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Using the mouse grimace scale and behaviour to assess pain in CBA mice following vasectomy

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ABSTRACT

Mice used in biomedical research should have pain reduced to an absolute minimum through refinement of procedures or by the provision of appropriate analgesia. Vasectomy is a common and potentially painful surgical procedure carried out on male mice to facilitate the production of genetically modified mice. The aim of our study was to determine if 0.05 mg/kg buprenorphine would ameliorate pain associated changes following abdominal vasectomy and to determine if the mouse grimace scale is an appropriate tool for the assessment of pain in this model. Eight male CBA mice underwent abdominal vasectomy as part of a genetically modified mouse-breeding programme. Here we assessed pain using a previously validated behaviour-based method and the mouse grimace scale. All mice received buprenorphine (0.05 mg/kg s.c.) pre-surgery. Behaviour and grimace scores were compared between baseline (pre-surgery), 30 min, 5 h, 24 h and 25 h post surgery. Following 24 h post-op, all mice were administered 5 mg/kg meloxicam (s.c.) as additional analgesia. Significant increases in specific pain behaviours and mouse grimace scale score were found 30 min post surgery. At 5 h post surgery, scores were returning to baseline levels. Frequency of rearing was significantly decreased at both 30 min and 5 h post surgery compared to baseline, demonstrating a longer lasting change in normal exploratory behaviour. Buprenorphine (0.05 mg/kg) was ineffective at ameliorating these pain-associated changes in CBA mice and should be considered inadequate at this dose. By 24 h post surgery, pain associated behaviours, grimace scale and rearing had all returned to baseline levels. There was no change in pain behaviours or MGS following administration of meloxicam indicating that an additional dose of meloxicam does not appear to offer benefit at this point. Using the mouse grimace scale to assess pain in mice, appeared to be effective in the immediate post vasectomy period in CBA mice demonstrating the same duration of increased score as the pain associated behaviours.

1. Introduction

Prevention or alleviation of pain in laboratory animals is a fundamental requirement of in vivo research. In 2013, 3.08 million mice were used in regulated procedures in the UK, with over 449,000 undergoing general anaesthesia with recovery (Home Office, 2014), often for the purposes of surgery. The production of GM mice requires the use of vasectomised males to induce pseudo pregnancy (Ittner and Götz, 2007) and this provides a useful model for assessing the pain associated with surgery. A number of previous studies have assessed pain following both scrotal and abdominal approach vasectomy and have identified key changes in behaviour considered to be pain related (Leach et al., 2012; Miller et al., 2012; Wright-Williams et al., 2007). Although vasectomy via the scrotal approach was predicted to be less painful (Robinson et al., 2003), data has shown that there is likely no significant advantage to one approach over the other (Miller et al., 2012). A number of other studies have used this model, with the behaviour-based scoring system, to evaluate analgesic efficacy (e.g. Wright-Williams et al., 2013).

Behaviour-based scoring is very time consuming to carry out, so novel methods of assessing pain and analgesia efficacy that take less time to implement would offer a distinct advantage. The mouse grimace scale (MGS), devised by Langford et al. (2010) shows promise in this area as it is described as accurate and reliable with scoring requiring significantly less time than full behavioural analysis. The

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MGS comprises five facial action units (FAUs), orbital tightening, cheek bulge, nose bulge, ear position and whisker position. These FAUs are scored separately on a 3 point scale and then combined to produce an overall 'grimace score'.

To date this method has undergone initial validation in the assessment of scrotal approach vasectomy in CD1 mice (Leach et al., 2012), demonstrating a significant increase in MGS score following surgery that could be reduced by the administration of either 20 mg/kg (sc) meloxicam or 5 mg/kg local infusion of the scrotum of bupivacaine. This pattern was also demonstrated when manually scoring key validated pain associated behaviours, with a high positive correlation between the two methods.

Here we aimed to study a different yet common strain of laboratory mouse, CBA, to determine if the MGS may also be an effective method of pain assessment in this strain. Previous work has demonstrated that neither isoflurane anaesthesia nor subcutaneous administration of buprenorphine results in any changes in MGS score or presence of 'pain behaviours' in control CBA mice that have not undergone a painful procedure (Miller et al., 2015). Consequently, any changes that are demonstrated here are likely due to the presence of pain. The dose of buprenorphine selected was 0.05 mg/kg. This dose has been previously shown to be the lowest dose required to significantly reduce MGS score in CD1 mice following laparotomy (Matsumiya et al., 2012) and significantly reduce pain specific behaviours in C57Bl/6 and C3H mice (Wright-Williams et al., 2013).

2. Materials and methods

2.1. Ethical statement

All procedures were conducted in accordance with the Animals (Scientific Procedures) Act 1986, European Directive EU 2010/63 and with the approval of the Animal Welfare and Ethics Review Board at Newcastle University. All mice that were vasectomised in this study were required for use in the university's genetically modified mouse production programme; consequently, no animals underwent surgery solely for the purpose of this study. Previous studies have utilised similar numbers of mice following appropriate power analysis (Leach et al., 2012). This study employed a strict 'rescue' analgesia policy. If any animal was deemed to be in greater then mild pain (assessed by an independent veterinarian), then buprenorphine (0.1 mg/kg sc) was to be immediately administered and the animal was removed from the study. No animals were deemed to require any additional analgesia. Animals acted as their own controls to remove the effect of within group variation and reduce the total number of animals used. Previous study (Wright-Williams, 2007) has indicated that vasectomy results in post-operative pain and therefore it was decided a control group with no analgesia was not appropriate in this case. A sham group was also not included as this has been carried out previously and no change in pain associated behaviours were found (Wright-Williams, 2007).

2.2. Animals

Eight CBA male mice (Charles River Laboratories Inc, Kent) weighing 25.6–28.7 g at the start of the study were used. Mice were housed in groups of 4 in individually ventilated cages (IVCs) (Type 2–Arrowmight, Hereford, UK) with autoclaved Aspen bedding (Datesand Ltd, UK) and nesting material ('Sizzle Nest', Datesand Ltd, UK). Environmental enrichment was provided in the form of chew blocks and cardboard tubes (Datesand Ltd, UK). A seven-day acclimation period was given prior to the start of the study. The animal room was maintained at 23 °C ± 1 °C, 50% ± 10% humidity and on a 12/12 h light dark cycle (lights on at 07:00). Food (CRM(P), SDS Ltd., Essex UK) and tap water were provided ad libitum. Cages were cleaned weekly, ensuring cleaning was not carried out on the day prior to surgery or the day of surgery. Some bedding from the dirty cage was always transferred to the new clean cage. The animals were free from any common pathogens in accordance with the FELASA health monitoring recommendations. Animals were maintained as specific pathogen free according to the FELASA Guidelines (Mähler et al., 2014) and sentinel mice were screened at least quarterly by diagnostic specialists using FELASA approved heath monitoring reports.

2.3. Baseline recordings

One week prior to surgery, mice were filmed twice, consecutively, each time in a slightly different set up to allow A) collection of close up HD images of their faces and B) HD footage of the behaviour of the individual mouse within a standard size home cage. A) Mice were placed individually into small custom made chambers (80 × 80 × 80 mm) and close up, high definition (HD) images of their faces recorded during a 3-min session a high definition camera (Casio EX-ZR100, Casio Computer Co., Ltd., Japan). B) Mice were then immediately placed individually in clear plastic cages (350 × 200 × 140 mm) (Techniplast UK Ltd,UK) that contained only sawdust bedding (DBM Ltd, UK). The behaviour of each individual was recorded, in high definition (HD), for 10 min using a video camera (Sony High Definition HandyCam model HDR-XR155, Sony, Japan) positioned at a fixed distance from the cage. The fixed distance was to ensure the whole cage remained in screen shot at the maximum possible resolution throughout. Following filming the mice were returned to their home cages.

2.4. Surgery

Thirty minutes prior to the individual's surgery start time, mice were weighed and administered 0.05 mg/kg buprenorphine subcutaneously (Vetergesic', Reckitt-Coleman, Hull, UK). This dose and route were chosen based upon recommendations of Dobromylskyj et al. (2000), Robinson et al. (2003), Flecknell (2009). Surgery began at 09:00 h, with the same surgeon operating on all mice. Anaesthesia was induced in a perspex anaesthetic induction chamber (VetTech Solutions Ltd, Cheshire, UK) with isoflurane in oxygen (induction 5%, 2 L/min) for approximately 2 min. The mice were then placed on bedding (VetBed, Kennel Needs and Feeds, Morpeth, UK) on a heating blanket (Harvard Apparatus, Edenbridge, Kent, UK) to maintain body temperature and anaesthesia was maintained using a facemask at 2.5%, 0.5 L/min. The lower abdomen was shaved and the skin sprayed with chlorhexidine (Hydrex Derma spray, Adams Healthcare, Leeds, UK) when loss of the pedal reflex was confirmed. Surgery involved a 1 cm transverse incision made through the skin and abdominal wall. The testes were exteriorized, vas deferentia were located and a small piece removed using cautery, the testes were then returned to the abdomen. The incision in the abdominal wall was closed with Vicryl 5.0 (Johnson & Johnson, Belgium). Tissue glue (Nexaband, Abbott Laboratories, Chicago, IL, USA) and sutures (Vicryl 5.0) were used to close the skin. Anaesthesia lasted 10 ± 2 min, following which the mice recovered in an incubator maintained at 30 °C for 30 min. They were then transferred to a quiet room for filming. No intraoperative complications were reported and all mice recovered from anaesthesia uneventfully.

2.5. Post surgery filming

Thirty minutes, five hours and twenty-four hours following the individual's surgery time, the process of recording footage for facial