Evaluation of the tail biopsy procedure on the behaviour and wellbeing of mice - a pilot study.

by

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Abstract

Mice constitute more than half of all the laboratory animals used in the world. The use of transgenic mice has greatly increased during the last years. To determine their genetic setup a tailbiopsy is often taken and the question arises whether this is a painful procedure that requires anaesthesia and/or analgesia. The tailtip of the mouse contains bone and skin and the periosteum is well supplied with nervous tissue. Today there are no recommendations from the Swedish National Board for Laboratory Animals about anaesthesia or analgesia in conjunction with the tailbiopsy. To evaluate the effects of tail biopsy on behaviour of mice, an automatic system for behaviour recognition was used. Differences were found between these mice and control mice and these differences might be caused by pain or discomfort.

Contents

Introduction

Transgenic or genetically modified mice are created by introducing foreign DNA into the genome. Production of transgenic animals is expensive and time consuming, therefore healthy animals with a short lifespan are preferred (Öbrink & Waller, 1996). For transgenic experiments the mouse is the primary choice since it is relatively easy to manipulate the adults and their embryos because of the extended knowledge of the murine genetics (Fox et al., 2002). The mouse is considered a good breeder and the embryonic stemcells needed for one of the methods (targeted gene transfer) for inducing transgenesis, is only available for the mouse (Van Zutphen et al., 2003). Transgenic mice are produced to serve as models of human disease, toxicology, models for transgenic livestock protocols and as *in vivo* systems for investigation of genetic expression of other species (Fox et al., 2002). They also have an important role in increasing knowledge of biological processes and physiology (Van Zutphen et al., 2003).

In Sweden alone the approximate number of mice used for scientific purposes in 2002 was 163,000 (CFN, 2003). In recent years the number of genetically modified animals produced has increased dramatically (Van Zutphen et al., 2003).

When producing genetically modified mice a biopsy is often taken from the offspring to determine their genetic composition. According to recommendations from the Swedish National Board for Laboratory Animals (CFN, 2000) the biopsy, with a maximum length of 5 mm, should be taken from the tip of the tail at the age of 9-16 days. However in practice tail biopsies are commonly taken at three weeks of age either shortly before or after weaning (Robinson, 2003). The age recommended by the Swedish National Board for Laboratory Animals is based on the fact that young animals usually bleed less and seem to be less affected by the procedure. There is no recommendation from CFN about the use of anaesthesia or analgesia in conjunction with the tail biopsy and the question arises whether this is a painful procedure that requires anaesthetic and/or analgetic treatment.

The ossification of the caudal vertebrae starts between the age of two to three weeks and there is clear evidence of vertebrae and bone mineralization in the last millimeter of the tail (Robinson, 2003). The removal of even a small piece of the tailtip is likely to be painful since the skin and the periosteum is well supplied with nervous tissue (Robinson, 2003).

The sixth report of the BVAAWF/FRAME/RSPCA/UFAW (British Veterinary Association Animal Welfare Foundation/Fund for the Replacement of Animals in Medical Experiments/Royal Society for the Prevention of Cuelty to Animals/Universities Federation for Animal Welfare) Joint Working Group on Refinement recommends that tail biopsies are taken from mice between three and four weeks of age. They also recommend some kind of analgesia to be used. When the mouse is approximately two weeks old, five millimetres of the tail is a great portion of the total length of the tail and there is a difficulty to administer analgesia to such young mice. Mice over four weeks of age have fully ossified tails and there is a risk of greater trauma and more pain (Robinson, 2003).

Up till today there has been no research published on the impact of tail biopsy on the behaviour of mice. Thus, the purpose of this study was to evaluate whether

there was a difference in behaviour between mice that had undergone tailbiopsy and mice that had not.

Assessment of pain

Depending on innervation, type- and the amount of nociceptors, different organs have different pain sensitivity. Pain from skin is usually strong and well localised. Bone and joints are relatively insensible except when in pathological state, the periost on the other hand has many nociceptors. Stimulation of nerves and periferal nociceptors gives rise to strong pain sensation.

When working with laboratory animals it is very important to avoid stress and pain since this can jeopardize the results.

Depending on the amount of pain that the animal experiences, there might be a difference in behaviour. (Öbrink & Waller, 1996)

According to Flecknell (2000) it might be difficult to assess pain in small mammals because their pain associated behaviours are more subtle and their normal behaviour is not familiar to most people. Acute pain causes the animal to try to get away from the painful stimuli, the animal may vocalise or become aggressive. Mice and other rodents are able to vocalise at frequencies that humans can not hear. Small mammals may reduce their activity level after injury or surgical trauma. They may hide in a corner, under the bedding or under the food hopper. They may also show a reduced frequency in certain behaviours, for instance grooming and rearing. Their gait can be altered and during resting they may show muscle twitching. When in pain rodents might get a dirty coat or show chromodacryorrhea (red staining around the eyes and nose) due to lack of grooming. The appetite may be reduced and less urine and faeces may be produced. (Baumans et al., 1994) It is important to remember that these animals might mask or change their behaviour when an observer is present. (Flecknell, 2000). When in pain mice may become immobile and increase their sleeping time. They can show piloerection and a hunched up appearance. Sick mice often isolate themselves from the rest of the group and they might show a decrease in body weight (Flecknell, 2000, Öbrink & Waller, 1996, Baumans et al., 1994).

Materials and Methods

Animals

To avoid using animals only for this study we used mice that underwent tailbiopsy in another research project.

A total of fifteen mice were studied. The animals were between 27 and 34 days old. The weight of the female mice was between 13.9 and 17.3 grams and the weight of the male mice was between 14.1 to 23.4 grams. The mice were transgenic galanin over-expressing (GOE) (Hygge Blakeman et al., 2001) and wild type (WT) littermates of C57BL/6 (table 1).

	Transgenic (n=8)	**Wild Type (n=7)**
Female (n=7)	3 biopsy 1 control	1 biopsy 2 control
Male (n=8)	2 biopsy 2 control	1 biopsy 3 control
Total	5 biopsy 3 control	2 biopsy 5 control

Table 1: Animals used for the study (n=15).

Galanin is a neuropeptide built up by aminoacids. It seems to be predominantly inhibitory and is distributed throughout the nervous system. Studies of galanin deficient and over-expressing mice have shown that galanin has a role in important body functions such as feeding, cognition, endocrine modulation and nociception. (Hygge Blakeman et al. 2001)

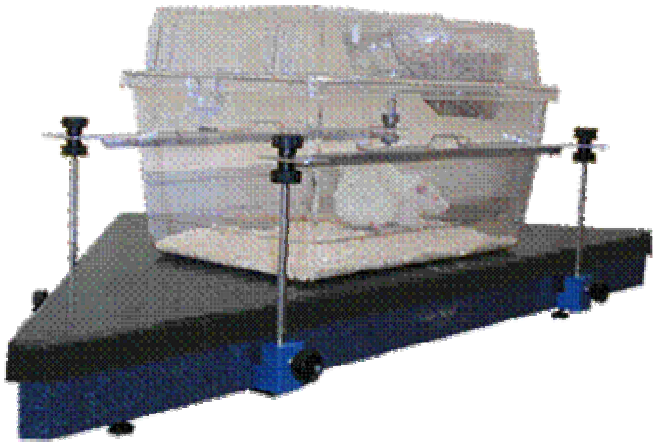
The mice were created and bred at Karolinska Institutet for a separate project that included tail biopsy for the identification of the genetic set-up. The age at tail biopsy was chosen to fit the behaviour analysis and registration system (LABORAS) that requires animals with a certain minimal bodyweight. The mice were kept together with their mother in open Macrolon type II cages (24 x 18 x 13 cm) with aspen woodchip bedding (Finn Tapvei, Finland) and additional Kleenex tissues as nesting material. The cages were cleaned once a week (SOP). Animals were barrier housed in rooms with racks behind sliding curtains. The day/night light cycle was 12/12 hours, the temperature was 20 ± 1 ° C and the relative humidity 50 ± 20 %. Food pellets (R34, Lactamin, Sweden) and drinking water were available *ad libitum*. The barrier-protected animal colony was free from all microbiological agents included in the FELASA (Federation of European Laboratory Animal Science Association) list of agents to be monitored.

FELASA is a European organisation established in 1978. It is composed of independent European laboratory animal science associations. FELASA has published recommendations on education of people working with laboratory animals, on the health monitoring of these animals, on the accreditation of laboratory animal diagnostic laboratories and on the detection, relief and control of pain and suffering. (FELASA, 2003)

Experimental design

The mice were randomly distributed to one of two groups (B=Biopsy, C=Control). Each mouse was weighed and placed in an anaesthesia induction chamber filled with 4.6 % isoflurane (Forene, Abbott, Sweden) for approximately one minute

(Univentor 400, AgnTho´s, Stockholm, Sweden).When the mouse did not respond when lifted, it was removed from the chamber. From each mouse in group B, approximately five millimetres of the tail tip was removed with a scissor and an ear tag (AgnTho´s, size 1, Monel, Stockholm, Sweden) was placed in the left ear. Thereafter the mice were returned to the cage. Mice in group C were anaesthetised in the same way and allowed to recover in the cage without biopsy or ear tag. The mice were then brought to a separate room in the animal departement for the five hour behavioural study. Each mouse was placed singly in a modified Macrolone type II cage (equipped with aspen wood chips, food pellets and tap water ad lib.) for automatic registration of behavioural parameters in the LABORAS system (picture 1).



Picture 1: LABORAS, for automatic registration of behavioural parameters.

Three to four animals were monitored simultaneously. The mice were visually observed for 5 minutes before the automatic monitoring was started and again one hour later to notice any signs of great discomfort (e.g. bleeding, violent movements, vocalizing). No observers were in the room during the automatic monitoring and any visual observation was performed through a small window in the door to the study room. After completion of monitoring the animals were weighed and thereafter all the animals were returned to their home cage in the animal room.

Behavioural testing

The behavioural monitoring was done in an automatic system for behaviour recognition and tracking of small rodents called LABORAS (Laboratory Animal Behaviour Observation Registration and Analysis System). Based on animal weight displacement LABORAS can detect locomotion, grooming, immobility, rearing, drinking, climbing and eating in an automated way. It also tracks the position of the

animal, speed, maximum speed, travelled distance and position distribution (Metris, 2003).

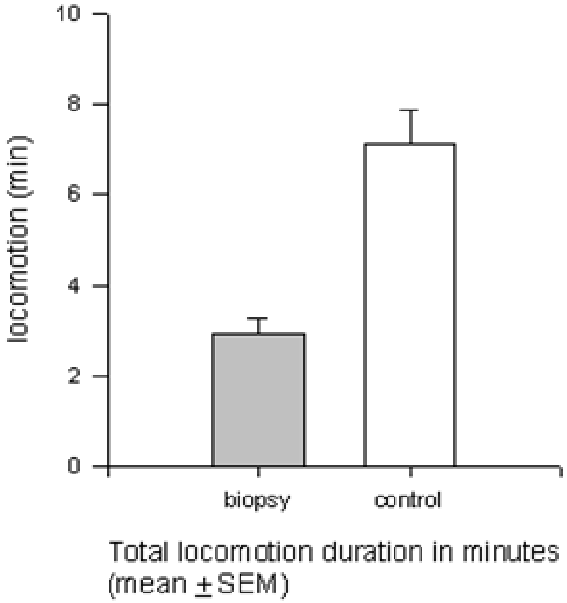
Statistics

The data was analysed with Sigma Stat version 3.0. The Students T-test was used and the level of statistical significance was set at $P<0.01$. The level of significance was chosen because several behaviours were compared between the groups. Closer analysis with repeated measures ANOVA is to be completed.

Results

Of all the behaviours measured, all mice spent most of their time resting and grooming. Mice in group B spent least time drinking and eating. Mice in group C spent least time drinking and climbing. The greatest differences in behaviour were seen during the first three hours and also during the last hour. Over the five hours mice in group B spent significantly less time on locomotion. Group B also travelled a significantly shorter distance and their average speed was significantly lower. (figure 1a-d)

A trend was seen among the mice in group B. They climbed less ($P=0.025$), were more immobile ($P=0.047$), and travelled at a lower maximum speed ($P=0.016$) than mice in group C. (figure 2a-c)



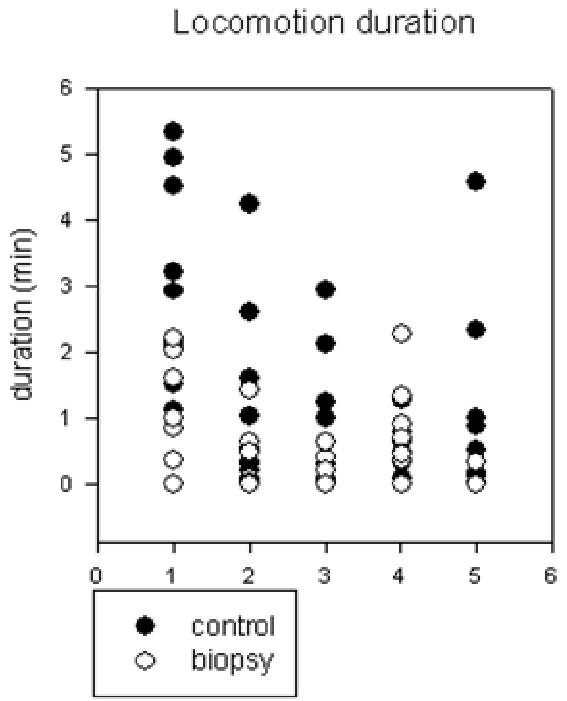
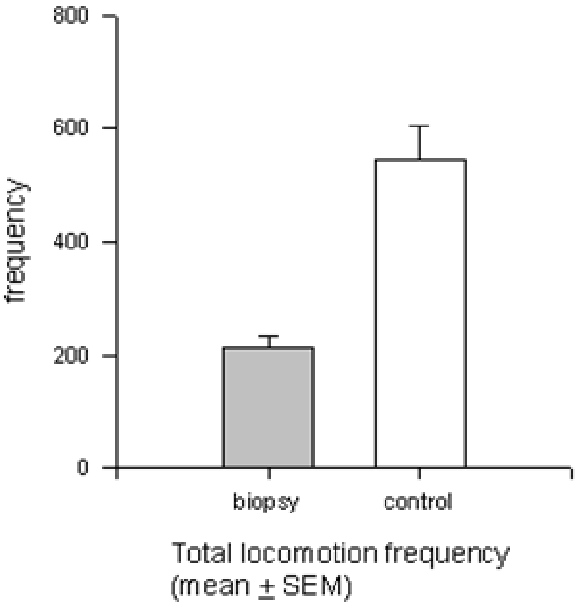


Figure 1a: Differences in behaviour between control group, C, (n=8) and biopsy group, B, (n=7) during the five hour LABORAS study. $P<0.01$.



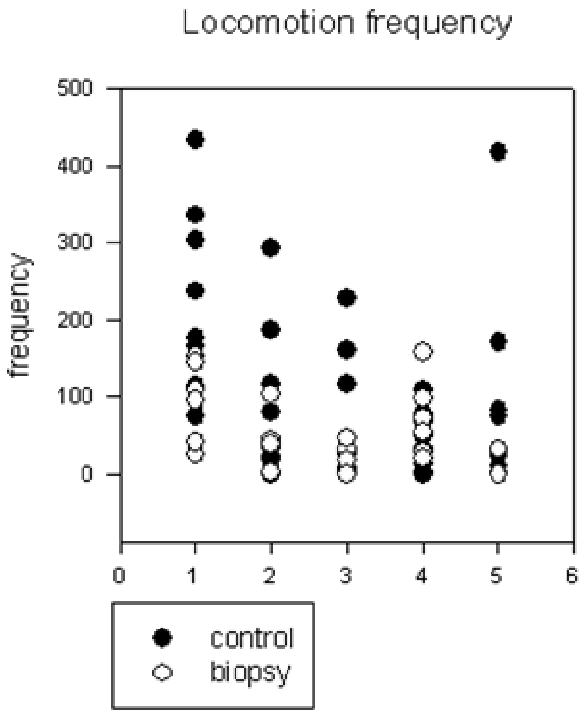
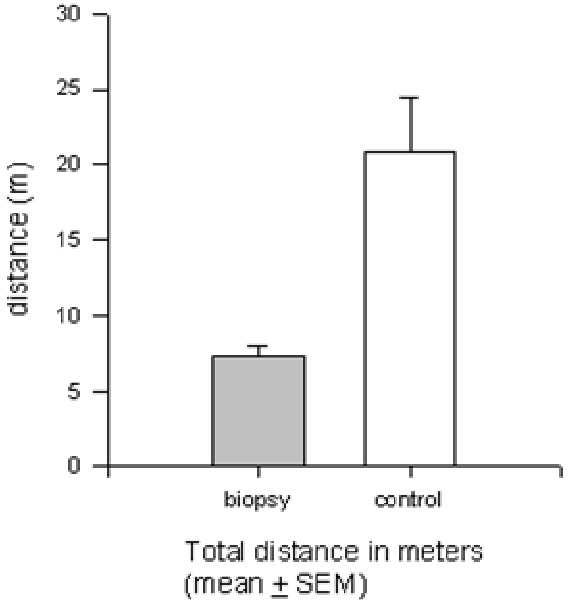


Figure 1b: Differences in behaviour between control group, C, (n=8) and biopsy group, B, (n=7) during the five hour LABORAS study. P<0.01.



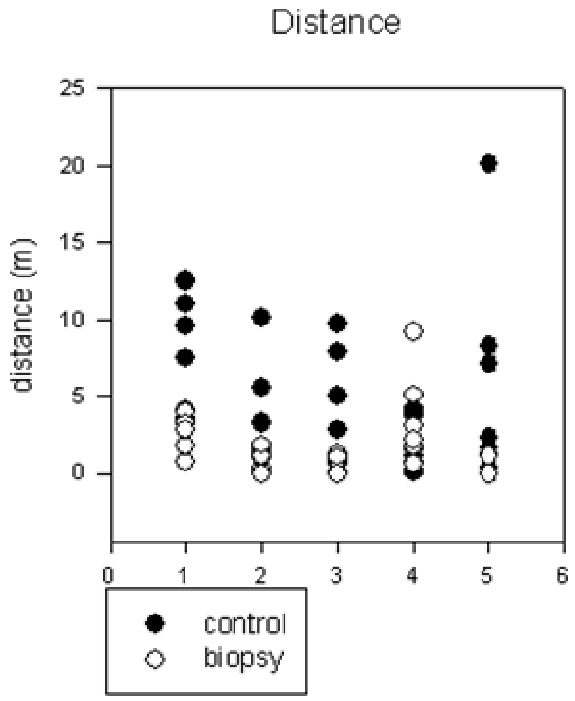


Figure 1c: Differences in behaviour between control group, C, (n=8) and biopsy group, B, (n=7) during the five hour LABORAS study. P<0.01.

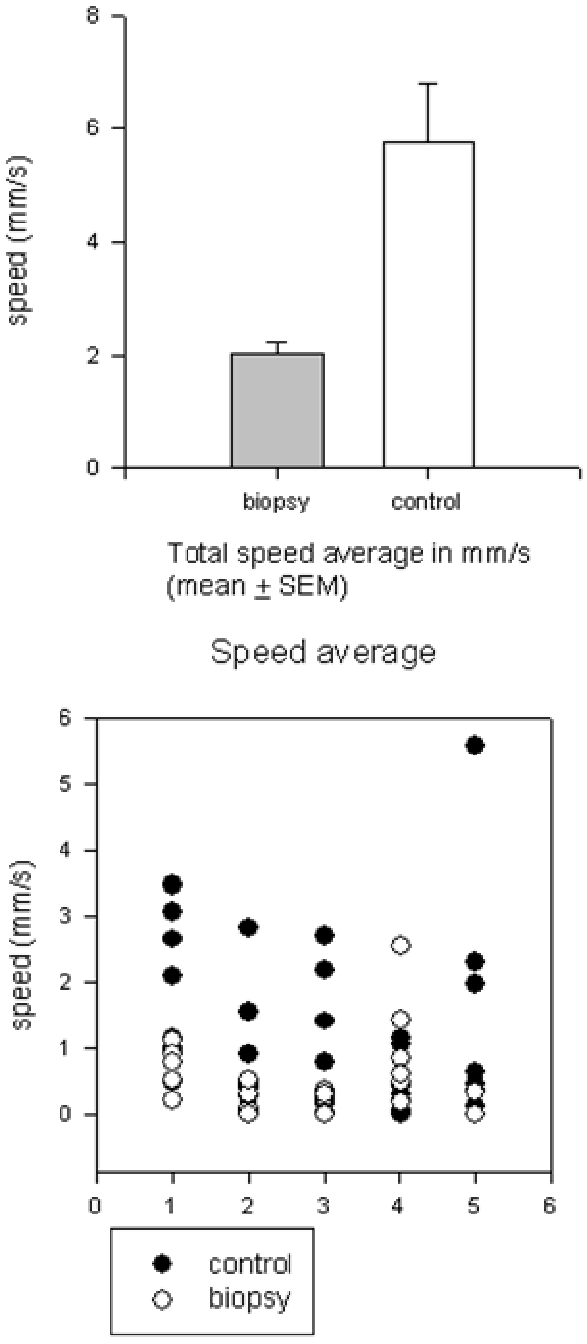


Figure 1d: Differences in behaviour between control group, C, (n=8) and biopsy group, B, (n=7) during the five hour LABORAS study. $P<0.01$.

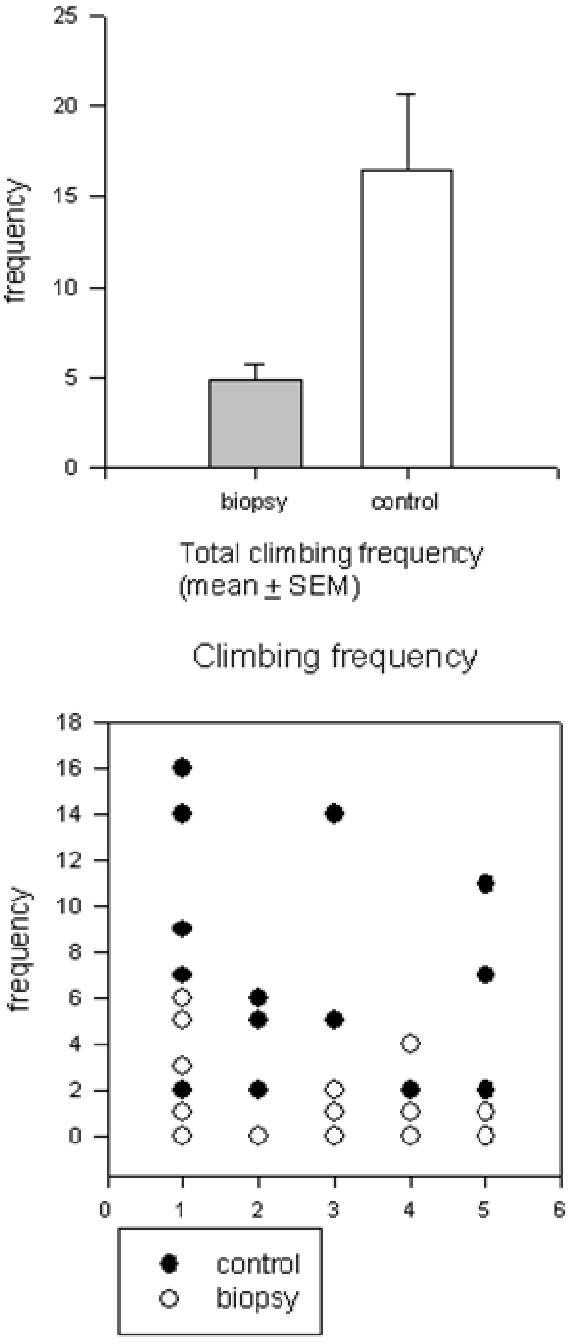


Figure 2a: Differences in behaviour between control group (n=8) and biopsy group (n=7) during the five hour LABORAS study.

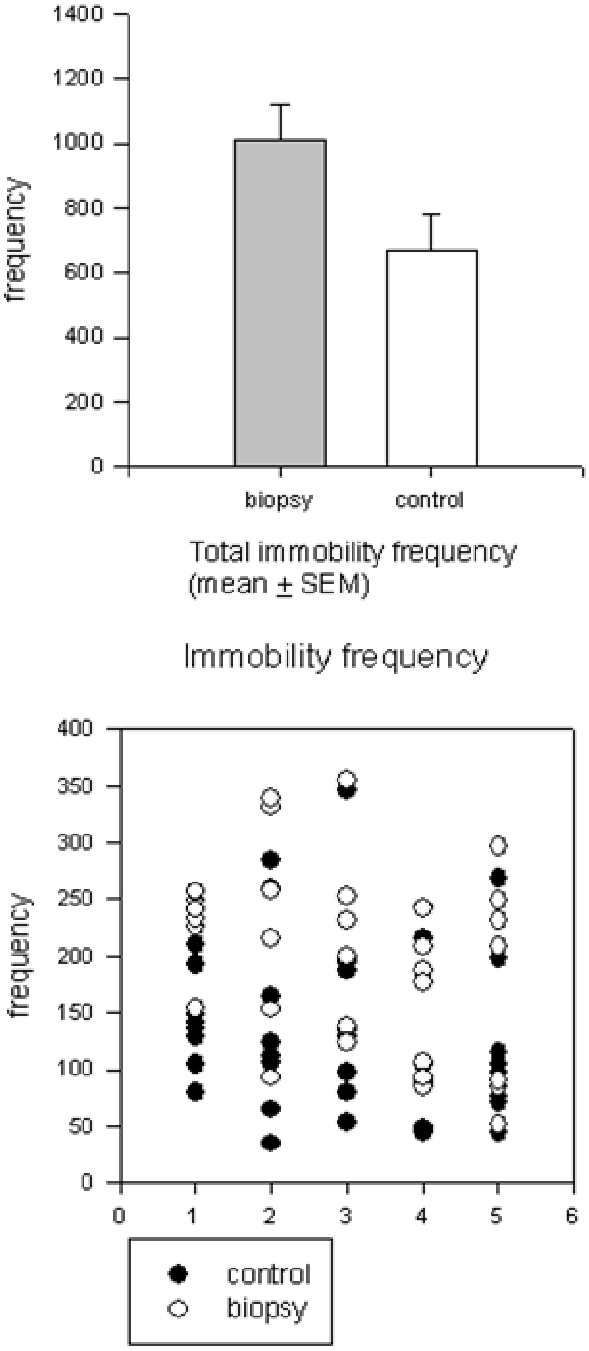
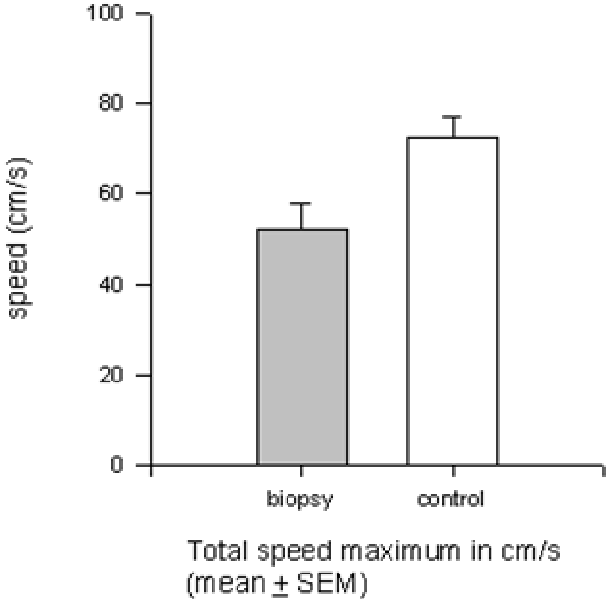


Figure 2b: Differences in behaviour between control group (n=8) and biopsy group (n=7) during the five hour LABORAS study.



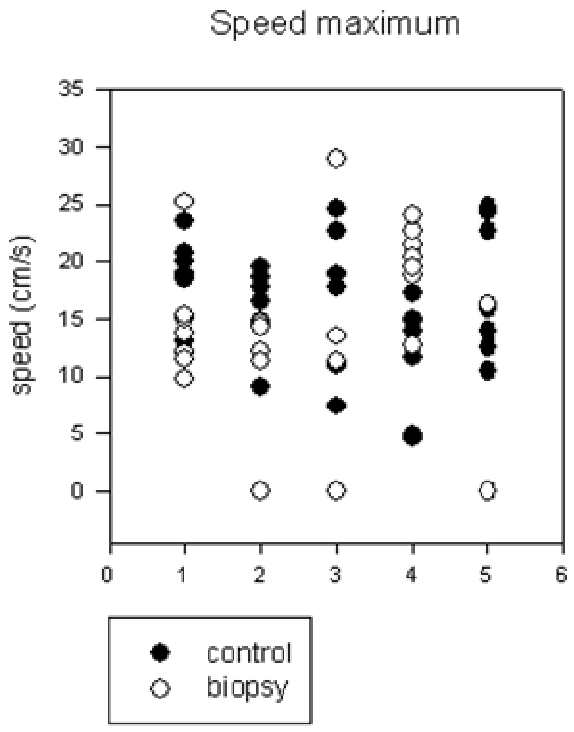


Figure 2c: Differences in behaviour between control group (n=8) and biopsy group (n=7) during the five hour LABORAS study.

Body weight

All mice in group B (n=3) lost weight (2.9%-5.0%) during registration. In group C (n=6) two mice gained weight (2% and 3.4%) and four mice lost weight (3.4% - 4.0%).

Discussion

The results indicate that tail biopsy has an effect on the behaviour of mice, in particular in that it reduces locomotion.

Experiments have shown that GAL-OE mice show an increased nociceptive threshold to heat stimulation compared to WT mice. This suggests that galanin has an inhibitory action in the spinal cord of the mouse (Hygge Blakeman et al. 2001). Based on this it may be assumed that the galanin overexpressing mice are less affected by tail biopsy compared to wild type mice. This pilot study contained too few mice to elucidate how galanin overexpression may have influenced the behaviour after tail biopsy.

The mice seemed to be irritated by the ear tag which they showed by scratching the tagged ear, however, this seemed to be a transient irritation. It cannot be excluded that the ear tagging may have contributed to the changes seen in the behaviour. To conclusively determine the effect of tail biopsy on behaviour, future studies of behaviour in mice that have undergone tail biopsy only, will be necessary.

The pups were weaned at the day of the study and this might have affected the behaviour.

To further elucidate if the differences in behaviours are caused by pain, a group of mice receiving analgesia in conjunction with tail biopsy, should be included. It is important that the administration of analgesics itself, does not lead to more distress or pain than does the tail biopsy procedure.

A long-lasting local analgesic would probably provide good analgesia during and after biopsy. The injection of a local analgesic could be performed after induction of general anaesthesia with a volatile agent. The local analgesic bupivacaine, has a duration of several hours. Topically applied local analgesics may be an alternative, but are not long lasting. The optimal analgesic drug should be given once pre-emptively before biopsy and last until the pain has ceased. The NSAID carprofen might fit this description. It is administered subcutaneously or orally and is believed to have effect for 24 hours (Flecknell, 2000).

Considering the preferred anaesthetic for tail biopsy, the primary goal is to select a safe and reliable technique. Inhalation anaesthesia with isoflurane fits this description and may be used for tail biopsy of young mice.

It would be of interest to videotape animals after tail biopsy in order to observe their posture and study behaviours that cannot be detected by the LABORAS system. In our study the differences in behaviour were seen during the first, second, third and fifth hour of the study, therefore an extended study may be needed in order to estimate how long the differences last. The tailbiopsy should be examined

histologically to evaluate the state of ossification. A clinical examination of the animals might give some additional information.

The results of this study suggest that there is a need for further studies in this area. Further studies are important for the well-being of the mice and thereby the research results.

My opinion is that taking a tail biopsy is painful for the mice. My hope is that further studies will be made and lead to a change in recommendations about anaesthesia and analgesia in conjunction with tail biopsy.

Sammanfattning

Möss utgör mer än hälften av alla djur som används till forskning. Andelen transgena möss som används i djurförsök har ökat under de senaste åren. För att ta reda på deras genuppsättning tas ett vävnadsprov från svansen och frågan uppstår huruvida detta är ett smärtsamt ingrepp som kräver anestesi och/eller analgesi. Musens svansspets innehåller ben, periost och hud. Periostet är rikligt innerverat med nerver. Idag saknas svenska rekommendationer om anestesi eller analgesi i samband med svansbiopsi. För att utvärdera effekter på beteendet hos möss från vilka ett vävnadsprov tagits, användes ett automatiserat system för beteendestudier. Skillnader påvisades mellan dessa möss och kontrollmöss, vilka kan ha orsakats av smärta eller obehag.

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