

URC RESEARCH REPORT

Name of Researcher: Mark Farnworth

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Project Title: “Behavioural and physiological indicators of analgesic efficacy in rabbits” and “Behavioural and physiological indicators of analgesic efficacy in rabbits”

RMOL#: R108217 and R109004

1. What is(are) the research question(s)?

- How effective is analgesia administration for experimental/companion rabbits?
- Are there behavioural and physiological correlations indicative of pain when analgesia is withdrawn or exceeds its half life (effective period)?
- What is the best method of analgesia administration to prevent unnecessary suffering and/or welfare compromise in laboratory/companion rabbits?
- Is there need for additional post operative analgesia, beyond the coverage supplied by peri-operative care?
- Are there differences in the behaviour of rabbits supplied additional pain relief?

2. Rationale

The welfare of laboratory animals is of particular importance to New Zealand and the wider global community. Information leading to the betterment of animal welfare is vital, especially as animal usage is increasing in the domestic market (NAEAC; Annual Report 2006). Currently rabbits within the Auckland University (AU) Department of Physiology are undergoing important and ethically approved surgical procedures. These research projects have demonstrated that pain, in rabbits specifically, can be quantified and identified using behavioural markers. Analgesic practices, post surgery, have been experimentally verified as failing to ameliorate all signs of suffering and pain. To minimise welfare compromise of animals it is was important to look at the application of different regimens of pain relief in rabbits under the same

experimental conditions. Much of the current research concentrates on clinical application of analgesia (Johnston 2005) and, as yet, none has been found to combine behaviour, physiology and analgesia into a welfare framework.

This novel experiment seeks to identify:

- How welfare compromise in rabbits undergoing invasive surgery can be further addressed through clinical procedures
- Best practice for administering post-operative pain relief

Such information is not solely concerned with the welfare of laboratory rabbits but will inform veterinary practice regarding the administration of post-operative analgesia to companion rabbits. This information is novel and will serve to support teaching in both the Bachelor of Applied Science (Welfare Major) and the Diploma of Veterinary Nursing.

The work will also support the School of Natural Sciences in increasing its research output in previously published areas and in line with the goals of the Research and Advanced Practice Plan

3. Methodology

Ultimately 20 rabbits (7 in study 1 and 13 in study 2) were observed within these 2 experiments, which is lower than previously expected. This resulted from changes within Auckland University's programme of study which could not be altered by Unitec research staff. However results remained of significant value.

Surgical Procedure

Rabbits were housed at AU in home cages in a purpose built, temperature controlled laboratory under a light dark cycle of 12/12. After 7 days of housing each rabbit underwent surgery to implant a telemetric device to measure blood pressure and renal nerve activity. Both surgeries are performed by AU collaborators under anaesthesia using a sterile surgical procedure and prophylactic antibiotics (Baytril 2.5%; 0.2ml/Kg) are administered. For experiment 1 all rabbits received peri-operative analgesia. For experiment 2 analgesia was provided in two treatment groups. The first received analgesia via injection 2 hours prior to surgery and the second received analgesia via injection 2 hours prior to surgery and then an immediate post-operative booster of analgesia. In addition all animals had analgesia administered twice post operatively at 24 and 48 hours (Ketofen 1%; 0.2ml/Kg). After surgery the animals were returned to their home cages constantly monitored for heart rate and arterial pressure via the telemetry device until it stabilised (~4 days post surgery). The above procedure is fully approved by the Auckland Ethics Committee (AEC Number: AEC/05/2007/R577) as of 07/05/07 and expires 02/07/10.

Behavioural Data

Behavioural data was collected remotely using digital camera footage. Data was collected for 24 hours post surgery. This time will allowed behavioural change to be established following surgery for the 2 different analgesic regimens.

Footage was analysed in real time using Observer XT (a software package used for logging behavioural data) and transferred to SPSS for statistical analysis. Analysis included identifying changes, if any, in behaviour post surgery, changes, if any, in behaviour during the first post-operative analgesic period. This behavioural protocol will not require additional ethical approval as it is non-contact and will not change any procedures currently in place.

Physiological Data

Physiological data was collected remotely, by AU collaborators, from the telemetric devices every 5 minutes for 72 hours post-surgery. These data were automatically logged into a data storage device. This data is still in storage and may be used in future work.

4. Outcomes / findings

Results of study 1 indicate there are several behaviours of interest as potential indicators of post-surgical pain in rabbits. These findings are contained within the abstract supplied in appendix 1. The second study indicates that current post-operative care of rabbits may not be optimal and makes recommendations for multi-modal pain relief to be considered. The abstract for this is contained in appendix 2.

5. Publications and dissemination

The research group anticipates providing full copies of peer-reviewed publications to the Research Committee in due course

Project 1:

Peer-reviewed abstract and platform presentation:

- Proceedings of the 43rd International Congress of the International Society for Applied Ethology, Cairns (2009). Farnworth, M.J., Walker, J., Chuang, C-L., Barrett, C., Malpas, S., Waran, N.K. Post-operative behavioural change in rabbits undergoing abdominal surgery: potential indicators of pain. p.103.

Submitted (Nov 09) to *Animal Welfare* one of the leading journals in the field (see Appendix 1):

- Farnworth, M.J., Walker, J.K., Schweizer, K.A., Chuang, C-L., Guild, S-J., Barrett, C., Leach M.C., Waran N.K., (2009) Behavioural indicators of post-operative pain in male laboratory rabbits following abdominal surgery. *Animal Welfare* **In Submission**.

Included in Unitec's *Advance* magazine and published in MAF quarterly *Pulse* for those working in Animal Welfare

Presented to Biology teachers at the "Biology in Context Day", Unitec Nov 09.

Project 2

Presented as part completion of the Master's Degree *Animal Behaviour and Animal Welfare (MSc)* for visiting student Katharine Schweizer. Student was hosted by the research group on behalf of Edinburgh University (see appendix 2).

Submitted for consideration by 44th International Congress of the International Society for Applied Ethology, Upsalla, Sweden.

In preparation for submission to peer-reviewed journal, likely to be *Animal Welfare*

Appendix 1: Paper in Submission to *Animal Welfare*

Potential behavioural indicators of post-operative pain in male laboratory rabbits following abdominal surgery

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Abstract

This study aimed to identify behaviours that could be used to assess post-operative pain and analgesic efficacy in male rabbits. In consideration of the “3Rs” behavioural data were collected on seven male New Zealand White rabbits in an ethically approved experiment requiring abdominal implantation of a telemetric device for purposes other than behavioural assessment. Prior to surgery rabbits were anaesthetised using an isoflurane/oxygen mix and given Carprofen (2mg/kg) as a peri-operative analgesic. Rabbits were housed individually in standard laboratory cages throughout. Data were collected at three time periods: 24-21h prior to surgery (T1) and post-surgery 0-3h (T2) and 3-6h (T3). Behavioural changes were identified using ObserverXT, significance of which was assessed using a Friedman’s test for several related samples. The frequency or duration of numerous pre-operative behaviours was significantly reduced in T2 and T3, as compared to T1. Conversely novel or rare behaviours had either first occurrence or significant increase in T2 into T3 as compared to T1, these include “full-body-flexing”, “tight-huddling”, “hind-leg-shuffling”. We conclude that reduced expression of common pre-operative behaviours and the appearance of certain novel post-operative behaviours may be indicative of pain in rabbits. Behaviours identified as increased in T2 as compared to T1 but not consistently elevated into T3 were considered separately due to the potentially confounding effect of anaesthesia recovery. These included lateral lying, “drawing-back”, “staggering” and “closed eyes”. We postulate that for effective application of best-practice post-operative care, informed behavioural observation can provide routes by which carers may identify requirements for additional post-operative analgesia. Additionally, improvement of the peri-operative pain management regimen may be required to ameliorate the immediate effects of abdominal surgery. Comparisons with other studies into post-operative pain expression in rabbits suggest behavioural indicators of pain may differ dependant on housing and surgical procedure.

Key Words: Analgesia, Animal Welfare, Behaviour, Rabbit, Pain, Peri-operative

Appendix 2: MSc thesis successfully completed and now in prep for publication.

Behavioural indicators of analgesic efficacy in rabbits under two different analgesic regimens.

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Abstract

Rabbits are one of the six most used animals in laboratory practice, yet little is known about behaviours exhibited in response to pain. Even less is known about the efficacy of analgesics used routinely in surgical procedures which relies on processes extrapolated from similar species. This study investigates the effects of additional analgesia on the frequency and duration of previously established pain behaviours in 12 New Zealand White rabbits (*Oryctolagus cuniculi*). Rabbits were recorded following abdominal surgery to implant telemetry devices. Recorded behaviour was then analysed to assess efficacy of Carprofen (0.08ml/kg) on individuals that received standard peri-operative analgesia (PO) and those that received an additional post-operative boost prior to recovery from anaesthesia (PO+). Hours of observations were separated into four time periods: T1 immediately post-surgery (0-4hrs); T2 (4-8hrs) T3 (21-24hrs) and T4 (24-27hrs). All individuals were administered a carprofen boost (0.08ml/kg) between T3 and T4. Analysis of video recording was performed using Observer XT software, and statistical analysis was performed using SPSS v.17. A Kruskal- Wallis test was used to determine differences between the treatment groups, and Friedmans was used to assess between time periods.

The results show no significant behavioural differences between PO+ and PO groups in T1-T4, suggesting that an additional carprofen boost does not sufficiently ameliorate the frequency or duration of pain behaviours. The posture 'tight huddle' has been found to be indicative of abdominal pain following surgery, the frequency of which decreased with analgesia in T2 and increased in T3 (no analgesia present) ($p < 0.007$). Several 'abnormal' behaviours (tight huddle, hind-leg shuffle – remained constant throughout observation, partial hop) appeared post-surgery indicating these are linked with post-operative pain, and pre-operative behaviours (eating, grooming, rearing, dog-sitting) increased gradually over the observation period, however did not return to normal levels within the 27 hour period, suggesting that pain was still present despite analgesic intervention.

The additional post-operative analgesic protocol used here appears not to provide additional pain relief beyond that provided by the standard peri-operative protocol. However, due to the half-life of carprofen (estimated at 8hrs), it may be necessary to investigate further with a boost administered 8 hrs post-surgery. Results from T2 to T3 period confirm this as a significant increase in 'tight huddle' was recorded, suggesting that pain has increased in this time. Research into multimodal analgesia to address the different types of pain present (visceral and somatic) may prove beneficial for abdominal surgery in the rabbit.