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Impact of inhalation anaesthesia, surgery and analgesic treatment on home cage behaviour in laboratory mice

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ABSTRACT

Anaesthesia and analgesia are used frequently in laboratory routine to ensure animal welfare and good scientific outcomes in experiments that may elicit pain or require immobilisation of the animal. However, there is concern regarding the effect of these procedures on animal behaviour in subsequent experiments. Our study determined the impact of short inhalation anaesthesia (sevoflurane, 15 min, 4.9%) and minor surgery (one-sided sham embryo transfer in females, one-sided sham vasectomy in males) with or without pain treatment (carprofen, 5 mg/kg, bid) on spontaneous species-specific home cage behaviours in inbred mice. Analysis of 18-h continuous video recordings showed clear post-procedural changes in spontaneous home cage behaviours, with changes of a moderate level after anaesthesia being marked after surgery. Self-grooming, resting and locomotion were the most important behaviours for group separation. Analysis of the temporal distribution of behavioural changes revealed that resting behaviour was altered contradictory to its circadian rhythm as it was decreased in the light phase and increased in the dark phase. Also, locomotion was decreased in the dark phase at 12 to 18 h after surgery and anaesthesia. In contrast, self-grooming was increased independently of circadian rhythm, being increased for up to 18 h after surgery and anaesthesia. Following surgery, there was no significant difference in duration of behaviours between animals that were treated with carprofen or left without pain relief. In conclusion, it can be assumed that the changes observed in home cage behaviours hint at reduced animal well-being. However, pain or the efficacy of post-operative pain treatment could not be discriminated reliably from the impact of the surgical procedure including inhalation anaesthesia by observing animals' home cage behaviour. However for the interpretation of behavioural research data, the distinct impact of anaesthesia, surgery, pain treatment and other experimental procedures has to be considered. Our results highlight the requirement for knowledge of species-specific circadian rhythms of behaviours as well as the importance of determining the appropriate time of day for behavioural and welfare assessment.

1. Introduction

Laboratory mice are currently the most widely used mammal species in biomedical research (Baumanns, 2004). Due to their manageable size, a wealth of inbred or genetically modified strains and plenitude of established

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experimental protocols, mice are used increasingly in complex investigations. These often require induction of general anaesthesia for performing special diagnostic manipulations (e.g., imaging procedures, endoscopy, blood collection), or surgical procedures that in turn require peri- and/or post-operative pain treatment. Analgesic treatment would seem necessary after invasive procedures like laparotomy, but has been omitted frequently in the past (Richardson and Flecknell, 2006; Stokes et al., 2009). Reasons may vary from concern that analgesic use may compromise the data obtained from the proven model to the difficulties of detecting and interpreting signs of pain after minor surgery in mice (Richardson and Flecknell, 2006).

In contrast to most physiological and clinical parameters, behaviour can be recorded easily in a non-invasive manner and can provide a sensitive correlate of the internal state of an animal. Alterations in the frequency of, or in the latency to display, spontaneous and species-specific behaviours, like rearing, sniffing, walking or burrowing behaviour (Roughan et al., 2009; Jirkof et al., 2010), as well as the quality of nest construction and structuring of cage territory (Arras et al., 2007; Jirkof et al., 2013b) are recent examples of behavioural indicators of well-being or distress and also pain in mice. Thus, behavioural indicators are frequently used not only in the clinical assessment of laboratory animal well-being but also in basic pain research.

Recently, it has become apparent that the physiological and behavioural changes induced by minor to moderate surgery can last up to 24–48 h (Arras et al., 2007; Matsumiya et al., 2012). Moreover, it has been shown that changes induced by anaesthesia, and possibly also by treatment-related procedures (e.g., handling, transport to operating theatre etc.), may affect physiology and animal well-being for several hours (Cesarovic et al., 2010; Jirkof et al., 2013b). It can be assumed that, in some situations, the effects of anaesthesia may overlap and to some extent mask the post-operative effects of pain and/or analgesic treatment. In addition, although the impact of volatile anaesthetic agents on learning, memory, solving of spatial tasks and activity has been studied recently (Petrenko et al., 2008; Valentim et al., 2008; Mena et al., 2010), the effects of anaesthesia, as an integral part of standard surgical procedures, on spontaneous home cage behaviours have been described only rarely.

Thus this study aimed to deepen the knowledge on mouse behaviour in the period following short anaesthesia or minor surgical procedure.

Further, there is concern regarding not only animal welfare but also the reliability of data obtained from research using animals that have undergone procedures that may elicit pain and/or involve analgesic and/or anaesthetic treatment. In some areas of biomedical research like in sepsis or acute brain injury models experimental read-out is recorded shortly post-procedural (Baracchi et al., 2011; Khatibi et al., 2011; Kuroki et al., 2013). For such procedures inhalation anaesthesia seems to be the protocol of choice. Hence, questions regarding the duration and persistence of long-lasting anaesthetic or procedural effects are rising into focus not only in behavioural welfare assessment but also basic and preclinical research (Stokes et al., 2009).

To our knowledge there is only one publication presenting changes in home cage behaviours after surgery and anaesthesia over a period of 24 h in rats (Roughan and Flecknell, 2000), while in mice observations of natural home cage behaviour after surgery and / or anaesthesia have been recorded for periods of less than 1 to 6 h only (Wright-Williams et al., 2007; Jacobsen et al., 2012; Jirkof et al., 2012, 2013a; Leach et al., 2012).

Therefore, this study aimed to determine the effects of minor surgery (one-sided sham embryo transfer in females, one-sided sham vasectomy in males) with or without pain treatment, as well as the impact of standard, short inhalation anaesthesia alone on spontaneous and species-specific home cage behaviours in two common inbred mice strains. To this end, the overall temporal distribution of the animals' natural behaviours was investigated according to their circadian rhythmicity in order to identify whether specific behaviours are altered significantly after surgery or inhalation anaesthesia.

2. Methods

2.1. Ethics statement

The animal housing and experimental protocols were approved by the Cantonal Veterinary Department, Zurich, Switzerland, under license no. ZH 120/2008, and were in accordance with Swiss Animal Protection Law. Housing and experimental procedures also conform to European Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals used for Scientific Purposes and to the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, 2011).

2.2. Animals

A total of 64 C57BL/6J and DBA/2J mice of both sexes were obtained from our in-house breeding facility at the age of six to eight weeks. The health status of the animals was monitored by a health surveillance program according to FELASA guidelines throughout the experiments. The mice were free of all viral, bacterial, and parasitic pathogens listed in FELASA recommendations (Nicklas et al., 2002), except for Helicobacter species.

All animals were housed in groups of three to eight animals of the same sex for at least three weeks prior to testing in our animal room. Animals were kept in type 3 clear-transparent plastic cages (425 mm × 266 mm × 155 mm) with autoclaved dust-free sawdust bedding and two nestlets™ (each 5 cm × 5 cm) consisting of cotton fibres (Indulab AG, Gams, Switzerland) as nesting material. Additionally, animals were provided with a transparent plastic shelter (Mouse house™, Indulab, Gams, Switzerland). They were fed a pelleted and extruded mouse diet (Kliba No. 3436, Provimi Kliba, Kaiseraugst, Switzerland) ad libitum (provided in the food hopper continuously throughout the entire duration of the experiment) and had unrestricted access to sterilised drinking water. The light/dark cycle in the room consisted of 12/12 h with artificial light