Stress response of mice under different volatile anaesthesia

Schlichting A¹, Haberstroh J³, Tsai PP¹, Stelzer HD¹, Hackbarth H¹
¹Institute of Animal Welfare and Behaviour, University of Veterinary Medicine Hannover, Germany
²Experimental Surgery, BioMed Centre, University Medical Centre Freiburg, Germany
Corresponding author: Anja Schlichting, Email anja.schlichting@googlemail.com

Abstract
Anæsthesia is often recommended to minimise discomfort caused by experimental procedure, e.g. blood sampling. However, it has been reported that anæsthesia with diethylæther causes a pronounced endocrinæal stress response in rats. Thus, it is important to determine whether anæsthesia itself will cause stress. The present study focused on the stress response of different anæsthetics (sevoæloræna, isofloræna, ether and CO₂). Corticosterone concentration and Open Field Test were measured as indicators of stress response.
In total, 60 inbred BALB/cOlaHsd mice, obtained from Harlan Winkelmann (Borchen, Germany), were randomly allotted to six experimental groups, in groups of five. With the exception of the control group mice were anaæsthetised with sevoæloræna isofloræna, ether or CO₂. The control and sham group (not anaæsthetised) were also transferred into the same box before blood sampling, but only the sham group received an airflow (same speed as under anaæsthesia). Blood samples were collected twice, one week before the experiment and 15 minutes after the experimental procedure. The Open Field test was performed immediately after the second blood sampling. Significant increased corticosterone levels were found for sham and different anaæsthetic groups after the experimental procedure. Compared to the control group corticosterone concentration increased significantly after anaæsthesia. In the Open Field test significant differences between groups were found for the total distance mainly due to the differences between groups under isofloræna and ether anaæsthesia, while for the crossing frequency the main difference was found between the sham group and groups with isofloræna & CO₂.

Keywords: volatile anaesthesia, stress, mice, corticosterone, open field

In laboratory animals inhalation anæsthesia is often used for short-acting anaesthesia to minimise stress caused by experimental procedure e.g. blood sampling. A closed anaæsthetic system is commonly used for the induction of inhalation anaæsthesia. However, it has been reported that some anaæsthetics such as ether can cause endocrinæal stress because it is highly irritating to the respiratory system. The use of CO₂ as inhalation anaæsthetics is also discussed very controversially. Various authors measured a variety of behavioural and physiological responses as indicators of pain and stress in studies with CO₂ such as hyperventilation or escape behaviour. Other authors prefer CO₂ as an anaæsthetic as well as for euthanasia. In a previous study we found that volatile anaæsthesia (ether and CO₂) caused more stress than retro-bulbar blood sampling without anaæsthesia. Other possibilities for inhalation anaæsthesia are sevoæloræna and isofloræna. Both demonstrate a very rapid induction and recovery from anaæsthesia and are non-irritant. Nonetheless, there are no results for endocrinæal stress during or after anaæsthesia with these agents.

Thus, this study focuses on the stress response of different short-term inhaled anaæsthetics (sevoæloræna, isofloræna, ether and CO₂), which are performed for rapid routine procedures such as blood sampling. Corticosterone (CORT) concentration and the Open Field Test were measured as indicators of stress response.

Materials and Methods

Animals: In total, 60 female BALB/cOlaHsd mice, specific pathogen free according to the FELASA recommendation, about 6 weeks old, obtained from Harlan Laboratories, were marked by ear puncturing and randomly allotted to six experimental groups. The animals were distributed to 12 Makrolon type III cages, in groups of five. At the beginning of the study the mice had an average weight of 18.7 g.

Environment: All animals were maintained in a scantainer (Scanbur A5 Denmark), with air exchange 10-16 times per hour. The room temperature was 22 ± 2°C and 50 ± 10% relative humidity, with a 12/12 hour
light/dark cycle (light on at 6:00) and at a light intensity of 50±10 lux inside the scantainer.

Food and water: Tap water in drinking bottles and pelleted food containing 19.0% protein, 4.0% fat, 6% fibre and 7% ash (Altromin No. 1324, Altromin GmbH, Lage, Germany) were given ad libitum.

Bedding and nest material: As bedding, soft wood shavings were provided for each cage (Altromin Type 5, Altromin GmbH, Lage, Germany). Cage and bedding were changed once a week (always on Thursdays). For environmental improvement nest material (Nestlets, EBECO) was placed in the cages after cage changing.

Experimental groups: In total 60 animals were tested in 6 groups with 10 female mice aged 8 weeks. Four different volatile anaesthetics (ether, CO₂, isoflurane and sevoflurane) were examined. Besides the groups controls and sham, anaesthesia was performed.

Anaesthesia: All animals were placed in a Perspex box (15 cm x 13 cm x 28 cm, designed for anaesthesia). In this box anaesthesia was induced either with 8% sevoflurane vaporised with oxygen (5 l/min) or 4% isoflurane vaporised with oxygen (51/min). For CO₂ anaesthesia, a special particularly developed lid with a CO₂ inflow of 4.6 l/min was introduced into the box. The lid can provide a very even CO₂ distribution in the box and therefore can prevent severe distress or pain. Ether anaesthesia was initiated with 8 ml ether inside a gas-washing bottle, which was vaporised with 5 l/min oxygen. All animals were taken out of the box when breathing was clearly depressed. Sham and control animals were placed in the same box for 78 sec (mean time of induction for all anaesthetics) without anaesthesia, but the sham group received an air-inflow of 5 l/min. An overview is given in Table 1.

Corticosterone analysis: For determining the basal value of corticosterone 150 µl blood was taken one week after arrival by retro-bulbar venous puncture in a Natrium Heparin capillary.

A second blood sample (150 µl) using the same method during the experiment without anaesthesia was taken from each mouse 15 min after anaesthesia. Blood plasma was collected after centrifuging for 4 min (12000 rpm). The corticosterone concentration was analysed using a competitive corticosterone ELISA (IBL) and measured with a microtiter reader at 450nm.

Open Field Test: Following the blood collection the mice were introduced into the open field (60 cm x 60 cm) for 5 min. During the test the animals were videotaped by a camera (Kegani). The video was analysed with Etho Vision (Noldus, version 3.1). The total distance and the frequency animals crossed between the outer and inner zones were recorded.

Experimental design

During 12 days of adaptation the animals were handled daily over 7 days. Each animal was put on the arm for at least 1 minute to minimise stress during the experiment. For measuring the basal corticosterone value blood was taken one week after arrival. At 8 weeks of age the experiment was performed and finished.

All tests were performed within six days between 9:00-12:00 in the morning, 10 mice per day. Only one animal per cage was taken out each day to avoid additional stress by opening the cage a second time. The animal was taken out of the cage and transferred into a transport cage and taken to the experimental room. Immediately after arriving the animal was, depending on the test group, anaesthetised. Following the anaesthesia the mice stayed in the transport cage for 15 min. From each mouse a blood sample was taken by retro-bulbar puncture without anaesthesia for corticosterone measurement. Finally the animals were introduced into the open field for 5 min and then were returned to their home cages.

Statistics

All data were analysed using StatView software (version 5.0, SAS Institute Inc., Cary, NC, USA, 1998). The data were analysed for normal distribution followed by ANOVA and a Scheffé-test with a significance level of 5%. Non-normal distributed data were analysed by non-parametric test (Kruskal-Wallis test or Mann-Whitney U test).

<table>
<thead>
<tr>
<th>Table 1. Overview of doses and gas inflow</th>
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<tbody>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>Sevoflurane</td>
</tr>
<tr>
<td>Isoflurane</td>
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<tr>
<td>Ether</td>
</tr>
<tr>
<td>CO₂</td>
</tr>
<tr>
<td>Sham</td>
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<tr>
<td>Control</td>
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</tbody>
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Results

Blood corticosterone concentration
In comparison to the basal value corticosterone concentration rose significantly after anaesthesia (sevoflurane, isoflurane, CO₂, ether: p<0.001) as well as after sham procedure (p=0.009) (Figure 1). Even though the corticosterone concentration of control group increased, it did not reach a statistical difference.

Significant differences were found for the change in the corticosterone concentration (F(3,54)=11.954, p<0.0001). The increased corticosterone levels of each group were: isoflurane (1816.2±386.8 nmol/l), sevoflurane (1527.2±397.9 nmol/l), ether (1444.3±405.0 nmol/l), CO₂ (1440.7±337.2 nmol/l), sham (773.5±535.1 nmol/l) and control (358.2±790.2 nmol/l). Compared to the control group corticosterone concentration increased significantly after anaesthesia (p=0.0004 for sevoflurane, p<0.0001 for isoflurane, p=0.0012 for ether and p=0.0012 for CO₂). There were no significant differences between groups anaesthetised by ether, CO₂, isoflurane and sevoflurane, nor between the sham and control groups (Figure 2).

Locomotor activity in the Open Field test
Significant differences were found for the Open Field test in locomotor activity (F(3,54)=3.528, p=0.0086 for total distance; F(3,54)=2.868, p=0.0243 for frequency). For the total distance the statistical difference is mainly due to the difference between groups under isoflurane and ether anaesthesia (p=0.0581), while the main difference was found between the sham group and groups of isoflurane & CO₂ for frequency (Table 2).

Discussion

The aim of this study was to compare four volatile anaesthetics (CO₂, ether, sevoflurane, isoflurane) commonly used for minor procedures, determining their impact on the animals after short exposure. The secretion of glucocorticoids is a classic endocrinial response to stress. The results show that corticosterone concentration of all anaesthetised mice is higher than the basic value of non-stressed mice, this being independent of the anaesthetics. There is nearly no difference between the corticosterone levels following the different anaesthetics. Animals with sham anaesthesia also show a higher corticosterone level in
Table 2. Travel distance (cm/5 min) and frequency of crossing (mean ± SD) in the Open Field Test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total distance</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sevoflurane</td>
<td>2033.5±445.2</td>
<td>15.8±10.7</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1556.1±598.5</td>
<td>10.8±9.9</td>
</tr>
<tr>
<td>CO₂</td>
<td>1612.4±948.6</td>
<td>10.0±10.0</td>
</tr>
<tr>
<td>Ether</td>
<td>2805.2±155.4</td>
<td>12.0±7.0</td>
</tr>
<tr>
<td>Sham</td>
<td>2503.4±227.2</td>
<td>23.2±8.0</td>
</tr>
<tr>
<td>Control</td>
<td>2322.2±498.1</td>
<td>17.5±7.3</td>
</tr>
</tbody>
</table>

comparison to the control group. This indicates that
the gas inflow alone into the box may also raise the
corticosterone concentration. Thus, the manipulation
of animals and the anaesthesia itself are both stressors.

In a previous study4 we showed that short-term
volatile anaesthesia (ether and CO₂) caused more
stress than retro-bulbar blood sampling without
anaesthesia, but there was no difference between
both anaesthetics. The present study also found no
difference between the corticosterone levels following
the different anaesthetics. This suggests that all
the inhalation anaesthesia performed in the present
study led to a similar level of stress, even though the reason
causing stress may differ to ether (highly irritating to
the respiratory system).

Quartermain et al.11 showed that stressors reduce
locomotor activity in the open field test. In the present
study animals which had received ether anaesthesia
travelled significantly more than animals which had
received isoflurane anaesthesia. Comparable results
were also found by a previous study4, mice given ether
anaesthesia travelling more than after CO₂ anaesthesia.
During the experiment all animals, independent of
treatment, showed a similar crossing frequency.
The results indicate that the impact of short-term
inhalation anaesthesia in the open field test may be
mild after 15 min even though the residual anaesthesia
could still play a role. Thus, it may not be ideal timing
to use the open field test to determine the stress
response of short-term inhaled anaesthesia.

Based on our data the corticosterone level rose
significantly after short-term volatile anaesthesia
and there were no significant differences between
the four anaesthetics used. It can be concluded that
all inhalation anaesthetics used in this study cause
temporary stress in mice and that there is no relevant
difference between the different anaesthetics.
Therefore, it should be taken into consideration
whether anaesthesia used for short experimental
procedures such as blood sampling reduces stress or
actually causes more stress than the experimental
procedure itself.

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