How does different fresh gas flows affect stress in rats during anaesthesia induction with sevoflurane in a chamber?

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Independent Project • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Veterinary Medicine and Animal Science
Veterinary Medicine Programme

Uppsala 2023

How does different fresh gas flows affect stress in rats during anaesthesia induction with sevoflurane in a chamber?

Hur påverkar olika färskgasflöden stress hos råtta vid anestesiinduktion med sevofluran i kammare?

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Credits:	30 credits
Level:	Second cycle, A2E
Course title:	Independent Project in Veterinary Medicine
Course code:	EX1003
Programme/education:	Veterinary Medicine Programme
Course coordinating dept:	Department of Clinical Sciences
Place of publication:	Uppsala
Year of publication:	2023
Copyright:	All featured images are used with permission from the copyright owner.

Keywords:	Rat, behaviour, stress, anaesthesia, inhalation chamber, excitement, induction, recovery, fresh gas flow, sevoflurane

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Abstract

The rat is one of the most used laboratory animals in Europe today. When replacement of live animals in research is not feasible, the aim is to improve the welfare of those animals by reducing stress and suffering. Anaesthesia in laboratory rats is a common procedure, and induction with inhalant agents is often associated with stress and discomfort in the animals. Therefore, one way to improve welfare would be to improve the induction quality. Additionally, research is lacking in the area about which factors influence stress in rats during inhalant anaesthesia induction.

The behaviour of ten Wistar rats was studied during induction of anaesthesia with sevoflurane using an inhalation chamber. The rats were randomized into two groups, receiving a fresh gas flow of three and six litres per minutes, respectively. The vaporizer was set to 8%. The behaviours were recorded inside the chamber during preoxygenation and during the induction and recovery. Latency to, frequency and duration of several behaviours were observed and compared between the two groups. Also, time to recumbency, first movement and regain of righting reflex was noted and compared between both groups.

No significant differences were found in frequency, latency or duration of the observed behaviours between groups, except for latency to ‘lie’ being longer in the low flow group. Stress-related behaviours were frequent in all rats during preoxygenation and induction. Rats induced with higher flow had a significantly faster induction than those in the low flow group.

Keywords: Rat, behaviour, stress, anaesthesia, inhalation chamber, excitement, induction, recovery, fresh gas flow, sevoflurane.

Sammanfattning

Råttan är ett av de vanligaste försökdjuren idag i Europa. I de fall det ännu inte finns metoder som kan ersätta djurförsök strävas det efter att förbättra djurens välfärd genom att minska stress och lidande. Ett sätt att göra detta är att förbättra induktionskvaliteten vid gasanestesi, vilket är ett vanligt förekommande moment och som ofta kopplas till stress och obehag hos djuren. I nuläget saknas det däremot forskning kring vilka faktorer som har betydelse för stress hos råtta vid induktion av gasanestesi.

Tio Wistar-råttor inducerades med sevofluran i inhalationskammare för att studera beteenden. Råttorna randomiserades till två grupper som inducerades med olika färskgasflöden på tre respektive sex liter per minut. Förgasaren ställdes in till 8 %. Råttorna filmades i kammaren under preoxygenering före gasen sattes på samt under induktion och uppvakning. Därefter utvärderades ett flertal olika beteenden där frekvens, latens och duration jämfördes mellan grupperna. Även tid till medvetslöshet och vaket tillstånd noterades och jämfördes mellan grupperna.

Inga signifikanta skillnader kunde ses mellan grupperna gällande frekvens, latens och duration av de beteenden som studerades, förutom att latensen till ”ligg” var signifikant längre i lågflödesgruppen. Stressrelaterade beteenden förekom hos alla råttor både under preoxygenering och induktion. Råttorna som inducerades med det högre flödet hade en snabbare induktion än de som inducerades med lågt flöde.

Nyckelord: Råtta, beteende, stress, anestesi, inhalationskammare, excitation, induktion, uppvakning, färskgasflöde, sevofluran.

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1. Introduction

The history of rats and humans goes centuries back in time. The rat was the first animal to become domesticated for the use of experimental science and appears for the first time in a documented experiment year 1856 (Modlinska & Pisula 2020). In 2019 the rat was the third most used laboratory animal in the European Union and Norway, with a population of approximately one million individuals (European Commission 2022). In other words, humankind can thank the rat for great success in biomedical research, but how do we show our gratitude?

In the year of 1986, the first legislation that aimed to protect animals used in experimental sciences was implemented in Europe. In 2010, the new updated Directive 2010/63/EU replaced the old one. This had even more emphasis on improved welfare for laboratory animals, implementing the principles of the "Three Rs"; Replace, Reduce, Refine (Directive 2010/63/EU). This EU Directive became world-unique since its ultimate goal is to completely replace the animals in experimental research over time (European Commission n.d.-a). However, on the occasions when animals still need to be used in research, Directive 2010/63/EU states that the welfare must be improved as much as possible for those animals, as in the third R: Refine. This is made by reducing factors causing stress, pain, and suffering.

Anaesthesia is commonly used within research to enable performance of invasive procedures on animals (Cicero *et al.* 2018). Therefore, one way of refinement could be to minimize stress during anaesthesia. This can be achieved by using anaesthetic protocols that promote animal well-being and reduce feelings of discomfort. One common way to induce anaesthesia in laboratory rats is with an inhalation chamber. For this, general recommendations are lacking, and there is a strong need for specific guidelines to be developed (Cicero *et al.* 2018). To enable this, firstly, factors affecting stress associated with anaesthesia in laboratory animals must be identified. In this study, the behaviour of ten rats is evaluated during induction and recovery of anaesthesia with sevoflurane, while comparing two different fresh gas flows. This study is a pilot to a greater research project that will evaluate several stress factors, and if the use of premedication can decrease stress in laboratory rodents during anaesthesia.

1.1 Aims of the study

This study is a pilot study that aims to investigate whether different fresh gas flows (FGF) influence the quality of chamber induction with sevoflurane in rats. Stress-related behaviours, time to recumbency during the induction, and time to first movement during the recovery will be evaluated.

2. Literature review

2.1 The Three Rs

The principle of the "Three Rs" were first mentioned by W.M.S Russel and R.L. Burch in 1959 when they published their book "The Principles of Humane Experimental Technique". The three Rs representing Replace, Reduce, Refine were intended by the authors to lead the way to humane research methods. Russel and Burch stated that the first R, Replace, should be the ultimate long-term goal. However, when live animals must be used, the researchers should aim to reduce the number of individuals as much as possible and at the same time refine these animals' environment to improve animal welfare. For example, refinement may involve reducing pain and distress among the animals or improving husbandry to make the animals feel more comfortable (European Commission n.d.-b). As a result of reducing stress, refinement can also improve the quality of data collected from the animals, and therefore also contribute to reducing the number of animals being used.

2.2 Stress-related behaviours in rats

Stress-related behaviours are commonly associated with fear or anxiety (Lezak *et al.* 2017). Examples of these behaviours are increased vigilance, hypoactivity and suppressed food consumption (Dess *et al.* 1989; Lezak *et al.* 2017). Behavioural defensive responses to fear differ between species and level of fear (Fanselow 1994). When encountering a predator or threat the brain promotes behaviours that reduce the likelihood of contact with that threat (Perusini & Fanselow 2015). The rodent, being relatively weak and slow, has evolved the freezing behaviour as an effective strategy to not be detected by predators (Fanselow 1994; Perusini & Fanselow 2015). When freezing, the rat stands completely still minimizing its movements. This freezing mechanism quickly is abandoned if the predator proceeds to make contact, where the rat instead tries to escape, jump, or may even vocalize or bite (Perusini & Fanselow 2015). One study by Sturman *et al.* (2018)

showed that rearing could indicate stress in mice, both males and females. In this study the authors differentiated supported from unsupported rearing, meaning whether the animals were touching anything with their front paws or not while standing on the back legs. The conclusion was that unsupported rearing seemed to be sensitive to acute stress. Also, urination and defecation can be a sign of stress (Tsukamoto *et al.* 2016).

Stress can also affect behaviours that are not primarily associated with fear or anxiety. For example, the rat's grooming behaviour is normally performed in a specific pattern, with the beginning of cleaning the nose, face, and ears (Whishaw & Kolb 2020), whereupon the body is groomed in a caudal direction, finishing with the genitals and tail. However, stress can trigger a form of grooming that is more extensive than normally and does not follow the regular pattern (Giorgi *et al.* 2003).

Lowering stress-levels is not only beneficial for the animals themselves but may also be crucial when it comes to collecting scientific data. Stress causes many physiological changes, such as elevated stress hormones, increased heart rate and blood pressure (Chu *et al.* 2022). It has been suggested that these changes may even affect the results of the research (Bailey 2017).

2.3 Anaesthesia in laboratory rats

There are several ways to anesthetize rats and different methods have been used in research over time. Since methods for inhalant anaesthesia have progressed to become more cost-effective with modern delivery systems, many rats today are anesthetized by gas (Gaertner *et al.* 2008).

2.3.1 Advantages with inhalation anaesthesia

Inhalant agents have shown to be beneficial in several areas and is claimed to be a safer method for rodent anaesthesia compared with injectable agents. One main advantage is that it is possible to quickly and precisely alter anaesthetic depth since most of the drug is being eliminated via the airways (Brunson 2008; Steffey *et al.* 2015). This also enables fast induction and recovery (Gaertner *et al.* 2008; Lofgren *et al.* 2020). Since inhalant anaesthesia usually is delivered together with oxygen via a breathing circuit, this method of anaesthesia results in decreased morbidity and mortality as it facilitates lung ventilation and improves arterial oxygenation (Steffey *et al.* 2015). Inhalation anaesthesia has also been suggested to have less impact on the experimental results, especially when being used as monoanaesthesia (Flecknell *et al.* 1999; Gaertner *et al.* 2008).

2.3.2 Disadvantages with inhalation anaesthesia

Physiological disadvantages with inhalant anaesthesia are respiratory depression and decrease in arterial blood pressure, both being dose-related according to Steffey *et al.* (2015). Another disadvantage with inhalants is that operating room personnel often are exposed to low concentrations of the drugs due to for instance leakage from the breathing circuit (Steffey *et al.* 2015). This happens especially when using a mask or when the patient is disconnected from the anaesthesia machine without any flushing of the system, allowing the patient to breathe out the remaining anaesthetic from the lungs into the air. This is likely a minor problem in rodents because of their small expired volumes. One sensitive moment in rodent anaesthesia is when the animals are taken out of the induction chamber, as stated by Gaertner *et al.* (2008). At this moment the personnel become exposed to the drugs, even if the chamber is connected to a scavenger system.

It has been suggested that long-term exposure of inhaled anaesthetics in humans can be a health problem due to mutagenic, carcinogenic, and teratogenic potential (Steffey *et al.* 2015). The Swedish Work Environment Authority states that there is an increased risk for pregnant women to suffer from miscarriage if they are regularly exposed to anaesthetic gases occupationally (AFS 2001:7). It has also been reported that health care professionals working with anaesthetics more often experience headaches, nausea, and fatigue. These negative effects have however decreased since Sweden established stricter safeguards in the 1980's that demanded greater focus on minimal environment release of gasses.

2.3.3 Excitement as a stage of anaesthesia

The level of anaesthetic depth during inhalant anaesthesia is commonly divided into four stages (Tranquili & Grimm 2015). This was described for the first time by Dr Arthur Guedel during the First World War to safely administer diethyl ether to wounded soldiers. Even though the anaesthetic techniques have improved greatly since then, this classification is still being used in both human and veterinary medicine (Tranquili & Grimm 2015; Siddiqui & Kim 2022).

The first stage of anaesthesia (stage I), called "the stage of voluntary movement" is classified as the time from administration of the anaesthetic drug until loss of consciousness (Tranquili & Grimm 2015). When the animal enters stage I, it is still conscious and can experience anxiety that may result in release of epinephrine, causing increase of heart rate and contractility (Tranquili & Grimm 2015). Sometimes the animal may also struggle violently or hold its breath, and it can also be urinating or defecating. As the animal progresses to stage II, it becomes ataxic and finally recumbent as it loses the ability to stand.

When the animal becomes unconscious, it enters stage II of anaesthesia called "the stage of delirium or involuntary movement" (Tranquili & Grimm 2015), or also, the "Excitement stage" (McKelvey & Hollingshead 2003). In this stage, the higher centers of the brain release voluntary control of bodily functions. By now, the anaesthetic drug has reached the potential to depress neurons in the brain that normally inhibit neuromuscular activity, resulting in the rapid involuntary movements as a reaction to external stimuli (McKelvey & Hollingshead 2003; Tranquili & Grimm 2015). This is often referred to as "excitement". Also in this stage, reflexes become exaggerated, and the animal may vocalize, yawn or vomit (McKelvey & Hollingshead 2003; Tranquili & Grimm 2015; Siddiqui & Kim 2022). Stage II ends when the reflexes are starting to decrease, the breathing pattern to become slow and regular, and muscular tension relaxes (McKelvey & Hollingshead 2003; Tranquili & Grimm 2015). This is when the animal progresses to stage III which is known as the preferred stage of "surgical anaesthesia". Later, in the fourth and final stage, the animal is so deeply anesthetized that the cardiovascular system is at shock level, and if not being resurrected it will eventually die by circulatory collapse (McKelvey & Hollingshead 2003; Tranquili & Grimm 2015; Siddiqui & Kim 2022).

Both in stage I and II of anaesthesia, the animal can be seen to struggle, voluntarily respectively involuntarily, of which both may be referred to as "excitement". McKelvey & Hollingshead (2003) emphasizes that the goal of the induction is to make the passage from completely awake to surgical level of anaesthesia as smooth and quick as possible to avoid the stages of anxiety and excitement. The authors explain that this is preferred since the animal during these stages may hurt itself or the personnel, and also, complications like cardiac arrhythmia or arrest can occur due to extensive release of epinephrine. In modern veterinary medicine, premedication along with fast acting injectable agents provide safer inductions where the animal seems to pass directly from stage I to III.

2.3.4 Sevoflurane as an anaesthetic inhalant

Sevoflurane is one of the most commonly used inhalations agents in both human and veterinary practice today (Cesarovic *et al.* 2012; Steffey *et al.* 2015). Sevoflurane was first introduced in the late 20th century, and like almost every other inhalant anaesthetic it classifies as a vapor rather than a gas (Steffey *et al.* 2015). This means that it exists in liquid form at room temperature and must be delivered by a vaporizer to be dosed accurately. Sevoflurane is less soluble in blood than isoflurane, resulting in the drug being able to transfer from the blood into the brain and exhaled from the lungs very quickly (McKelvey & Hollingshead 2003; Brunson 2008). Because of this, the induction and recovery times are shorter with sevoflurane than with isoflurane.

To determine anaesthetic dosage for inhalant agents, the value of "MAC" is commonly used (McKelvey & Hollingshead 2003; Steffey *et al.* 2015). MAC is defined as "the minimum alveolar concentration of an anaesthetic at 1 atmosphere that produces immobility in 50% of subjects exposed to a supramaximal noxious stimulus" (Steffey *et al.* 2015 p. 310). This means that, at this concentration, half of the subjects do not react to the stimuli and the other half do. To achieve immobility in 95% of the subjects this represents an alveolar concentration of the anaesthetic that is 20-40% greater than MAC (Steffey *et al.* 2015). According to different studies, MAC for sevoflurane in rats lies between 2.3-3.0% (Steffey *et al.* 2015; Lofgren *et al.* 2020). This means that the alveolar concentration should be at least 2.8-3.6% to reach the level of 95% non-reactive subjects.

2.3.5 The concept of time constants

While working with anaesthetic gases it is not only important to have knowledge of how these drugs will affect the body pharmacologically but also to understand the concepts of how gasses behave physically. When the concentration of a gas changes in an enclosed space and the inflow rate is kept constant, this happens according to an exponential equation (AVMA 2020). This means that the concentration does not rise at the same rate at the beginning of the displacement as towards the end. The authors explain that the fastest change happens when the gas concentration is beginning to rise and progressively slows down as it reaches the concentration of the inflow gas. The time it takes for the concentration of a gas to rise inside an enclosed area depends on the flow and the volume of space, the authors continue. This is described as the time constant (τ) according to the following equation:

TC (τ) = volume/flow

One TC is required for the concentration of the gas in the system to rise to 63% of the inflowing gas concentration (Plunkett & Cross 2014; AVMA 2020). Two TC makes a 87% rise in the system and 3 TC results in a 95% change. Mathematically, 5 TC is required to reach approximately 100% gas concentration (Plunkett & Cross 2014).

2.3.6 Aversion in rats caused by inhalants

There are some differences between how inhalant anaesthesia is used in laboratory animals and pets. In research rodents, premedication is rarely used. Rather, anaesthesia is induced directly with inhalant inside a chamber (Gaertner *et al.* 2008; Cesarovic *et al.* 2012; Lofgren *et al.* 2020). It has been stated that premedication is avoided to minimize stress caused by the injection (Gaertner *et al.* 2008). On the

other hand, many different gases commonly used for anaesthesia and euthanasia, like halothane, isoflurane, enflurane, carbon dioxide and even argon have been shown to be aversive to rats (Leach *et al.* 2002; Burkholder *et al.* 2010).

Sevoflurane is said to be a less pungent gas, and it has been suggested that it therefore may be less aversive to laboratory rats (Brunson 2008; Lofgren *et al.* 2020). On the contrary, studies show that this might be false to assume. Boulanger Bertolus *et al.* (2015) investigated rat aversion to sevoflurane compared to isoflurane and found that the rats evaded the two gasses equally. The authors also discovered that the rats were half as likely to tolerate the gases the second time of exposure, suggesting that the rats developed learned aversion and remembered the gases as something unpleasant that should be avoided. Also noted in this study, the rats showed learned aversion to isoflurane, even if they had been exposed to sevoflurane the first time. This suggests that the less pungent property of sevoflurane has no value when it comes to level of aversion. Rabbits have also shown strong aversion to both isoflurane and sevoflurane. In a study by Flecknell *et al.* (1999) these animals exhibited breath-holding, trying to avoid inhaling the gas for as long as possible, and even made violent attempts to escape the induction chamber.

In both human and veterinary medicine, it is widely known that multimodal anaesthesia, referring to combining several anaesthetic drugs in lower dose, is beneficial. For example, previous studies have shown that injectable agents can lower MAC for inhalant agents such as isoflurane and sevoflurane, therefore reducing the required dose of these vapors (Criado *et al.* 2000; Cesarovic *et al.* 2012; Tsukamoto *et al.* 2016; Lipiski *et al.* 2017; Lofgren *et al.* 2020). This in turn leads to less negative cardiovascular and respiratory effects during maintenance of anaesthesia. Premedication have also been seen to result in smoother inductions with less adverse reactions such as urination or excitement in mice and rats (Tsukamoto *et al.* 2015; 2016; David *et al.* 2022).

This study is meant to contribute with valuable data to a more comprehensive study in which the effect of premedication on stress-related behaviours in rats and mice will be evaluated during induction of anaesthesia. An earlier student thesis has investigated stress in rats during induction of anaesthesia with isoflurane and found no difference between the two different FGFs compared (Lövgren & Lindh 2022). The authors also found the presence inside the chamber without anaesthetic gas to be stressful to rats.

3. Materials and methods

3.1 Study design

The practical parts of this study were divided into three trials. During the first trial, the equipment was tested by filling the induction chamber with sevoflurane while using different fresh gas flows (FGF) and vaporizer percentage settings, measuring time and sevoflurane concentration. During the second trial, four sets of combinations were tested on four rats to find the most optimal vaporizer percentage and which two different fresh gas flows to compare in the third trial. The aim for the second trial was to find the least stressful induction. Lastly, in the third trial, ten rats were anaesthetized. Induction and recovery was video recorded to compare stress-related behaviours between the two chosen fresh gas flows. This study was ethically approved by the authorities in Uppsala, Sweden (SLU-ID: Dnr 5.8.18-15533/2018).

3.2 Animals

Fourteen inbred Wistar rats (*Rattus norvegicus*), were provided by the Laboratory animal science facility at the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden. Four of them, three males and one female, were used for evaluating the anaesthesia settings in the second trial. Subsequently, the remaining ten rats, five males and five females (n=10) were used in the third trial. The female rats weighed between 187-229 grams and the male rats between 315-352 grams. All rats were 3-4 months old and housed pairwise in plastic cages with separate ventilation (model GM 1800 DD IVC). The room temperature varied between 22.2-22.8 degrees Celsius and a relative humidity of 31-46%. The brightness in the rat room was set to 50% during the day, and the rats were habituated to a normal light cycle, switching the lights on and off by 6 am and 6 pm. Prior to our study, six of the male rats had been exposed to handling while used for education. None of the other rats were regularly handled.

3.3 Equipment

For the induction, a transparent plastic cage (MAK II) holding 6 litres, with a matching plastic lid was used as a gas chamber (Figure 1). The lid was modified with three drilled holes, one smaller for connecting a gas sampling line and thermometer and two larger for connecting the inspiratory and expiratory breathing tubes. The smaller hole was sealed by using a tip of a latex glove together with tape to prevent leakage of gas. The induction chamber was placed on a down-flow ventilated bench and its position was marked with tape. Under the chamber, a heating pad (Harvard Apparatus, K 023648, US) was placed. This pad was set to 40 degrees Celsius, making the air temperature inside the middle of the chamber around 24-25°C, measured with an ATP Digital Thermometer DT-610B.

Next to the induction chamber, a second heating pad set to 35°C was placed on the same down-flow bench. This pad was connected to a CODA Non-invasive Blood Pressure Monitor (Kent Scientific Corporation, Torrington, USA), which was used for measuring blood pressure, heart rate and body temperature during maintenance of anaesthesia (results not included). Above this heating pad, a heating lamp was placed for additional warmth during maintenance to keep the body temperature between 38.5-39.5^{o}C, and tail temperature between 32-36°C (for optimizing blood pressure measurement). The tail temperature was measured with an infrared thermometer (Kent Scientific Corporation, Torrington, USA). A small breathing mask with connected breathing tubes and a sampling tube were also placed in this area. All the anaesthetic equipment was handled carefully on the down-flow bench to minimize gas leakage.

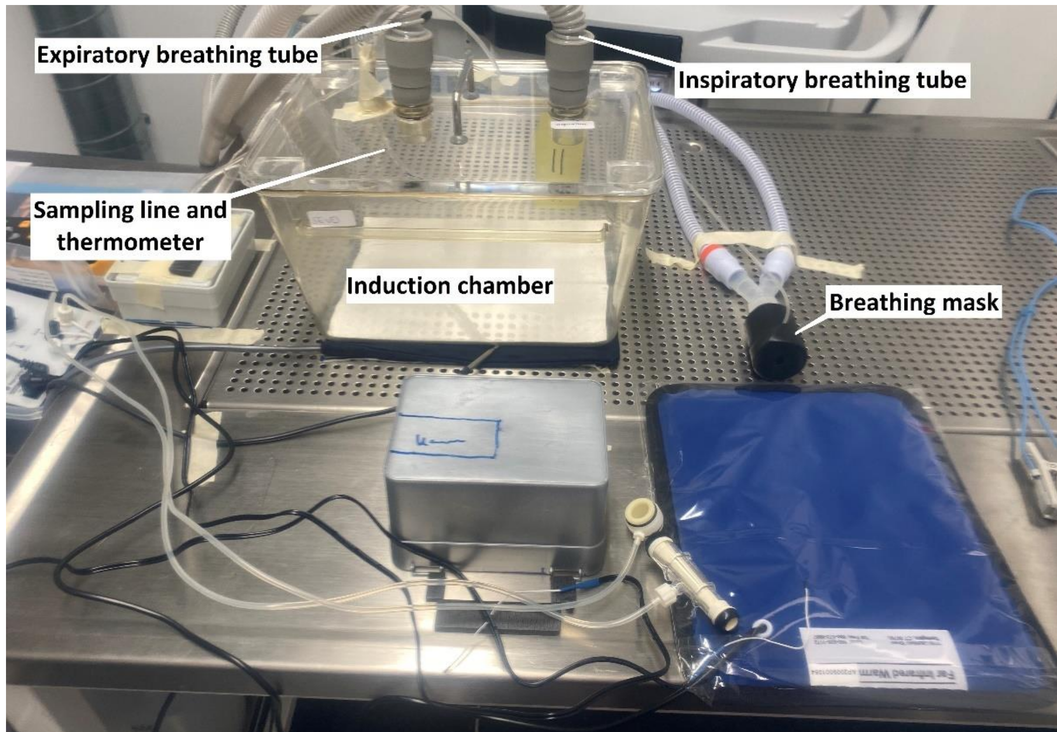

Figure 1. Induction chamber with connected breathing tubes and breathing mask set up (authors photograph).

An identical plastic cage to the one used for induction was used for recovery, but instead of a plastic lid, a metal grid was placed over this chamber, allowing air to flow in and out (Figure 2). This recovery chamber was placed on a sideboard, also with a heating pad under it. Two Lamax action cameras (X9.1 and X Thaurus) were used to record the induction and recovery from two different angles. The positions of the cameras were marked.

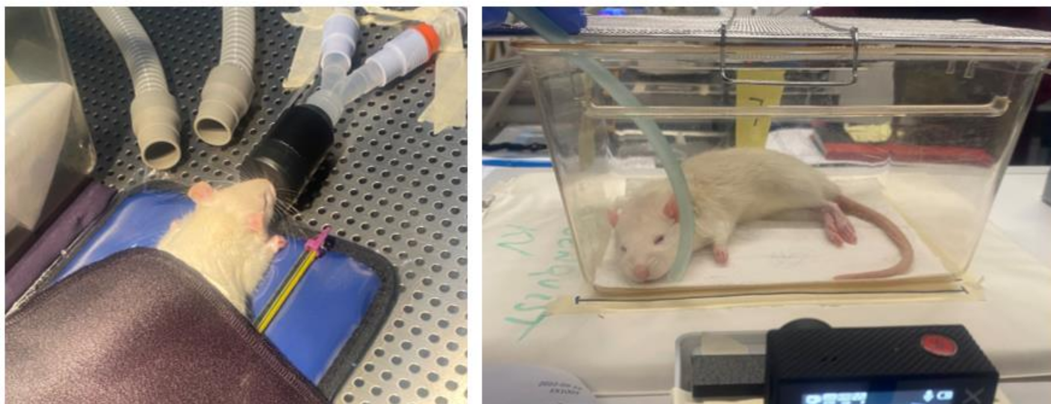

Figure 2. Left: Rat under maintenance via breathing mask. Right: Rat being recorded inside the recovery chamber (authors photographs).

The rats were anesthetized with sevoflurane (Sevorane®, AbbVie, Sweden), with a MAQUET FLOW-i® anaesthesia machine (Getinge AB, Sweden). This anaesthe-

sia machine was also used to measure oxygen and sevoflurane concentration via a sampling line. For measuring hemoglobin oxygen saturation (SpO_2) during maintenance of anaesthesia a LifeWindow multiparameter monitor (Digicare, US) was used.

3.4 Literature study

Literature was gathered from several databases (Web of Science, PubMed, Google Scholar) by performing searches using different combinations of the words “rat”, “behaviour”, “excitement”, “anaesthesia”, “induction”, “sevoflurane”, and “stress” as well as their variants. Additionally, more literature was found by reviewing the reference list in the studies collected from the initial searches. Also, regulation documents from Swedish authorities and EU Directives have been used.

3.5 First trial

In the first trial, the equipment was tested by filling the induction chamber with sevoflurane and measuring the rising concentration over time. The following different fresh gas flows were used; 1, 3 and 6 L/min (with 55% oxygen), together with the vaporizer being set to 4 and 8% (Table 1). Each combination was tested once except for FGF 1 L/min together with 4%, since the results of testing the other combinations showed that this would have resulted in an overly slow rise of sevoflurane in the chamber. The sevoflurane concentration was noted every thirty seconds from the anaesthesia machine until it had reached either 4 or 8% depending on the settings, or for a maximum of three minutes. Between tests, the chamber was flushed to reset gas concentrations.

Table 1. Combinations of FGF and vaporizer settings that were tested in the first trial.

Fresh gas flow (FGF)	Vaporizer settings
1 L/min	8 %
3 L/min	4 %
3 L/min	8 %
6 L/min	4 %
6 L/min	8 %

3.6 Second trial

In the second trial, four different combinations of FGF and vaporizer settings were tested on four rats, three males and one female (Table 2). The oxygen level was set to 55%.

Table 2. Combinations of FGF and vaporizer settings that were tested on four rats in the second trial.

	Rats (*n*)	Sex	Fresh gas flow (FGF)	Vaporizer settings
A	1	♂	6 L/min	4 %
B	1	♀	6 L/min	8 %
C	1	♂	10 L/min	4 %
D	1	♂	10 L/min	8 %

Before the day of anaesthesia, two of the four rats were trained for four continuous days. The first day, the rat was picked up by the handler from its home cage, still being inside the room where the rats were normally kept. The rat was handled for 2-3 minutes and was offered a snack (All Bran Flakes) before it was put back in its home cage. The second day the rat's home cage was moved into the operating room. For transportation, the cage was placed on a trolley and covered with towels to protect from bright lights. Inside the operating room, the light was set to the same setting as in the rat room. Here, the rat was picked up by the handler and put inside the induction chamber for two minutes, then picked up again and returned to its home cage. The rat was offered a snack while being handled as well as inside the induction chamber. The same procedure was repeated on day three and four. The rats were always handled this way by the same two persons during the whole experiment (author and assistant supervisor).

On the day of anaesthesia, each home cage was transported to the operating room by the same procedure as described above. The rat was picked up and placed inside the induction chamber. The rat was preoxygenated for 60 seconds upon which sevoflurane was started. When the rat had become recumbent, it was kept inside the induction chamber for approximately 0.5-2 minutes until it had a relaxed and slow breathing pattern. When this occurred, the sevoflurane gas was turned off and the rat was taken out of the induction chamber.

The rat was placed on the second heating pad and the snout gently pushed into the breathing mask. This was followed by instrumentation of monitoring equipment. SpO_2 was monitored by a pulse oximeter on the foot and sevoflurane concentration via a sampling line inside the breathing mask. Oscillometric blood pressure was measured on the tail and registered from the CODA monitor, and a thermometer (also connected to the CODA monitor) was inserted in the rectum and colon to

measure body temperature. Initial anaesthesia machine settings were a FGF of 1 L/minute and sevoflurane 4%. The vaporizer setting was adjusted according to anaesthetic depth, which was controlled by testing the pedal withdrawal reflex by a nociceptive stimulus with a Touch Test ® Sensory Evaluator (6,65, 300g, Exacta, US). Every five minutes, the following was registered in an excel sheet: SpO_2, pulse rate, systolic-, diastolic-, and mean arterial pressure, temperature of the tail and body, oxygen and sevoflurane % measured in the mask, respiratory rate, and nociceptive response.

After 30 minutes of anaesthesia, the gas was turned off and the rat was moved to the recovery chamber. Here, the rat received flow-by oxygen (2 L/min) until it was awake and was brought back to its home cage.

3.7 Third trial

In the third trial, ten rats were anesthetized as described above during three continuous days. First, the rats were randomized into a treatment group, day, and order, by using a modified block randomization in which sex and cage number were taken into account. This meant that two rats sharing home cage were never anesthetized during the same day. This consideration was made to avoid any stress caused by a rat being left alone in the cage. Group 1 was exposed to FGF 3 L/min and a vaporizer setting of 8% and group 2 with FGF 6 L/min and the same vaporizer setting (Table 3).

Table 3. Number of rats tested in each group in the third trial.

Group	Rats (*n*)	Fresh gas flow (FGF)	Vaporizer settings
1	5	3 L/min	8 %
2	5	6 L/min	8 %

Prior to the day of anaesthesia, the rats were equally trained for four days as described above, except that the scavenger system, bench ventilation and assigned FGF were started on the fourth day to simulate gas flow and noise.

All the rats were anesthetized between 8 am and 12 pm. The FGF was set to either 3 or 6 L/min with an oxygen level of 55%. Both the one minute of preoxygenation and the induction was recorded. Time to recumbency and sevoflurane % inside the chamber at recumbency was noted. The first five minutes of maintenance, the rat received 4% sevoflurane and FGF 3 L/min in the mask. Later, FGF was turned down to 1 L/min and the vaporizer settings adjusted up or down according to anaesthetic depth (judged by nociceptive testing) every five minutes by steps of 0.5%. During recovery, the rats were recorded until it was awake as judged by

return of the righting reflex and moving around. Both time to first movement and regain of righting reflex was noted from the recordings.

The rats were weighed on the day of anaesthesia and the day after for comparison, since body weight loss can be associated with stress (Dess *et al.* 1989). The rats were handled carefully and calmly to minimize stress. Between every rat both chambers were wiped with a wet paper towel.

3.8 Behavioural scoring

For behavioural scoring, an ethogram was created and modified from Burkholder *et al.* (2010) (Table 4) and used together with BORIS (version 7.13.8), a free event-logging software (Friard & Gamba 2016). All recordings were scored once by the same person (author) who was not blinded to treatment due to recognition of the rat's number placed on the chamber wall. Baseline behaviour was scored during one minute of preoxygenation. The induction was scored from when the vaporiser was turned on until recumbency. During the recovery, the rats were scored for one minute and 45 seconds from the first movement. This time limit, corresponding to the shortest video recording, was used so that all the rats had equal amount of time to perform behaviours during the scoring. Each behaviour received a duration, frequency, and latency.

Table 4. Ethogram used for behavioural scoring of the recordings. This ethogram is a modified version from Burkholder et al. (2010).

Behaviour	Definition
Walk	Quadrupedal ambulatory movement, with legs in contact with the ground
Crawl	Quadrupedal ambulatory movement, with legs and abdomen in contact with the ground
Free rearing	Raising of the upper body while standing on the two back legs without anything supporting the front legs
Wall rearing	Raising of the upper body while standing on the two back legs with something supporting the front legs
Jump	Vertical projection of body with all four feet off the ground
Dig	Digging motions with front paws towards the ground
Shake	Rapid rotation of the head or body about the central axis
Groom (face)	Scratching or rubbing the face with front paws
Groom (body)	Licking or scratching the body with front paws
Groom (genitals)	Licking or rubbing the genitals with front paws
Interrupted groom	Groom following the order face-body-genitals, but which becomes interrupted at any stage
Sniff	Rhythmic movement of the snout and head accompanied by rapid breathing and movement of vibrissae
Escape	Attempts to escape from the box by trying to move the lid with front paws or snout
Excitement	Uncoordinated and spastic movements of the body and legs and/or some of the following occurring: throws body against the wall, paddling with legs in the air, rolling around uncontrollably, losing balance and falling
Defecating	Defecating at any time in the chamber
Urinating	Urinating at any time in the chamber
Still	Standing still on all four paws during >2s, without the abdomen having contact with the ground
Lie	Laying still with the abdomen having contact with the ground

3.9 Collection of data and statistical analysis

Data were collected in Microsoft Excel ® (Microsoft 365, version 2209, 64-bit). Excel was also used for designing graphs. Statistical analyses were made in InVivoStat ® (version 4.6). Time to recumbency, first movement, regain of righting reflex and duration, frequency, and latency for all behaviours were analysed between groups non-parametrically with the Mann-Whitney test. A confidence interval of 95% was chosen and $p<0.05$ indicated statistical significance. If there was no difference between groups, data from both groups were pooled and compared with Friedmans test between time points (preoxygenation, induction and recovery). If the overall effect was significant, a post hoc test was performed with the Wilcoxon signed rank test. Data are presented as median (range) unless otherwise specified.

4. Results

4.1 First trial

The sevoflurane concentration in the induction chamber over time while using different combinations of FGF and vaporizer settings is shown in Table 5. The higher FGFs resulted in a faster rise in sevoflurane concentration than the lower, as expected from calculations of time constant.

Table 5. Sevoflurane concentration inside the induction chamber over time using different FGF and vaporizer settings.

Time (min)	*1 L/min 8%*	*3 L/min 4%*	*3 L/min 8%*	*6 L/min 4%*	*6 L/min 8%*
0	0	0	0	0	0
0.5	0	0	0	1	1.7
1	0	1.8	3.5	2.7	5.4
1.5	1.4	2.4	4.8	3.3	6.7
2	2.1	2.6	5.7	3.5	7.3
2.5	2.5	2.8	6.3	3.7	7.5
3	3	3	6.7	3.8	7.7
	Sevoflurane concentration (%)				

The air temperature inside the induction chamber increased and decreased during the filling with sevoflurane and flushing of the chamber from 24-25ºC up to 29-30ºC and back to 24-25ºC.

Figure 3 shows the rising sevoflurane concentration in the chamber with the FGFs 3 L/min and 6 L/min, and 8% sevoflurane. These values were compared to the calculated time constant. For example, using the FGF 6 L/min and the chamber having the volume of 6 litres results in TC = 6 L/min/6 L which equals 1 minute. This meant that the sevoflurane concentration inside this chamber with a flow rate of 6 L/minutes would reach 100% of the vaporizer setting after 5 TC = 5 minutes. By using half the FGF rate 3 L/min, the calculated time for filling the chamber with 8% sevoflurane equals 10 minutes.

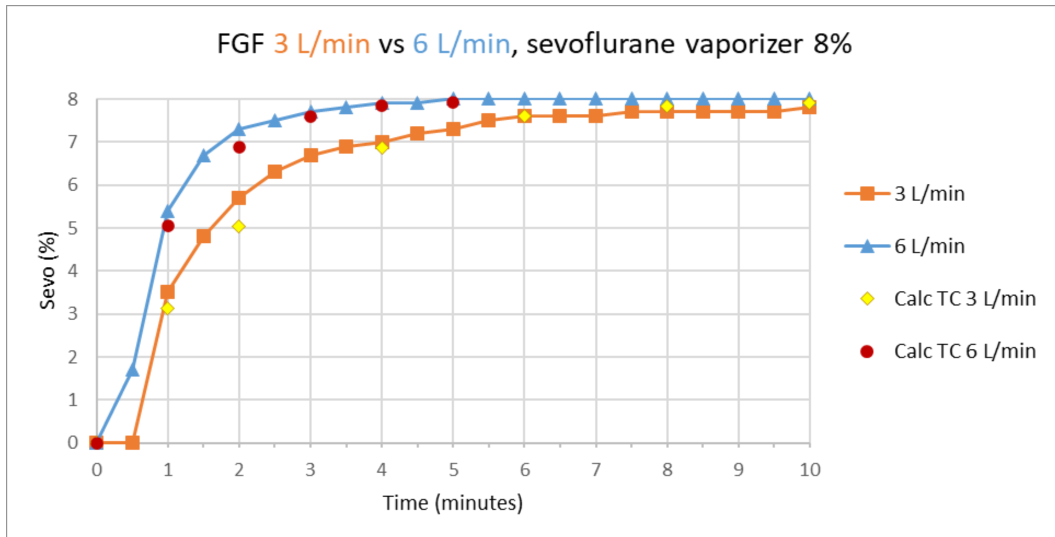

Figure 3. Rise of sevoflurane concentration inside the induction chamber over time with FGF 3 L/min and 6 L/min and vaporizer settings at 8%. Compared with the calculated time constant for each FGF.

4.2 Second trial

The results of the second trial are shown in Table 6. With FGF 1 L/min and vaporizer set to 4% for initial setting during maintenance, two of the rats (C and D) woke up in the mask during maintenance. This flow was therefore increased to FGF 4 L/min during the first five minutes of maintenance in the third trial, as described in materials and methods. This also resulted in the recovery not being recorded for rat D, hence no data was collected here.

The incidence of excitement behaviour during the induction and recovery is shown in Table 7. Since the first two combinations (A and B) showed excitement during the induction, the FGF was increased to 10 L/minute for the next two rats (C and D). However, since they also struggled during the induction showing excitement, it was decided to continue the third trial using the FGF 3 and 6 L/min and vaporizer set to 8%.

Table 6. Results of the second trial. Time to recumbency, first movement and regain of righting reflex using different combinations of FGFs and vaporizer settings. All rats were anaesthetized with sevoflurane by chamber induction.

	Rats (*n*)	Sex	FGF	Vaporizer	Time to recumbency (s)	Time to first movement (s)	Time to regain of righting reflex (s)
A	1	♂	6 L/min	4 %	94	70	80
B	1	♀	6 L/min	8 %	63	120	166
C	1	♂	10 L/min	4 %	64	172	172
D	1	♂	10 L/min	8 %	60	*Wakes up on mask*	*Wakes up on mask*

Table 7. Treatment combinations resulting in excitement behaviour during the second trial.

	Excitement (induction)	Excitement (recovery)
A	Yes	Yes
B	Yes	Yes
C	Yes	No
D	Yes	-

4.3 Third trial

4.3.1 Objective parameters

Time to recumbency from start of sevoflurane was significantly shorter (p=0.028) for the FGF 6 L/min (median 61s, range 56s – 78s), than for FGF 3 L/min (median 94s, range 66s – 106s) (Figure 4). The sevoflurane concentration at recumbency was significantly higher (p=0.016) with FGF 6 L/min (median 7.4%, range 6.7% - 7.5%) than with 3L/min (median 4.7%, range 3.4% - 6.7%) (Figure 4). There were no significant differences regarding time to first movement or time to regain of righting reflex between the two groups. The time to first movement in the recovery cage varied between 12s – 108s (median 101s) for FGF 3L/min and 43s – 143s (median 114s) for FGF 6 L/min, and time to regain of righting reflex varied between 15s – 129s (median 115s) and 55s – 147s (median 131s), respectively (Figure 5).

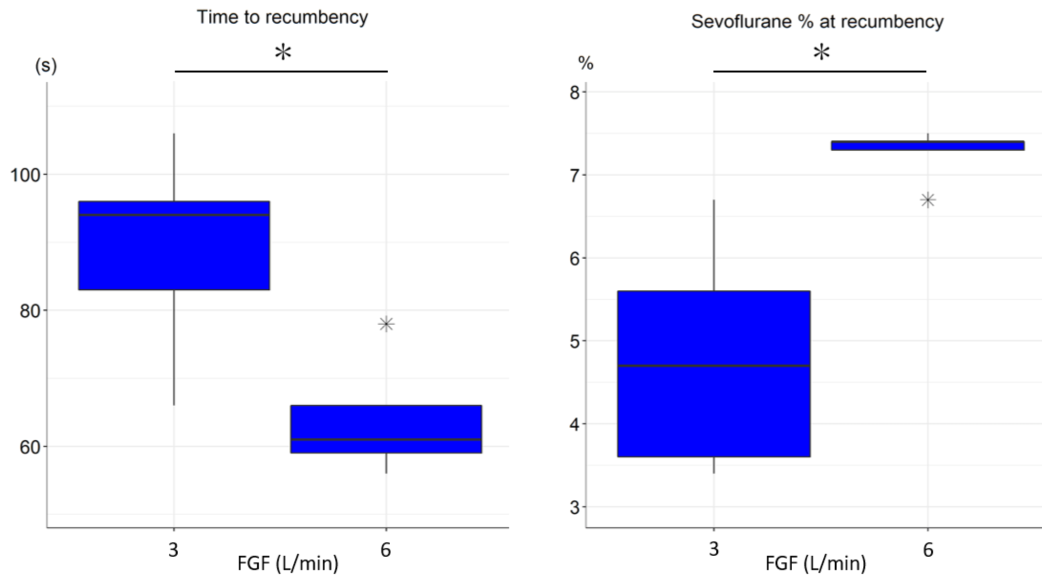

*Figure 4. Time to recumbency (left) and sevoflurane % in the induction chamber at recumbency (right). Vaporizer set to 8% sevoflurane. Boxes indicate the interquartile range, and the median is marked by a horizontal line. Vertical lines extend to the maximum and minimum value of the lower and upper quartile. Outliers are marked by a star inside the graph. (Mann-Whitney test, *p<0.05)*

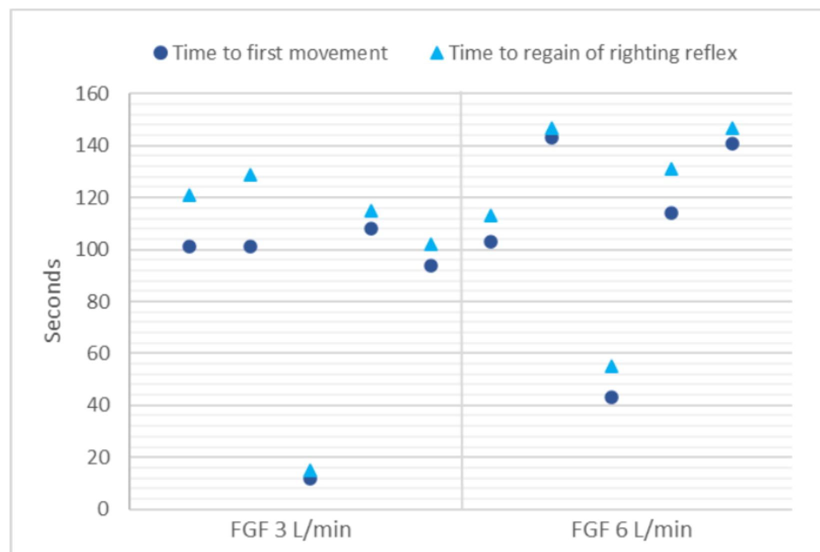

Figure 5. Time to first movement and regain of righting reflex during the recovery for each individual rat in both treatment groups.

When the weight of each rat was controlled the day after anaesthesia, some had lost up to 6 grams of weight (group 1: mean weight difference -2.2g, SD ± 2.8g; group 2: mean weight difference -2.6g, SD ± 2.3g). The one rat that lost 6 grams belonged to group 1 and had the overall highest frequencies of free and wall rearing during the preoxygenation. The next day none of the other rats had lost more weight and this rat had regained all the weight lost.

4.3.2 Behaviours

Differences between groups

During preoxygenation and induction, most time was spent sniffing, whereas during recovery, most time was spent lying and showing excitation (Figure 6, 7). None of the rats in any group or on any occasion jumped.

There were no significant differences regarding frequency or duration in any of the behaviours during the preoxygenation, induction or recovery between low FGF and high FGF. The duration tended to be longer for 'sniff' (p=0.095) during the induction in the low flow group (median 54.9s, range 32.3s – 82.5s) compared with the high flow group (median 31.7s, range 24.1s – 46.8s) (Figure 6, 7). The frequency of 'sniff' also tended (p=0.063) to be higher in the low flow group (median 3, range 3-11) compared with the high flow group (median 1, range 1-4) during the induction (Figure 8, 9).

No rat tried to escape the chamber or performed 'shake' or 'groom genitals' in either group during the induction. The total most frequent behaviour in both groups during the induction was 'free rearing' which was performed by all rats except for one in the low flow group. The second total most frequent behaviour during the induction was 'wall rearing'. One rat in the low flow group performed 'crawl' and one rat in the high flow group performed 'dig'.

During the recovery, no rat performed 'dig', 'shake' or any of the groom behaviours. Also, because the rats were still heavily sedated during recovery, no rat was able to perform 'walk' or 'still' properly. Instead, they crawled or lied. One rat in each group tried to escape the recovery chamber. The total most frequent behaviour during the recovery in both groups was 'lie', followed by 'sniff' and 'crawl'. Overall, the two groups were similar during preoxygenation, induction and recovery.

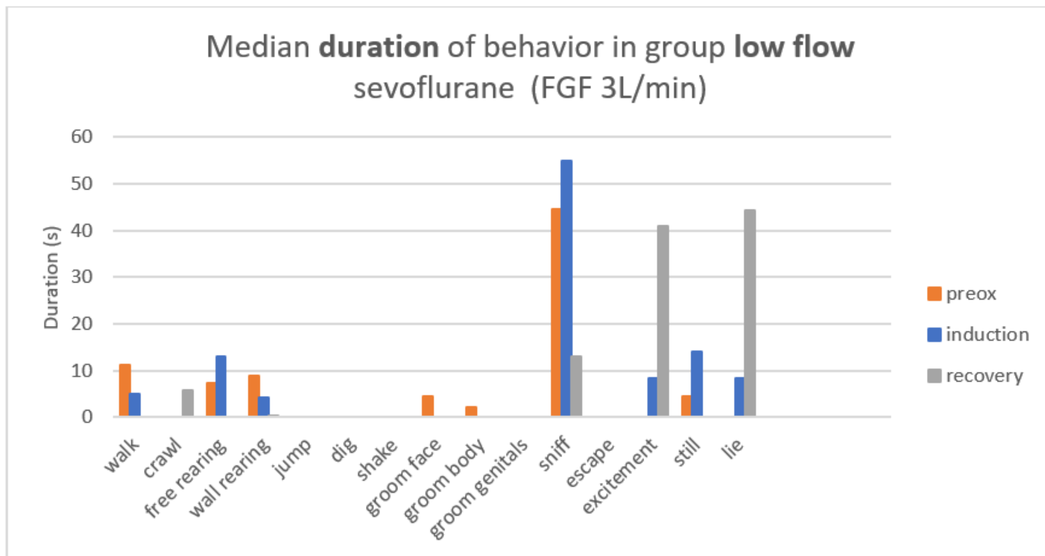

Figure 6. Median duration of behaviours in the low flow group induced with FGF 3 L/min.

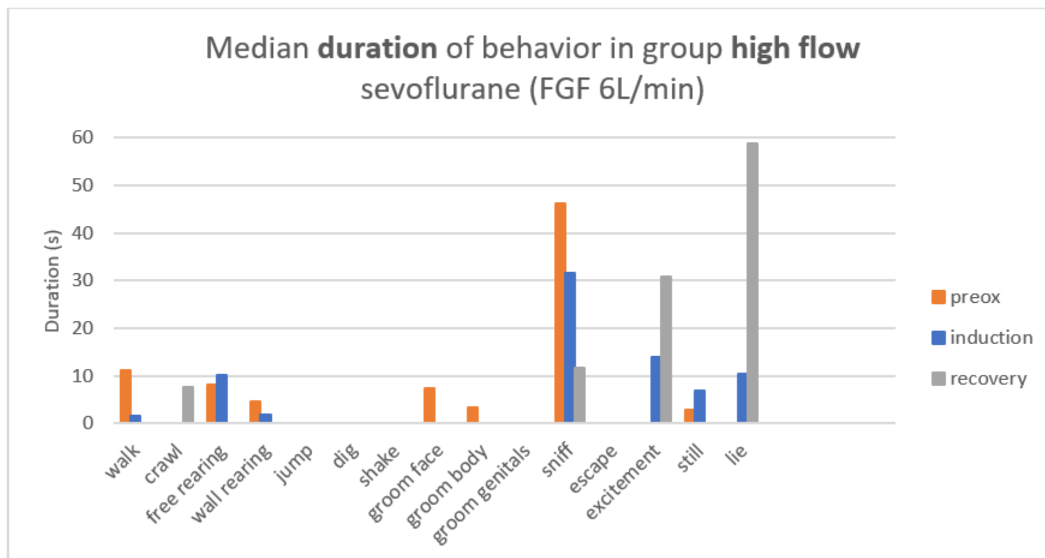

Figure 7. Median duration of behaviours in the high flow group induced with FGF 6 L/min.

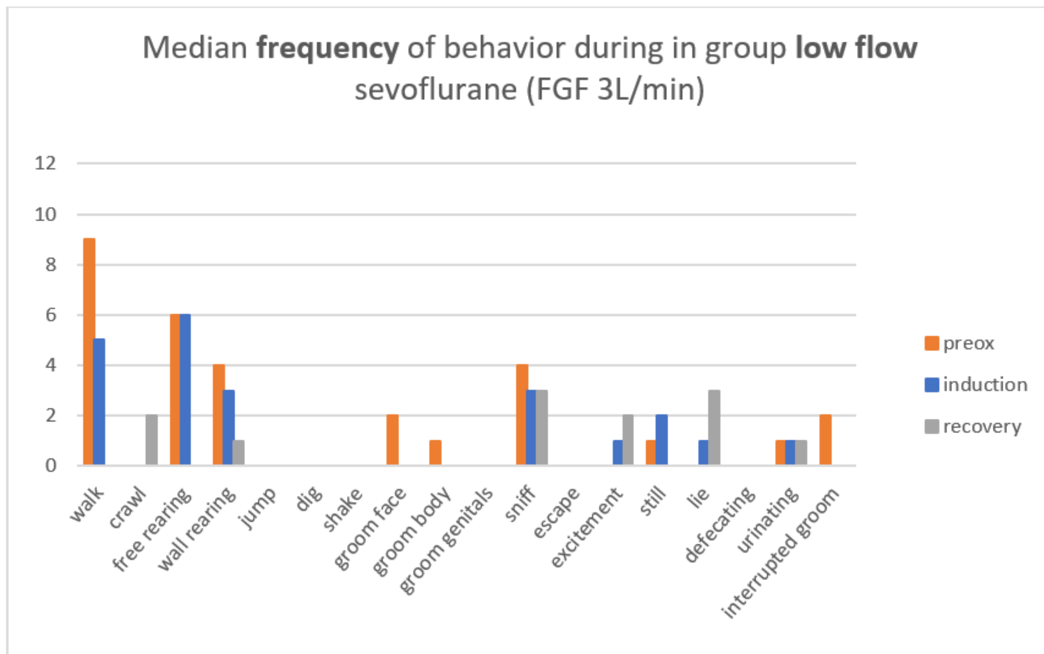

Figure 8. Median frequency of behaviours in the low flow group induced with FGF 3 L/min.

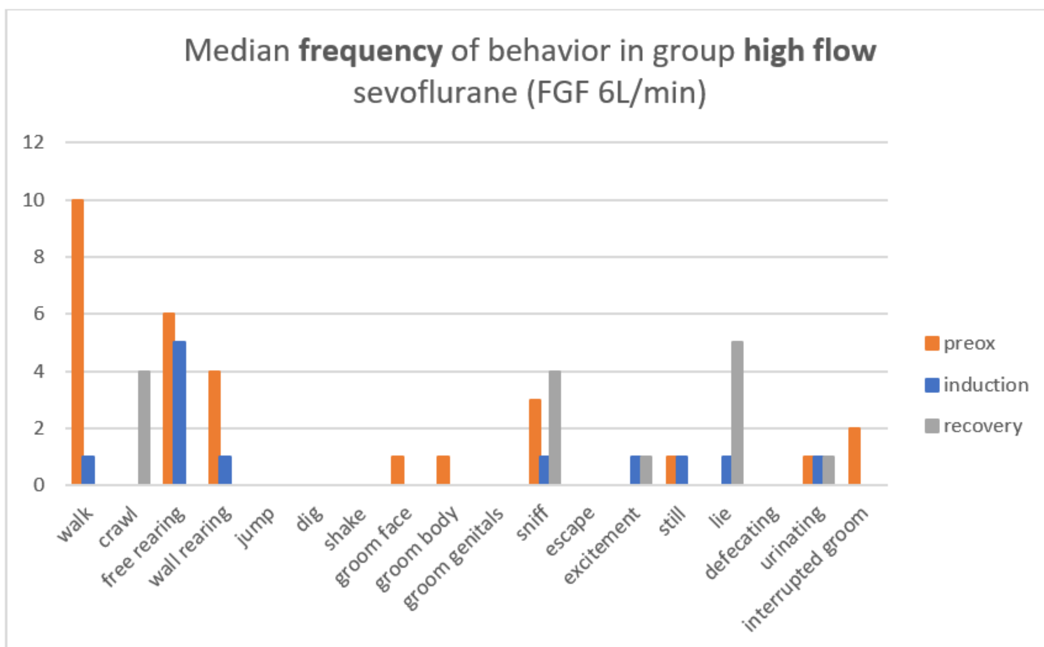

Figure 9. Median frequency of behaviours in the high flow group induced with FGF 6 L/min.

Regarding latency to the behaviours during the induction, there was a significant difference (p=0.008) between the groups in that the latency to 'lie' was longer in the low flow group (range 62.8s – 94.7s) compared with the high flow group (range 43.2s – 58.6s) (Figure 10). There was also a tendency (p=0.057) for the latency to 'excitement' being longer during the induction in the low flow group (median 59.7s, range 53.3s – 86.2s) compared with the high flow group (median 39.4s, range 33.9s

– 46.8s). The latency for behaviours during the recovery was very similar between groups (Figure 11).

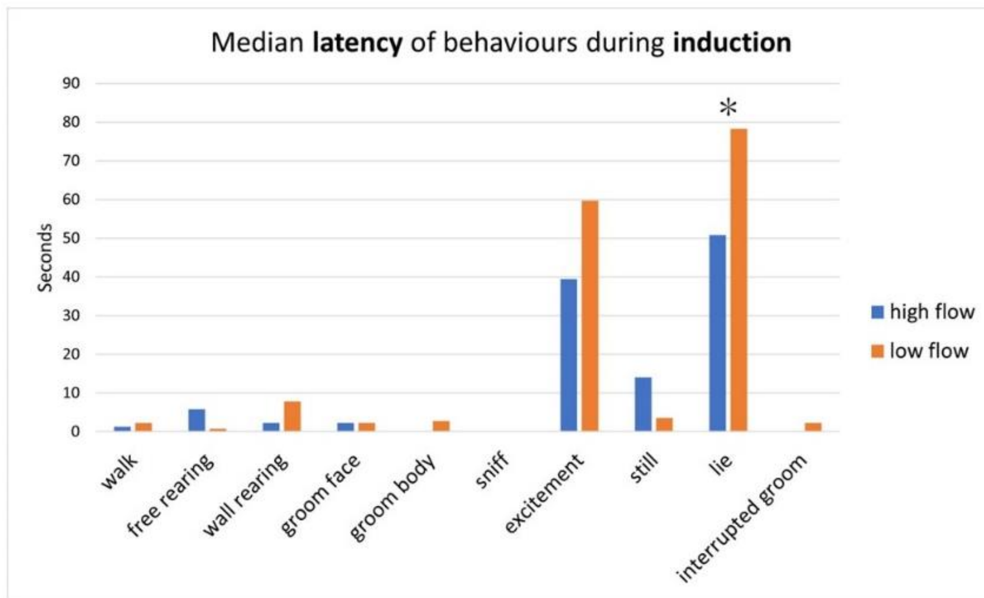

*Figure 10. Median latency of behaviours during the induction, comparing low flow with high flow. *p<0.05, Mann-Whitney test.*

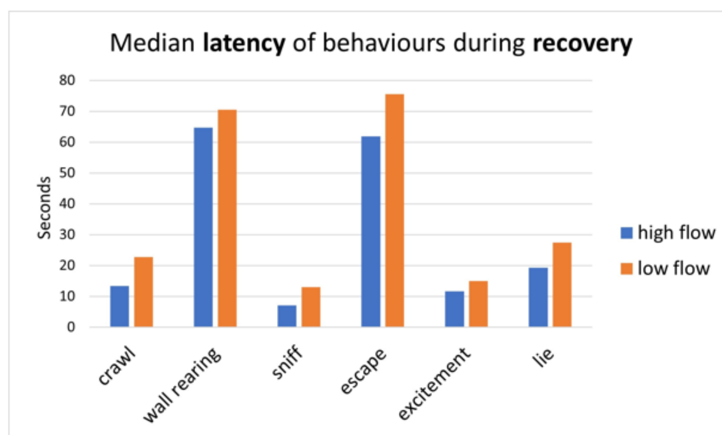

Figure 11. Median latency of behaviours during recovery comparing low flow with high flow.

During the induction, one more rat showed excitement in the high flow group compared with low flow (Table 8), but the rats in each group performing this behaviour during the induction had similar duration (low flow: range 8.5s – 25.3s; high flow: range 3.8s – 24.3s). The equal number of rats in each group showed excitement during the recovery (Table 8). For these rats, the duration range of excitement was 25.8s – 59.0s during low flow and 26.5s – 49.1s during high flow. Every rat except for one in the low flow group showed excitement at least once during either the induction or recovery.

*Table 8. Number of rats in each group to defecate, urinate or show excitement behaviour during induction and recovery in the third trial. *Defecation and urination were only noted if prevalent inside the induction chamber, not specifically if it occurred during the preoxygenation or induction.*

	Excitement		Defecation		Urination	
Group	Induction	Recovery	Induction*	Recovery	Induction*	Recovery
Low flow	3	4	0	2	3	4
High flow	4	4	0	1	4	4
Total	7	8	0	3	7	8

Differences over time

Compared with the preoxygenation, animals walked less and were more still during the induction with sevoflurane ($p_{walk}<0.01$, $p_{still}<0.05$) (Figure 12). They also tended to spend more time on grooming during the preoxygenation than during the induction, consisting mostly of interrupted grooming patterns. Only one rat performed a full grooming cycle from face to genitals, but this rat also performed five interrupted grooming cycles.

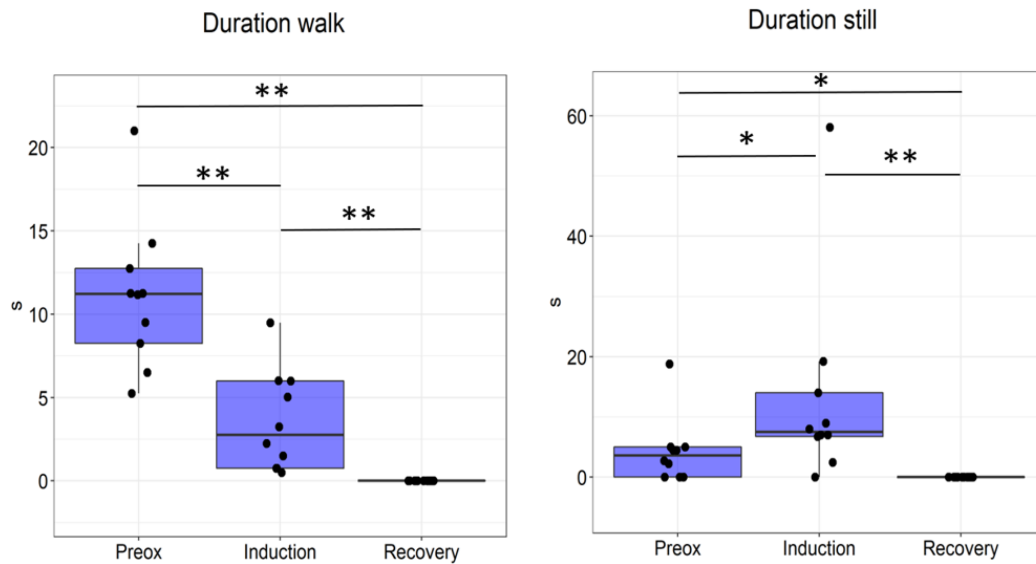

*Figure 12. Duration of walk (left) and still (right) of all rats across three time points. Boxes indicate the interquartile range, and the median is marked by a horizontal line. Vertical lines extend to the maximum and minimum value of the lower and upper quartile. Dots show individual data. Friedmans test: p=0.0001. *p<0.05, **p<0.01 (Wilcoxon signed rank test).*

5. Discussion

5.1 Behavioural differences between groups

Except for latency to 'lie', no significant differences in the behaviours during the induction were found between group 1 induced with low flow and group 2 induced with high flow. The fact that latency to 'lie' was longer in the low flow group could be explained by this group also having a significantly longer time to recumbency. Especially considering that 'lie' always was performed lastly before recumbency, making the latency to this behaviour practically equal to the total time of induction. This explanation also applies for the latency to excitement that tended to be longer in the low flow group, since the excitement behaviour always happened in the last part of the induction, before 'lie' and finally recumbency.

Another trend was that the rats in the low flow group had a longer duration and higher frequency of 'sniff', compared with the high flow group. This may also be due to the difference in time to recumbency, since the rats in the low flow group on average could perform behaviours during a longer period. Even though this behaviour tended to show a longer duration and higher frequency in the low flow group compared, it cannot be established whether this was due to the longer time span or to the lower flow being more stressful. Either way, one can argue that by using a high flow during chamber induction, the amount of stress related behaviours are somewhat reduced since the induction is faster. With this perspective, it could be motivated to use a higher flow during inhalation chamber induction.

To eliminate time to recumbency as a factor affecting the registered behaviours, all rats would have to be observed during an equal amount of time. To make this possible, the behavioural scoring would have to be limited by the overall shortest induction in one rat. This would on the other hand cause a lot of missed behaviours that may only be present at the later stages of induction when sevoflurane concentration is higher among rats that takes longer time to fall asleep. In future research, instead a suggestion could be to make analyses where the time to recumbency is taken into consideration by using a calculated form of ratio for every behaviour.

No significant differences were found between groups during the recovery, in contrary to Lövgren & Lindh (2022) who found that rats induced with a higher flow took longer time to recover. One possible explanation may be that in this study the rats were kept on maintenance for 30 minutes, receiving different FGF and sevoflurane concentrations to keep a surgical anaesthetic depth. Therefore, the flow used for induction may have had less impact on the recovery. More studies are needed to establish which factors influence the recovery from inhalant anaesthesia in rats.

5.2 Behavioural differences over time

In this study, the rats were acclimated to the induction chamber, including the air flow. This was done to minimize stress from being inside the chamber, as Lövgren & Lindh (2022) found being stressful to rats. After the 60 seconds of preoxygenation, when the sevoflurane vaporizer was turned on, several differences among the rats' behaviours appeared. Less time was spent on 'walk' and more on 'still'. This could indicate a freeze behaviour, meaning the gas was aversive. In this study, the criteria for the still behaviour did not completely match those for the freeze behaviour described by Fanzelow (1994), as the still behaviour could be scored even if the rat moved its head. In future studies, the freeze behaviour should be used during ethological scoring of rats since this behaviour is well developed and plays an important role in reaction to fear in the species.

In previous studies rats have shown aversion to both isoflurane and sevoflurane (Leach *et al.* 2002; Boulanger Bertolus *et al.* 2015). In these studies, the animals had the possibility to avoid or flee from the gas which many of the animals did. In the current study however, the rats had nowhere to escape, and therefore, the freeze behaviour might have been used as the best possible defence mechanism, emphasizing the importance of recognizing this behaviour.

The rats spending much time on grooming during the preoxygenation could indicate that the presence inside the induction chamber was stressful regardless of training, since extensive grooming can be triggered by stress (Giorgi *et al.* 2003). Additionally, most of the rats did not perform normal grooming cycles, but instead many interrupted ones. This also indicates the grooming behaviours as being a reaction to stress rather than being normal behaviour, usually performed when the animal is calm and relaxed. When the sevoflurane vaporizer was turned on, the duration and frequency of grooming behaviours decreased. Possibly, the gas might have induced more direct fear behaviours in the rats, such as freezing, hence the decrease of grooming. Another behaviour that had a slight increase in mean duration after the sevoflurane start was 'free rearing', which according to Sturman

et al. (2018) indicates acute stress in mice. In summary, these behaviours could be interpreted as being inside the induction chamber is stressful to rats, but the sevoflurane causes even more stress in the animals. To evaluate this, the behaviours would have to be graded to which behaviours that are more sensitive to stress than others. This would require more thorough studies of rat behaviour. Although, it can be concluded that the gas regardless of flow rate is stressful to the animals, and therefore it is essential to investigate how to decrease stress in rats during chamber induction. For example, the effects of premedication should be evaluated since the flow rate for gas delivery seems less important regarding stress.

5.3 Excitement

McKelvey & Hollingshead (2003) states that a faster induction could lower the risk of an animal to enter the excitement stage of anaesthesia. In this study, no significant differences were found in frequency or duration of excitement between the two groups, even though the high flow group had a significant faster induction. Instead, 4 of 5 rats in the high flow group showed excitement during the induction, but only 3 of 5 rats in the low flow group, which rather would indicate the opposite relation. However, McKelvey & Hollingshead (2003) do not only associate a fast induction with less excitement, but also the use of premedication before induction of anaesthesia. From the present study, it is concluded that more research with a larger study population is needed to see if faster chamber induction with higher flows lowers the risk of excitement, and how premedication affects the issue.

Another factor that may have great influence on the induction quality is the size of the induction chamber. In this study the induction chamber had a volume of six litres, allowing the animals quite much freedom of movement during the induction. Some other authors recommend the induction chamber to be smaller. The UBC Animal Care Committee (2017) states that the rat should be able to move around at some extent so that the anaesthetist can see when the righting reflex is lost. The authors recommend a chamber volume of two litres for rats weighing less than 400g. McKelvey & Hollingshead (2003) explain that the excitement behaviour during stage II of anaesthesia is an overreaction to external stimuli while inhibiting neurons are depressed by the anaesthetic drug. It could be possible that a large induction chamber, as the one used in this experiment, results in a greater amount of stimulus signalization reaching the brain since the rats were able to move up and down, back and forth at all times during the induction. Therefore, a larger chamber could perhaps promote excitement behaviour. Hence, research should be made to investigate the importance of the induction chamber size regarding induction quality. In the book *Laboratory Animals* published in 1992, the authors address the issue and states that a smaller size induction chamber results in a decrease in

excitement behaviour and ataxia during induction of anaesthesia in rats (Gwynne & Wallace 1992). Although this might be true, it is uncertain if the restraint only makes the animal physically unable to express ongoing excitement phase in the neurological system, or if it actually lowers the excitement activity. Even though the uncontrolled movements cannot be performed, there is a possibility that the rat still experiences excitement, and therefore still release epinephrine as a response to anxiety and stress. Being a stress hormone, epinephrine affects the body physiologically, and therefore also has a possibility to interfere with test results. To minimize excitement during induction of anaesthesia in laboratory animals therefore does not only benefit animal welfare but could also reduce research bias, making it an important consideration in future research.

6. Conclusion

From this study, it cannot be concluded that different fresh gas flows have an effect on stress-related behaviours in rats during chamber induction of anaesthesia with sevoflurane.

The rats that were induced with a higher flow of 6 L/min had a significantly faster induction than rats induced with a low flow of 3 L/min (vaporizer setting 8%). The higher fresh gas flow also resulted in a significantly higher sevoflurane concentration inside the induction chamber at time of recumbency. Rats in the low flow group tended to show longer median durations of stress related behaviours during the induction, but this could be due to the significantly longer time to recumbency in this group. However, if a quick induction is desired, a higher flow should be used.

There were no differences regarding frequency or duration of excitement behaviour between the low flow and high flow group. In other words, a faster induction may not reduce the risk of excitement during chamber induction with sevoflurane. More research is needed in this area to be able to understand which factors that influence excitement during anaesthesia induction and recovery in rats.

Comparing rat behaviour in the induction chamber, all rats performed stress-related behaviours both during preoxygenation and induction. This might indicate that both the induction chamber and the sevoflurane gas are stressful to rats. This study suggest that sevoflurane might be stressful to rats regardless of fresh gas flow. Further, the effects of premedication on stress and excitement during chamber induction in rats should be evaluated in future studies.

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Populärvetenskaplig sammanfattning

Råttor är vanliga försöksdjur och används ofta inom forskningen. Enligt rådande EU direktiv ska användningen av försöksdjur i möjligaste mån ersättas med andra metoder. I de fall detta ännu inte är möjligt ska i stället så få djur som möjligt användas och djurvälfärden ska ha högsta prioritet. För att förbättra djurens tillvaro bör stress och lidande minimeras.

Råttor kan reagera på stress med ett flertal olika beteenden. Dessa kan till exempel vara att ställa sig på bakbenen, tvätta sig frenetiskt, urinera och passera avföring. Gnagare har även utvecklat en försvarsmekanism som kallas frysning, där de står helt stilla för att inte upptäckas av potentiella hot. Kroppen reagerar även på stress genom att frisätta stresshormoner som ökar pulsen och höjer blodtrycket. Dessa fysiologiska förändringar skulle kunna påverka mätvärden i olika experiment och därför är gynnas även forskningsresultaten av att minska stress hos försöksdjuren.

Ett vanligt förekommande moment inom forskning där djur används är narkos för att kunna utföra operationer. När råttor sövs med gas sker detta ofta genom att djuret placeras i en gaskammare. Detta har visat sig vara kopplat till stress och obehag hos djuren, och studier har visat att råttor undviker narkosgaser om de får möjligheten att välja. En sådan relativt ny narkosgas som är vanlig både inom veterinär- och humanvården är sevofluran. Sevofluran har egenskapen att det lättare transporteras till och från hjärnan via blodet jämfört med tidigare narkosgaser. Detta medför att djur som sövs med sevofluran både somnar och vaknar snabbare.

Ett problem som kan uppstå när djur eller människor ska sövas är att individen hamnar i en så kallad excitationsfas. Detta innebär att narkosmedlet dämpar nerv-systemets naturliga förmåga att minska elektrisk signalering, vilket resulterar i att hjärnan överreagerar på intryck. I excitationsfasen kan djuret därför utföra krampaktiga okontrollerade rörelser trots att det förlorat medvetandet. Även vid excitation frisätts stresshormoner i kroppen som skulle kunna ha påverkan på forskningsresultat. Några sätt att undvika att djuret exciterar är att se till att insomningen går relativt snabbt samt att tillföra lugnande läkemedel, så kallad premedicinering, inför narkosen. Premedicinering är även vanligt inom den kliniska veterinärvården för att minska stress hos sällskapsdjur inför narkos. I dag är det

ovanligt att sådana läkemedel används på råttor inom forskningen som ska sövas med gas.

När det kommer till gasnarkos på råtta saknas det forskning kring sövningsmetoder som orsakar minst stress. För att ta reda på det krävs det först att de olika faktorer som påverkar stress hos råttor vid gasnarkos identifieras. I denna studie sövdes tio försöksdjursavlade råttor med sevofluran i en gaskammare för att studera stress-beteenden. Vid användningen av narkosgaser kan bland annat koncentrationen av gasen och hastigheten som gasen levereras (så kallat flödet) regleras för att djuret ska somna så lugnt som möjligt. I denna studie delades råttorna slumpmässigt in i två grupper, där ena gruppen sövdes med ett lågt flöde och andra med ett högt flöde, men med samma koncentration av sevofluran. Råttorna filmades under insom-ningen och uppvakningen för att undersöka huruvida olika flöden påverkar stressbeteenden hos råttorna. Det noterades även hur lång tid det tog för råttorna i de olika grupperna att somna och vakna, samt om råttorna exciterade. Därefter studerades filmerna utefter ett bedömningsschema där flera olika beteenden registrerades.

Resultatet visade att frekvensen, latensen till och durationen av olika stress-beteenden inte skiljde sig mellan de båda grupperna, förutom att latensen till beteendet ”ligg” var längre i lågflödesgruppen. Resultatet visade även att de råttor som fick det högre flödet somnade snabbare än de som fick det lägre flödet. Det sågs däremot inte heller några skillnader i frekvens och duration av excitation mellan grupperna, utan majoriteten av råttorna i båda grupper exciterade. Detta trots att den snabbare insomningen i högflödesgruppen borde minskat risken för excitation.

Genom att jämföra beteenden hos råttorna i gaskammaren både före och efter gasen sattes på sågs även att råttorna utförde flera stressbeteenden vid båda dessa till-fällen, men att beteendet tenderade att förändras över tid. Råttorna spenderade i större utsträckning mer tid på att tvätta sig och gå omkring i kammaren före, men var i stället mer stilla, som vid frysning, efter att sevoflurantillförseln påbörjades. Detta skulle kunna indikera att råttorna upplevde både narkosgasen och att befinna sig i själva kammaren som stressande, men att flödet som gasen levererades med hade mindre betydelse.

För att bättre förstå vad som orsakar stress hos råttor vid gasnarkos i kammare krävs att fler studier genomförs där större grupper av individer undersöks för att lättare kunna se samband. Det är även värdefullt att utvärdera effekter av premedicinering hos råttor inom forskningen som ska sövas med gas, då denna studie visat att excitation och stressrelaterade beteenden vid gasnarkos med sevofluran i kammare hos råttor inte verkar påverkas av de flöden som används i denna studie.

Acknowledgements

I would like to thank my supervisor Patricia Hedenqvist and assistant supervisor Vanessa Bettembourg for great guidance and support during the process of this paper. I also gratefully want to acknowledge the personnel at the Laboratory animal science facility for providing the animals, premises, and equipment.

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