Thermal Effect during Ketamine Anaesthesia in Laboratorial Mice

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SUMMARY

Ketamine is an anaesthetic and analgesic agent used frequently in research and clinical practice. However, this drug is related with memory deficits. These deficits are dependent of the temperature. So, body temperature is an important parameter to monitor during anesthesia. Determination of body temperature by traditional means, such rectal thermometer, is stressful to animals, and extremely time consuming. Thermography may be a rapid non-invasive method to determine mice superficial body temperatures without the need to insert thermometers, thermocouples or implantable microchips. Therefore the purpose of this study was to evaluate thermography as noninvasive method for monitoring thermal superficial changes during ketamine anaesthesia in laboratory animals. To achieve this aim, four adult mice were anaesthetized with ketamine (150 mg/kg) and their body temperature was measured continuously during anaesthesia and recovery of the animals. Thermal measures were conducted using a FLIR long infrared camera with a spatial resolution of 320x240 pixels, a thermal sensitivity of 68 mK. Our results showed that tail temperature decreased during anaesthesia, as we expected. In summary, this work showed that thermography showed to be a good, fast and easy method to evaluate the thermal distribution in living beings. Moreover, this work suggested that thermography can be used for developing better and more effective types of anaesthesia.

1. INTRODUCTION

Most clinical procedures in veterinary medicine as well as in humane medicine have to be performed using anaesthesia. Ketamine, a non-competitive glutamate N-methyl-d-aspartate acid receptor antagonist, is an anaesthetic and analgesic agent used frequently in research and clinical practice (6). More specifically, this drug is used, in human medicine, in painful diagnostic procedures, traumatic and hypovolemic shock and burn situations (1, 7, 8). In veterinary medicine and research, ketamine is frequently used as part of the anaesthetic protocol, combined with other drugs in high variety of surgeries and short procedures (5). However, this drug can trigger neuro-degeneration and memory deficits (4, 11). Cell death at a neurological level may have serious implications for the learning capacity and memory. It was reported that these deficits in memory are dependent of the temperature (3). Room temperature of the 21°C may exert neuroprotection but 25°C is a potential stressful event that increases brain vulnerability and may potentiate ketamine-induced deficit (3). In other way, hypo-thermia may lead to death of animals during anaesthesia. Furthermore, it was reported that high doses of ketamine may cause hypothermia, indicating an involvement of the N-methyl-D-aspartate receptor in thermoregulation pathway (10).

Body temperature is an important parameter to monitor during anesthesia. Determination of body temperature by traditional means is stressful to animals, and extremely time consuming. Conventionally the core body temperature of mice has been measured by either the insertion of a thermometer into the anus of the mice or insertion of a thermocouple via the anus into the large intestine (9, 13). Thermography is a rapid non-invasive method to determine mice superficial body temperatures without the need to insert ther-mometers, thermocouples or implantable microchips. Therefore the purpose of this study was to evaluate thermography as noninvasive method for monitoring thermal superficial changes during ketamine anaesthesia in mice.

2. METHODS

2.1 Animals

Four 12 months of age, male C57BL/6 mice bred in the animal facility of the institute (F1-F2 offspring of animals bought from Charles River, Barcelona, Spain) were used. The mice were housed with controlled temperature (21º) and relative humidity at 55%. Lights were on a 12/12h cycle, with lights off at 17.00h. The animals were housed in groups of three to five mice per cage (Makrolon type II cage, Tecniplast, Dias de Sousa, Alcochete, Portugal) (Fig. 1) and it received a commercial pellet diet (4RF25-GLP Mucedola, SRL, Settimo Milanese, Italy) and water ad libitum. Each cage was provided with standard corncob litter (Probiológica, Lisbon, Portugal), a piece of tissue paper and a cardboard tube. The mice were allowed to acclimate to the facilities at least one week prior to the commencement of the study.

2.2 Anaesthesia

Ketamine (Imalgéne® Merial, Portugal; 100mg ml-1) was used for anaesthesia. Standard physiological sa-line 0.9% (Soro Fisiologico, Braun Vet, Portugal) was used for diluting the drug (to ease handling small volumes).

Fig. 1 - Type II cage with mice.

The mice were weighed using an electronic scale and the drug dosage calculated for each animal. By holding the mice firmly by the base of the tail, the mice were placed on the lid of the cage. The thumb and index finger of the left hand secured the skin of the neck and lifted the animal while the palm and third finger of the same hand held the tail. The animals were maintained in dorsal recumbence during the administration of the drug. Ketamine was administered as a single intraperitoneal (i.p.) injection (150mg/kg). Intraperitoneal administration was per-formed lateral to the midline next to the umbilicus. The needle (15mm / 25 gauge) was inserted in an angle of 45º to the abdominal wall in the lower left quadrant of the abdomen (Fig. 2). Injection and restraint were always performed by the same person. After i.p. injection, each animal was placed alone on a blanket with circular acrylic protection until it lost its righting reflex. After this lost, the animal was placed in dorsal recumbence. The time to loss of righting reflex and duration of anesthesia were rec-orded. The time point to recovery of anesthesia was defined as a recovery of righting reflex.

2.3 Thermal Measures

Tail temperature was measured continuously during induction, maintenance and recovery of the anaesthesia.

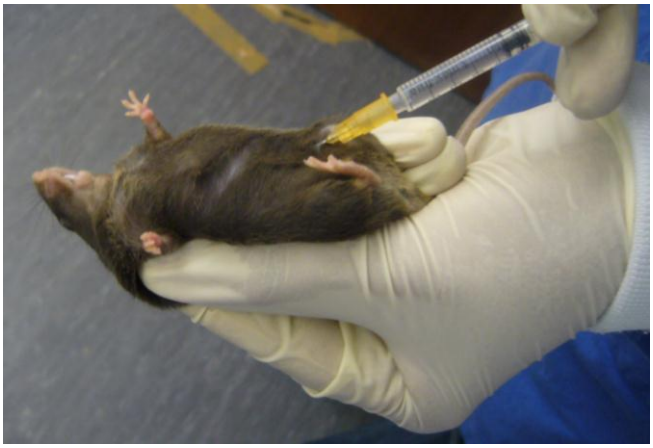

Fig. 2 - Intraperitoneal injection administration.

Thermal measures were conducted using a FLIR long infrared camera (A325) with a spatial resolution of 320x240 pixels and a thermal sensitivity of 68 mK (Fig. 3). All the measures were conducted re-cording one image per second and analyzed posteriorly.
During induction of anaesthesia animals were placed in a transparent cilinder. The room temperature was 21ºC.

Fig. 3 - Thermal image acquisition setup and camera view.

2.4 Data analysis

The recorded thermal sequences were processed with the ThermaCam™ researcher Pro 2.10 software from FLIR. In these sequences a straight line was drawn perpendicular to the mice tail (Fig. 4) and the maximum temperature along the line over the time was exported to Microsoft Office Excel 2003 (Microsoft Corporation, U.K.). Since the ambient temperature was always lower than the mice tail, the maximum temperature along the line was always correspondent to the mice tail.

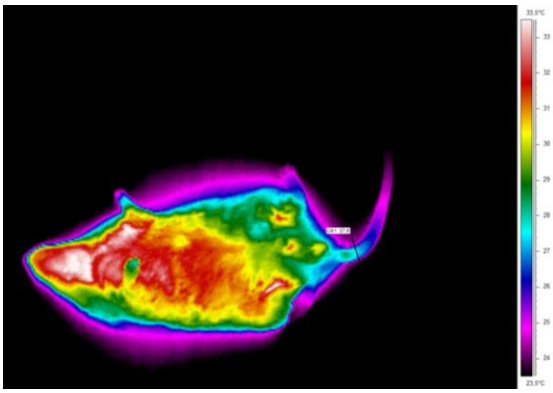

Fig. 4 - Thermal image processing with line maximum temperature, 10mm from the tail base.

Six time points were defined for temperature analyses: immediately after i.p. injection (time 0), 1, 5, 10 and 15 minutes after injection, and immediately before recovery. The temperatures measured in these time points were compared between them. All results were analyzed by using Microsoft Office Excel 2003 for data management and SPSS 16.0 for Windows (Apache Software Foundation, Forest Hill, MD) for statistical analysis. Firstly, data was tested for normality and homogeneities of variances. Para-metric tests (one-way ANOVA with Bonferroni post hoc tests) were used if data fulfilled this assumption.
Data was expressed as means ± standard deviations. $P<0.05$ was considered statistically significant.

3. RESULTS

All animals lost the consciousness. The time to loss of righting reflex was 1.83±0.75 minutes and mice were unconsciousness for 30.5±5.75 minutes. As it was expected, these results showed that ketamine is an effective anaesthetic agent for laboratory animals. The time that the animals were unconsciousness is sufficient to perform a several short procedures in mice, such as collect blood, biopsies, inoculations, surgical implantation, aseptically urine collection among other things. Ketamine is however routinely used combined with several drugs to achieve longer times of anaesthesia and reduce side effects.
This work showed that tail temperature decreased significantly during anesthesia, (Figs. 5, 6, 7). The temperature of the animals immediately after i.p. injection (32.35±1.60ºC) is significantly different of the temperature at times 5 (27.23±0.51ºC, $p<0.01$), 10 (25.19±0.52ºC, $p<0.01$) 15 min. (25.18±0.89ºC, $p<0.01$) after i.p injection, and at re-covery time point (25.39±0.80ºC, $p<0.01$). Moreover, no significant differences were observed between the temperature recorded at time 10 minutes and temperature immediately before animal recov-ery, showing temperature stabilization after that time point ($p=1$).

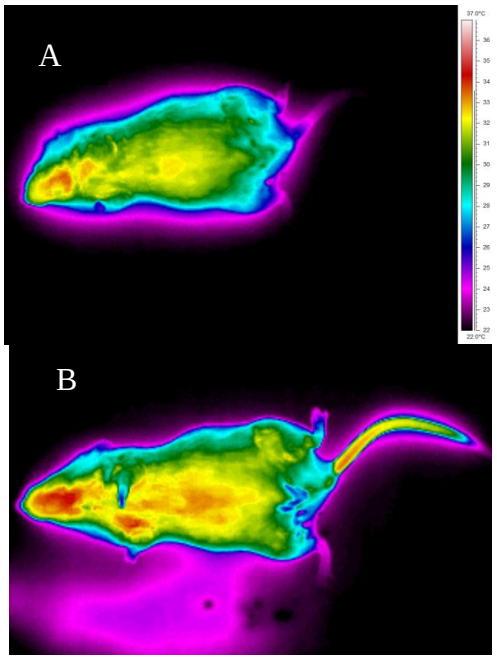

Fig. 5 - Thermal images:
A) after induction (time 0).
B) immediately before anaesthesia recovery.

In anaesthetic situations, animals decreased their metabolism and consequently the body temperature is reduced. It was reported that high doses of ketamine, an N-methyl-D-aspartate receptor antagonist, caused hypothermia (10). For thermography to be considered a good method for determining temperature in laboratory animals it should be able to detect the fall in body temperature caused by anaesthetic drugs. This paper showed that thermography can detect such drop in temperature after ketamine anaesthesia.

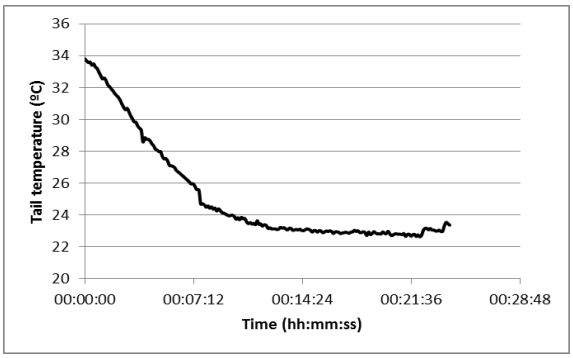

Fig. 6 - Tail temperature evolution during anaesthesia from one animal showed as an example.

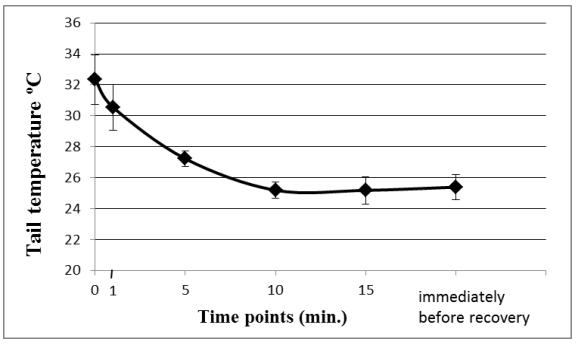

Fig. 7 - Tail temperature at different time points: 0, 1, 5, 10 and 15 minutes after i.p. injection, and immediately before recovery from all animals (n=4). Data are presented as mean±SD.

In this work we used tail temperature to evaluate the superficial body temperature. Tail is the major thermoregulatory organ in mice and it is a good indicator of superficial body temperature. Tail has a large surface to volume ratio, and it is perfused with many blood vessels, especially at the tail tip and midlength (2). More specifically, mice control their body temperature through their tails by dilating or constricting their tail blood vessels. When body temperature drops, the tail vessels shrink in diameter (vasoconstriction) thus restricting blood flow to the tail (12). Less blood flows into the tail for cooling, and body heat is conserved. This heat also can be channeled to the vital organs in order to compensate the reduction in internal body temperature caused by the slower metabolism induced by anaesthetics.

4. CONCLUSIONS

Anaesthetic agents, such as ketamine, produce a drop in superficial body temperature in mice. Thermography showed to be a valid, fast and easy method to evaluate the thermal distribution in living beings. Moreover, this work demonstrated that thermography can be used for developing better and more effective types of anaesthesia in laboratory an-imals.

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