Report on the Use of Anaesthesia for Tail Tattooing in Mice

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Introduction

Permanent mouse identification is essential in avoiding errors in the selection animals for breeding and to ensure accuracy in monitoring and sample collection during research. Many permanent identification methods are invasive, inconsistent in application, or difficult to read (Dahlborn K et al.[1]). While the Labstamp® automated tail tattooing system provides definite advantages in terms of consistency and readability, no studies have addressed the issue of whether the tattooing causes pain and stress when performed in conscious animals. This study aims to assess the pain and stress caused by tail tattooing with the Labstamp® automated tail tattooing system in order to determine if anaesthesia is required to optimize animal welfare. The study uses a combination of behavioural observations during and after the application of the tattoo, as well as glucocorticoid metabolite levels in faeces collected before and after the application of the tattoo (Touma C et al.[2]).

Methodology

Animals

A total of 85 BALB/cAusb and 85 C57BL/6JAusb female mice 3 weeks of age were used in the study. All mice were obtained from an SPF breeding facility. All mice were housed in passive exhaust IVC caging, at 20-22° C under 12 hour light: 12 hour dark cycle. All mice were placed in clean cages at least 3 days prior to the study to minimize the risk that stress associated with cage change would impact on results.

Materials

Isoflurane was used for anaesthetic induction and maintenance in anaesthetized groups. The isoflurance only group (1) was kept anaethetised for approximately the same time as the anaesthetic and tattoo group (2). The tail tattoo was performed using Labstamp® automated tail tattooing system. Two numbers were tattooed on the tail of each mouse in the tattoo groups using black ink for the BALB/c and green ink for the C57BL/6. The tails of mice in all groups were wiped with 70% ethanol followed by the application of lubricant tissue oil prior to tattooing.

Group	No. & Strain	Age	Treatment
1	17 x C57BL/6 17 x BALB/c	3-4 wk	Isoflurane only
2	17 x C57BL/6 17 x BALB/c	3-4 wk	Isoflurane and tattoo
3	17 x C57BL/6 17 x BALB/c	3-4 wk	Isoflurane, ear clip and tattoo. The ear clip is a small triangular piece of tissue cut from the lower ear pinnae for genotyping (approx. 2 x 2x 2mm)
4	18 x C57BL/6 17 x BALB/c	3-4 wk	Tattoo only (without anaesthesia)
5	17 x C57BL/6 17 x BALB/c	3-4 wk	Control for tattoo - Mice are placed in the tattooing restraint and the restraint is placed in the tattooing chamber. Mice are exposed to the noise of the tattooing machine for 30-40 sec but they are not tattooed

Table 1. Procedure and group assignments.

Behavioural observations & monitoring wellbeing

Pain was assessed by scoring behavior during the procedure (conscious groups 4 & 5), and during the first 2 minutes following the procedure. Mice were placed in the observation cage immediately following the procedure in the conscious groups (4 &5) and once the mice had regained consciousness and were freely moving about the cage, in the anaesthetized groups (1& 3). During the procedure the mice were recorded as active if they displayed any overt movement. In addition the number of tail looks, the number of times the mice attempted to turn and look at their tail was counted. During the two minutes post procedure observation period, the mice were observed for any signs of facial grimace (Matsumiya LC et al.[3]), the number of tail flicks and the number of tail licking and facial grooming sessions. Wellbeing was assessed by visual observation and weighing of the mice prior to and 24 hours after the procedure. The mice were then monitored daily for one week for general signs of ill health or inflammation of the tail.

Glucocorticosteriod metabolites in faeces

Faecal samples were collected from mice at 3-4pm the day prior to the procedure and at 3-4 pm the afternoon following the procedure. The procedure was carried out between 7-8am in the morning. This allowed post procedure faecal collection to occur 8 hours after the stressful incident (Touma C et al.[2]). Faecal collection was done by placing mice in a novel cage with Protowels® (Thermofisher) to absorb urine and facilitate faecal collection. The mice were left for 15-20 minutes to allow for defaecation in the novel environment. As 2-3 g of faeces was required to run the assay, the faeces from 3-5 mice/group was pooled. The samples were tested for the corticosterone metabolite (5α- pregnane-3β,11β,21-triol-20-one) using enzyme immunoassay (EIA) (Touma C et al.5). Samples were tested at the Wildlife Reproductive Centre, Taronga Conservation Society of Australia

Results

Behaviour during conscious tattoo (Group 4) vs conscious restraint (Group 5)

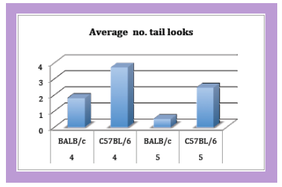

Figure 1.
The number of tail looks per mouse was also significantly higher (P= 0.004) in the conscious tattoo (4) than in the conscious restraint group (5). The average number of tail looks was significantly higher (P<0.005) in C57BL/6 (groups 4 & 5) compared to BALB/c (groups 4 & 5). The average number of tail looks was significantly higher (P=0.02) in BALB/c group 4 than BALB/c group 5. The average number of tail looks was not significantly higher (P=0.08) in C57BL/6 group 4 compared with C57BL/6 Group 5.

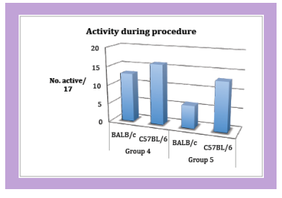

Figure 3. The percentage of mice showing *activity was higher in the conscious tattoo group (group 4) 85.3%* than in the conscious restraint group (group 5) 52.9%. Activity was higher in the C57BL/6 85.2% than in the BALB/c 55.8%.

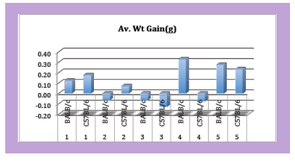

Figure 2.
Weights
Mice were weighed before the procedure and 24 hours following the procedure.
The combined tattoo groups (2 & 4) had significantly lower (P=0.017) average weight gain than the non- tattoo groups (1 & 5). However strain differences were noted. The BALB/c anaesthetic and tattoo group (2) had a negative average weight gain of -0.06g significantly less (P= 0.03) than the positive weight gain of 0.33 for the BALB/c conscious tattoo group (4). The C57BL/6 anaesthetic and tattoo group (2) had a positive average weight gain of 0.07g significant greater (P=0.01) than the negative weight gain of -0.25g for the conscious tattoo group (4). There was no significant difference (P>0.05) between the average weight gain of the non- anaesthetised groups 4 & 5 (0.18g) and the anaesthetized groups 1 & 2 (0.08g) when information from both strains was pooled. However the BALB/c anaesthetized groups 1 & 2 had significantly (P=0.01) lower weight gain than the BALB/c nonanaesthetized groups 4 & 5. While the anaesthetized & tattoo & ear clip group (3) was the only group with a negative average weight gain for both the BALB/c and C57BL/6 of -0.06 & -0.13g respectively this was not significantly different to the average weight gains of the other anaesthetized groups 1 or 2 (P=0.07, P=0.63).

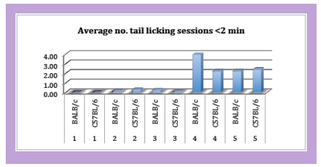

Figure 4.
Behaviour immediately after tattooing

Tail licking
There was no significant difference between the average number of *tail licking sessions in the tattoo* groups 2 & 4 and the non-tattoo groups 1 & 5 (P=0.09). The average number of *tail licking sessions* was however significantly higher (P<0.005) in the conscious groups 4 & 5 compared with the anaesthetized groups 1 & 2.

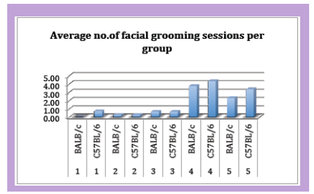

Figure 5.
Facial grooming
The number of facial grooming session in non- anaesthetized groups (4 & 5) was significantly (P<0.005) greater than in anaesthetized groups (1 & 2).The number of facial grooming sessions in the conscious tattoo group (4) was significantly greater (P=0.017) than in the conscious restraint group.

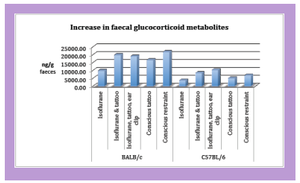

Figure 6.
During this study the BALB/c faecal glucocorticosteriod metabolite levels were significantly higher (P<0.005) than the C57BL/6. Because of this significant strain difference the between group results have been analysed within strain.

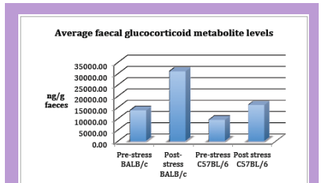

Figure 7.
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Additional Observations

- *Tail flicking:* None of the groups displayed any tail flicking.
- *Facial grimace:* Only two mice in group 4 displayed squinting eyes in the 2 minutes post tattoo. Squinting eyes is one of the behaviours on the facial grimace scale indicating pain. In one of these mice the tattoo was slightly blurred indicating the needle was blunt, and in the second mouse one toe was injured during restraint. No other mice in any groups display any signs of facial grimace.
- *Observation of the tail for 1 week post tattooing:* All mice were observed daily for one week post tattooing. One mouse had mild erythema near the tattoo 24 hours after the procedure but this disappeared by 48 hours. No other mice displayed any irritation or health problems during the seven days post procedures. All tattoos were clearly visible at seven days.

Conclusion

The behaviour of the mice during conscious tattooing indicated sensory perception, but it is not clear whether the tattooing was perceived as painful. As there was no evidence of pain in the two minutes after conscious tattooing when equipment use was optimal, any pain felt during conscious tattooing was likely to be either very mild or absent. There is evidence however that tattooing was stressful, both from the grooming behaviour of the mice and the faecal glucocorticosteroid metabolite levels. This stress does not seem to be effectively alleviated by isoflurane anaesthesia, as both anaesthetised and conscious mice had significantly elevated faecal glucocorticosteroid metabolite levels. An additional consideration is that the anaesthetic appeared to have a significant negative impact on the growth rate of BALB/c mice but not C57BL/6 mice. This study does not suggest that the use of isoflurane anaesthesia provided any significant animal welfare benefits during the tail tattooing of mice 3-4 weeks of age.

References

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