardiac output; treat cardiac arrhythmias; drain pneumothorax. In severe cases an inotropic agent such as dopamine 5-10 micrograms/kg IV infusion is indicated. Keep blood pressure charts.

Hypertension

Hypertension usually occurs as a response to noxious stimuli and also indicates inadequate anesthesia. Other causes include hypercapnia and hypoxemia, or fluid overload. The pressor response to tracheal intubation may be prevented by increasing the depth of anesthesia, although this may not be appropriate in ill animals. In severe cases one can administer 1.5mg/kg lidocaine intravenously. Generally hypertension should be treated by increasing the concentration of the volatile agent or by additional increment of an opiate.

Arrhythmias

Sinus tachycardia following the administration of atropine, pancuronium, gallamine, or ketamine is benign and requires no treatment. Sinus tachycardia in response to endotracheal intubation is a sign of inadequate anesthesia and will disappear when the depth of anesthesia is increased. In contrast, atrial flutter and fibrillation is rare but always serious. In the operating room, atrial flutter or fibrillation of acute onset should be treated by direct-current cardioversion. Generally a lower than normal heart rate is common in animals receiving halothane or large doses of morphine or fentanyl for maintenance. Treatment with atropine is usually not necessary, unless there is a concurrent fall in blood pressure.

Anesthesia Chart at MPIK

Premedication

- Administer 0.01mg/kg Robinul
- Wait 10 minutes
- Administer 15mg/kg ketamine
- Transfer the monkey into the NMR room
- Apply noninvasive monitoring

Induction

- Preoxygenation (mask respiration for 3-5 minutes)
- Administer fentanyl 3ug/kg 5 minutes before intubation (opiates in MICROgrams)
- Additional preoxygenation to lower CO_2 down to about 25-30mmHg
- Administer thiopental (Trapanal) 5mg/kg 5 minutes after fentanyl
- Administer succinylcholine chloride (Lysthenon) 3mg/kg immediately after thiopental
- Continue mask respiration and intubate after 30 seconds
- If intubation fails, do not reuse succinylcholine; it may cause ventricular fibrillation
- Check CO_2 waveform to ensure tube is in the trachea (value 25-30mmHg)
- The induction will keep the animal anesthetized for about 10 min. If the blood pressure starts to increase towards the initial value start with maintenance of anesthesia.
- Start isoflurane at 0.4%

Maintenance

- Administer a bolus of mivacurium chloride (10mg) before attaching the eye-specula
- Start infusion of mivacurium chloride 5mg/kg/h in 50ml syringe
- If isoflurane is used, then administer fentanyl 3ug/kg as needed during monitoring

Emergence

- Stop the opioids about 1hr before the end of the experiment
- Stop the paralytics when you bring the animal out of the magnet
- Keep isoflurane at about 0.4-0.45% for sedation, higher dose might be necessary
- Extubate when spontaneous breathing occurs and CO_2 is systematically below 45mmHg

Monitoring and Care during Anesthesia

- Maintain hydration with 10 ml/kg/h with lactated Ringer
- Administer analgesics as needed
- Tachycardia and hypertension may indicate respiratory problems; check CO2 and SpO2 and if everything OK, then check depth of anesthesia
- *Tachycardia* and *hypotension* may indicate hypovolemia; check the plethysmogram. Respiratory undulations on the plethysmogram indicate that the output of the heart fluctuates greatly from inspiration to expiration. Hypovolemia may be the cause.
- If no undulations occur but the pleth signal is low, then the monkey may have low hematocrit
- If the animal is hypovolemic administer 10-20 ml of hydroxyethyl starch (amyl) within 1-2 min and monitor plethysmogram, blood pressure and heart rate closely.
- If the effect persists after the end of infusion the amount of volume expansion was sufficient and adequate intravascular volume is achieved for the next 30-60 min.
- Check the respiratory gas monitor regularly, and apply blood gas analysis if needed

Direct Laryngoscopy and Endotracheal Intubation

<u>Before initiating the intubation procedure, make the following preparations</u>:

❑ Verify that the anesthetic machine is operational.
❑ Select correct endotracheal tube size and verify integrity of cuff.
❑ Verify that laryngoscope is operational.
❑ Obtain cuff inflator, forceps, stylet, spray and lubricant.
❑ Verify means of pharyngeal suction.

<u>The procedure for endotracheal intubation is as follows:</u>

❑ Position the monkey's head such that the long axes of the oral cavity, of the pharynx and of the trachea are in a straight line. Provide 100% oxygen for 2 minutes so that the monkey can tolerate apnea during intubation without becoming hypoxic.
❑ Hold the laryngoscope in left hand and introduce the blade into right side of the monkey's mouth. Advance the blade posteriorly and toward the midline, sweeping the tongue to the left, out the visual path.
❑ When the epiglottis is in view, advance the tip of the laryngoscope blade into the vallecula formed by the base of the tongue and the epiglottis.
❑ Lift the laryngoscope upward and forward, in the direction of the long axis of the handle to bring the larynx into view. If the epiglottis is seen to overhang the larynx, advance the tip of the blade further into the vallecula. If the esophagus is seen, withdraw the blade until the larynx falls into view.
❑ When the larynx is in view, introduce the endotracheal tube from the right with its concave curve facing downward and to the right. Have the assistant retract the angle of the mouth on the right side if maneuvering room is required.
❑ If only the posterior portion of the glottis (the arytenoids) is in view, have the assistant apply gentle backward pressure on the thyroid cartilage so that the larynx can be brought into full view.
❑ Once the endotracheal tube is in place, apply positive-pressure ventilation while the assistant inflates the cuff gradually.
❑ Continue to ventilate the lungs, and rule out intubation of the esophagus by auscultation over the epigastrium.
❑ Rule out bronchial intubation by checking movement and air entry at the apices of the lungs.
❑ Fasten the endotracheal tube if correctly positioned.

<u>The extubation routine should be as follows:</u>

❑ Verify that the monkey is recovering from anesthesia and breathing spontaneously with adequate volume.
❑ Allow the monkey to breath 100% oxygen for 5 minutes to wash out nitrous oxide.
❑ Suction accumulated pharyngeal secretions.
❑ Verify that the monkey is not semiconscious; extubation at this plane can elicit laryngospasm.
❑ Lift up monkey to sitting position slightly leaned forward while securing the tube
❑ Deflate the cuff and remove the endotracheal tube quickly and smoothly during inspiration.

ANESTHESIA EQUIPMENT & MONITORS

The Siemens Respirator

Trigger Sensitivity below PEEP :
value of negative pressure that the animal must produce (relative to PEEP) in order to trigger a breath
In Pressure Control mode set to -14, for emergence in Press.Support and CPAP mode start with low Trigg.Sens. (-2) and increase according to the reaction of the animal (self-respiration, watch CO2 curve !!!)

Inspiration time :
always constant at 33%

Respiration rate :
always constant at 24 breaths/min

Pause time :
always constant at 0

PEEP:
Positive End-Exspiratory Pressure, keeps the lungs constantly inflated to prevent atelectasis, usually at 4cmH$_2$O

Switch between infants (Kinder, monkey anesth.) and adults (Erwachsene, standby mode with high Insp.Press.Level)

Insp.Pressure Level above Peep:
Constant inspiratory pressure that is added to PEEP , usually in a range between 6 and 12cmH$_2$O depending on the exsp. CO$_2$ level and the tidal volume V$_T$

Mode Selection :
Regular operation in Pressure Control mode (Druckkontrolle), Pressure Support (Druckunterstuetzung) for induction of emergence, CPAP (Continous Positive Airway Pressure) for continuation of emergence from anesthesia

Parameter selection :
value in display, most important parameters are Exp.Tidal Volume V$_{TE}$, Insp.Tidal Volume V$_{TI}$ and Exp.Minute Volume, difference V$_{TI}$-V$_{TE}$>10ml indicates insufficiently inflated or defective tube and requires immediate action

Medical Gas Systems

Main Source of Gases

The main sources of medical gases originate from banks of compressed gas cylinders. Each cylinder has a characteristic size (or volume), weight, and a gas-specific service pressure, which is the maximum pressure to which the cylinder can be filled at a temperature of 20° C. High-pressure cylinders are connected to the piping system via pressure regulators, often called the pressure reduction valves, which usually have add-on pressure gauges, and a safety relief mechanism, preventing explosion of the cylinder in the case of abrupt, great changes in the cylinder's pressure, as for instance in the case of fire. At MPI our main cylinders have a capacity of 50L, and are located in the basement in a room labeled *Gasversorgung*. They are arranged in a row, with a left and a right bank, each connected to a single central manifold control box. At any given time, one bank is active (the primary bank) while the other (the secondary or reserve bank) is shut off. Changeover from the primary bank to the reserve bank takes place automatically when the pressure in the primary bank drops below a preset level. Each bank has 2 pressure regulators for the 1-stage of pressure reduction, and 1 pressure regulator for the 2-stage. In addition each bank has 2 spring-type pressure gauges (Bourdon gauges). The gauge closer to the cylinder shows the cylinder's pressure, while the other shows the pressure of the pipeline after the 1-stage reduction. The cylinder pressure gauge is an indicator of the amount of gas available if the gas can be stored in gaseous form. Otherwise the 'cylinder plus gas' weight is the only indicator for the remaining amount of gas.

Table 1 Gas Source System at MPI

Oxygen cylinder pressure	200 bar (approx. 2940 psig)
Oxygen pressure reduction (Stage 1)	200 bar to 10 bar
Oxygen pressure reduction (Stage 2)	10 bar to 5 bar
Oxygen check valve will signal alarm at	12 bar
Nitrous oxide cylinder pressure	51 bar (approx. 750 psig)
Nitrous oxide pressure reduction (Stage 1)	51 bar to 10 bar
Nitrous oxide pressure reduction (Stage 2)	10 bar to 5 bar
Nitrous oxide check valve will signal alarm at	12 bar
Nitrous oxide weight	30 kg
Nitrous oxide weight plus cylinder weight	53.1 kg
Air pressure	5 bar
Vacuum pressure	10 bar (max)

DEFINITIONS

Distress – A state in which an animal is unable to adapt to an altered environment or to altered internal stimuli.

Pain – An unpleasant sensory or emotional experience that results from potential or actual tissue damage. In 1987, under the auspices of the Institute of Medical Ethics in England, a group of people consisting of scientists from academia and industry as well as campaigners for animal welfare, philosophers and lawyers published a report that spells out the criteria by which policy-makers can rigorously judge the animal suffering that may be involved in any particular research project. According to this report an animal can feel pain if it meets the following criteria:

- ❑ **It has receptors sensitive to noxious stimuli, which are present in functionally useful positions on/in the body.**
- ❑ **Its brain contains structures analogous to the human cerebral cortex.**
- ❑ **It has nervous pathways link receptors sensitive to noxious events and the higher brain.**
- ❑ **It has receptors in the central nervous system, especially the brain, that are activated by opioid substances implicated in pain control.**
- ❑ **Painkillers modify the response to noxious stimuli and are chosen by an animal given access to them when the experience is unavoidable.**
- ❑ **The animal responds to noxious stimuli by avoiding them or by minimizing the damage to its body.**
- ❑ **The animal's avoidance of noxious stimuli is relatively inelastic. The response is largely unchanged irrespective of how much the animal is rewarded for a particular behavior.**
- ❑ **The animal's response to noxious stimuli persists and it learns how to associate neutral events with noxious stimuli.**

Analgesia – A neurologic or pharmacologic state in which painful stimuli are so moderated that, though still perceived, they are no longer painful.

General Anesthesia – A state induced by one or a combination of agents to provide controlled, reversible depression of CNS function; a state the basic elements of which include unconsciousness or sleep, amnesia, analgesia, muscle relaxation, diminished motor responses to noxious stimuli, and reversibility.

Local Anesthesia – Local or regional anesthesia consists of techniques that depend on a group of drugs (called local anesthetics) that produce transient loss of sensory, motor, and autonomic function in a discrete portion of the body. Local anesthetics typically block impulse conduction in nerve fibers. Many substrates have local anesthetic properties, but only those that produce a transient and completely reversible inhibition of spike propagation are clinically useful.
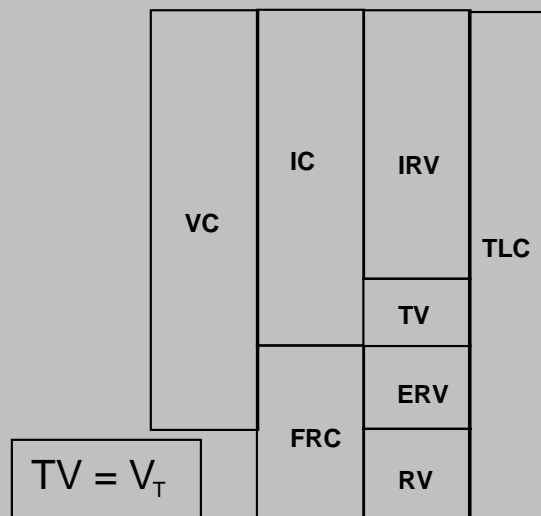
LUNG VOLUMES AND CAPACITIES

Lung Volume (% TLC)

IRV = 45-50%
TV   = 10-15%
ERV = 15-20%
RV   = 20-25%

$$TV = V_T$$

IC	IRV
VC	TLC

Volumes/Capacities in mL	Label	Human (70 kg)	Monkey (4-10 kg)
Vital capacity	VC	4800	230 - 580
Inspiratory capacity	IC	3800	170 - 360
Functional residual capacity	FRC	2400	61 - 115
Inspiratory reserve volume	IRV	3500	140 - 290
Tidal volume	TV	500	35 - 70
Expiratory reserve volume	ERV	1200	40 - 85
Residual volume	RV	1200	30 - 65
Total lung capacity	TLC	6000	265 - 700

Preparation of an MRI Experiment

Mydriatic Cyclopentalate,
one drop in each eye in the
beginning for pupil dilation

Special cloth for
lens cleaning
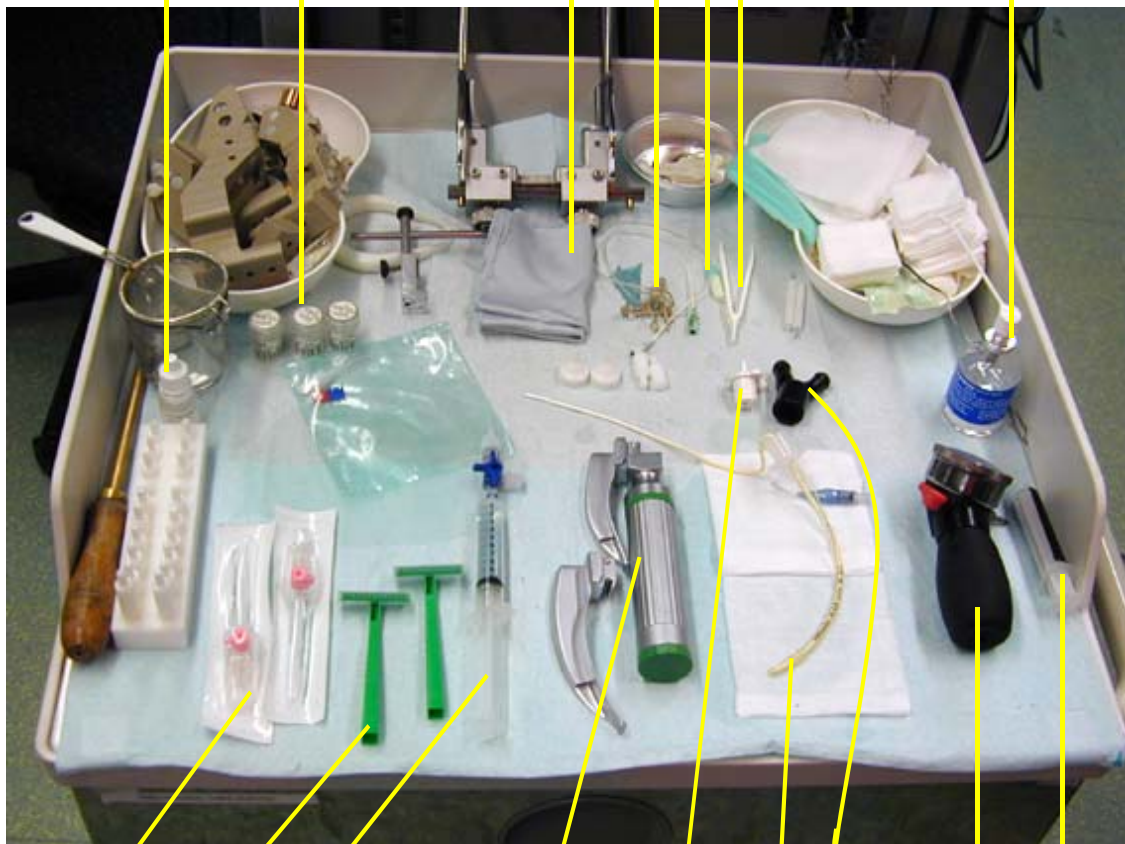
Xylocain spray

Optical lenses,
correct size given in the last
Mri experiment drug chart

Lid specula

Lens suction

Plastic forceps

IV cannulas

Razors

10ml saline
syringe with 3-way
stopcock and
extension

Laryngoscope
with spatula

Tube connector
(appropriate size)

Y-connector

Endotracheal
tube with
mandrin (guide)
inside

Syringe for
cuff deflation
before
extubation

Cuff pump

Preparing the MRI chair

IV line (300cm)

Eye irrigation lines
(2x (300cm+150cm
infusion line+3-way
stopcock))

Water hose for heating pad
(not kinked !)

ECG electrodes

Blood pressure
cuff

Water trap
between
breathing tube
extensions

Warm air supply

HP SpO$_2$ sensor

Nonin SpO$_2$
sensor

Breathing tubes
with artificial lung
and gas sensor
line

Respiration
masks
(different sizes)

b
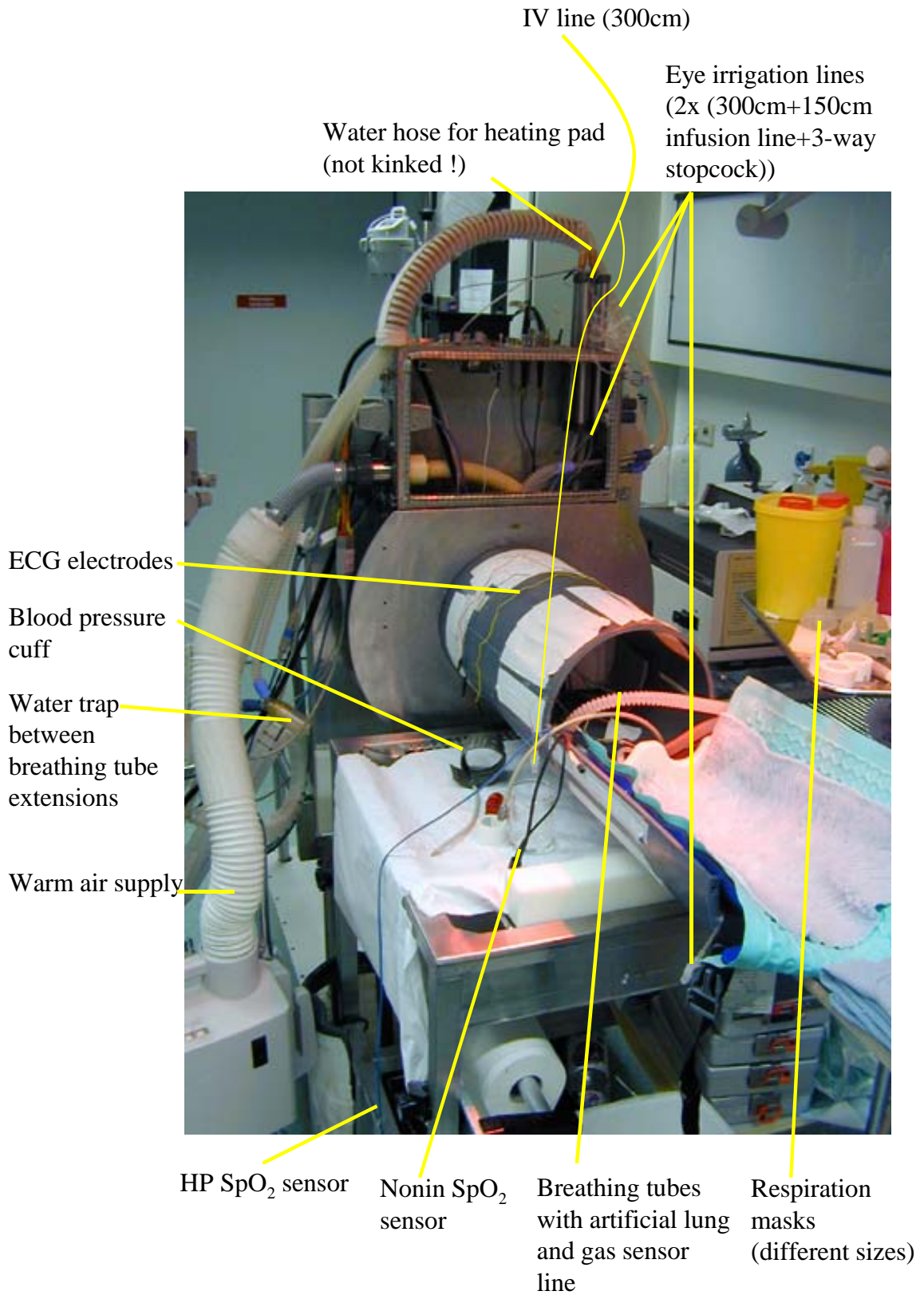
Preparing the IV stand
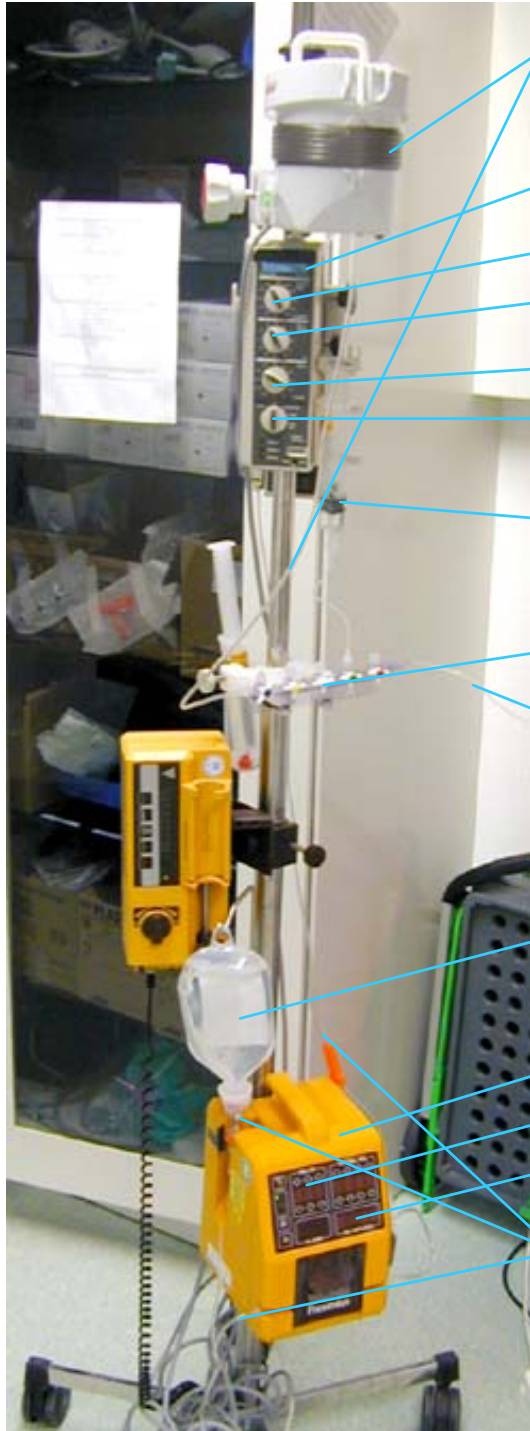
ASTOTUBE infusion extension wrapped around the infusion warmer starting with the white stopper on top

Muscle relaxant (Mivacron) infusion pump

Mivacron infusion rate, usually 0.75 ug/kg/h

Body weight, constantly set to 100kg

Bolus size, set to 0.15 ug/kg

Switch to "Infuse" and open stopcock at stopcock manifold to start Mivacron infusion

60ml syringe with 100% Mivacron + 15cm extension to the stopcock manifold

Stopcock manifold, input from infusion warmer (Astotherm, from left side), output 300cm infusion extension into chair

500ml lactated Ringer's solution

Infusion pump

Infusion rate

Total infused Ringer's solution

Intradrop infusion set, starting with the reservoir at the 500ml bag, running through the pump and the bubble detector on the right side of the infusion pump to the infusion warmer

MRI Anesthesia - Important devices and values

Timecourse of exp. CO$_2$

Blood oxygen saturation in % and Pulse rate

Nonin fibre-optic SpO$_2$ sensor

Exp.CO$_2$ (ideally constant at 32mmHg, keep >28 and <40) an Insp.CO$_2$ (2-4mmHg)

HP Anesthesia Gas Monitor

Insp. and exp. O$_2$, Insp. and exp. N2O, Insp. and exp. Isoflurane

Water heating pad control

Pulse curve from HP SpO$_2$ sensor

Infusion pump for eye irri-gation

Pulse rate and SpO2 i

Blood pressure Systolic/Diastolic (Mean)

HP Viridia Anesthesia Monitor

Isoflurane vaporizer with adjustment of Isoflurane level in %

Siemens Servo control panel

O$_2$ in the insp.gas in % (normally 21%, 100% during mask respiration, induction and emergence) and N$_2$0 level

Heart rate

HP Anesthesia Monitor

ECG