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**Title:** Feasibility of pair housing of rats after cranial implant surgery

**Short title:** Pair-housing in rats with cranial implants

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**Keywords:** cranial implant, pair-housing, headstage, rat model

**Abstract:**

Rat models employing cranial implants are increasingly employed to facilitate neural stimulation and recording in freely moving animals. Due to possible damage to wound, implant or attached devices, rats with cranial implants are traditionally housed singly, and little information is available on group or pair-housing. Here we describe a protocol for pair-housing rats following cranial implant surgery and describe our experience with pair-housing during post-surgical recovery and up to 16 weeks following surgery.

Thirty-six adult Wistar rats of both sexes were implanted with deep brain stimulation electrodes. Ten rats were equipped with an additional wireless headstage. Rats were housed in stable pairs before surgery and re-introduced 0-18 hours post-surgery. Rat grimace scores did not indicate pain after conclusion of the analgesia protocol, physiological parameters were in the normal range 3 days post-surgery and weight loss did not exceed 10%. Rats with a cement cap only were pair-housed continuously without damage to the headcap. Rats carrying an additional fragile headstage had to be separated during lights-off periods to prevent headstage damage but could be pair-housed during lights-on periods.

Pair-housing is a feasible and effective method to facilitate the rats' need for social companionship following cranial implant surgery.

## Introduction

Wireless telemetry systems and cranial implants in rodents for neural recording, stimulation, optogenetics, intra-cranial catheterisation and combinations thereof, are increasingly used in experimental protocols in neuroscience. These systems typically consist of a cranial implant embedded in dental acrylic cement, held in place by bone screws, with a neurostimulator or amplifier in the form of a headstage(1), rucksack(2) or implant under the skin(3), temporarily or continuously attached.

In these types of experiments, individual housing of animals remains standard practice due to the risk of damage to the device or wound during interactions between animals(4, 5). However, social isolation is long known to reduce animal welfare(6, 7), affecting stress hormone levels(8), anxiety(9), social interaction(10), brain metabolism and structure(11), blood pressure and heart rate(12), and food intake(13). Group-housing has been shown to prevent these detrimental physiological and behavioural effects of social isolation and increase animal welfare.

Although research studies often do not report housing conditions in rodents, pair-housing is possible in non-human primates(14-16). In addition, two groups have reported pair-housing of rodents with cranial implants. Pinnell *et al.* presented a headstage purposefully covered with a protective cap to allow pair-housing and behavioural assessment in the water(17, 18). Koletar *et al.*, reported co-housing of rats after creation of a cranial window for two-photon fluorescence microscopy. Neither reported damage to the device or wound. In addition, Koletar *et al.* reported improved handling in group-housed rats compared to individually housed rats. Norecopa, Norway's National Consensus Platform have published limited

descriptions of experiences with pair-housing of rodents with cranial implants while highlighting the lack of literature on the topic(19).

The aim of this article is to present our protocol for pair-housing of rats with cranial implants and report our experience with the protocol in the period immediately post-surgery and up to 16 weeks after surgery. The housing protocol was developed in the context of a project to examine the effect of chronic deep brain stimulation on the electrode tissue interface and surrounding brain parenchyma. Two different types of head-mounted devices were used: (1) a basic cement cap and (2) a larger headstage continuously connected to the top of the cement cap for deep brain stimulation of the basal ganglia (S-series, Triangle Biosystems).

## **Methods**

### **Animals**

Thirty-six adult male (n=34) and female (n=2) naïve Wistar rats (weight:  $404\text{g} \pm 70\text{g}$  (SD); age:  $9.8\text{ weeks} \pm 2.6\text{ weeks}$ , breed in the local facility using Charles River breeders) that underwent successful cranial implant surgery for DBS in the time period 2017-2021. The animals were divided into two groups: 26 rats had a cranial implant secured by a cement cap and 10 had an additional headstage attached throughout the protocol. No control group was included because pair-housing was required by the licensing body. Welfare assessments were conducted twice daily for the first 4 days and 3 times per week thereafter by the same researcher at the same time in no set order. The rat grimace score(20) ranging from 0 (no pain) to 2 (severe pain) depending on four facial features was rated by one observer in real-time. A facility specific welfare score, including general appearance, behaviour, respiration, weight, state of the surgical site and adverse effects was also filled scoring each point from 0 (normal) to 3 (supplemental data). Training was provided by the facility beforehand. Rats

were assessed in no set order in the home cage. After 8 or 16 weeks rats were perfused with formalin and brains harvested for histological assessment. All experiments were approved by the UCD Animal Research Ethics Committee (AREC 17-22/20-02) and licences by the Health Product Regulatory Authority Ireland (AE18982-P122/P190).

The primary welfare measures reported for each animal (~~experimental unit~~) here are adverse effects, welfare scores, rat grimace scores and weight development.

### Cranial implant surgery

Stereotaxic surgery was performed under inhalation anaesthesia (induction: 4.5% isoflurane (Iso-Vet, Chanelle Pharma, Loughrea, Ireland) in 4 l/min oxygen; maintenance: 1.2-1.8% isoflurane in 1 l/min oxygen) in a procedure room. In addition, local anaesthesia at the skin incision (maximum 0.05 ml 0.5% lidocaine, diluted from Lidocaine 1%, Hameln Pharmaceuticals Ltd, Gloucester, UK) and anaesthetic cream at the non-rupture ear bars (Emla 5% cream, AstraZeneca, Cambridge, UK) were used. The depth of anaesthesia was monitored continuously using the pedal withdrawal reflex and corneal reflex. Surgical practice included temperature control using a heating blanket, aseptically prepared surgical field, subcutaneous (s.c.) antibiotics given pre- and 4 days post-op (metronidazole (20mg/kg, B. Braun Medical Ltd, Dublin, Ireland) and gentamicin (6mg/kg, diluted from Gentaject 10%, Alpha Med Ltd, Fermoy, Ireland) QD), tear replacement ointment (Vidisic, Dr. Gerhard Mann, Chem.-pharm. Fabrik GmbH, Berlin, Germany) and intra-operative fluid. Buprenorphine (0.05 mg/kg s.c., Buprenodale, Dechra, Shrewsbury, UK) was given as analgesia 1 hour before surgery and post-operatively two to four times per day for 3-4 days.

All rats underwent craniotomy, electrode insertion into the basal ganglia area and formation of a cement cap (Dentalon plus, Heraeus Kulzer GmbH, Hanau, Germany) held by four bone

screws (1.59 mm, Stoelting). The skin was re-aligned cranially and caudally using an intradermal suture (Vycyl 4-0, Ethicon Inc., Somerville, USA). Ten rats had a wireless and inductively powered headstage for deep brain stimulation (S-series, Triangle BioSystems Inc., Durham, USA) continuously attached. In the other 26 rats the connector in the cement cap was accessed for stimulation or recording for short time periods.

#### Animal husbandry

The general housing conditions in a specific pathogen free facility following the FELASA recommendations(21) included a 12/12 hour light-dark cycle in a temperature ( $21.8^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$ ) and humidity ( $49\% \pm 4.1\%$ ) controlled environment and access to water and standard rodent diet (209/18 Tekland irradiated diet, Envigo, Indianapolis, USA) *ad libitum*. Individually ventilated rat cages (size: 34cm x 46cm x 29cm) were adapted for housing. The metal food dispenser and water bottle holder were removed to increase headspace and remove possible sites for entanglement or headstage damage. For the same reason, initially no ~~houses, rolls or hammocks were~~ other enrichment was supplied. Hammocks were supplied for the last 10 rats to undergo surgery. Cages contained woodchip bedding (Gold Chip Original, LBS, London, UK), paper shreds (Enviro-Dri, LBS) as nesting material and enrichment in form of wooden balls or sticks. Rats were fed on the ground and water bottles fed through the ceiling. Cages were cleaned 2 or 3 times per week.

Rats were housed in stable pairs for a minimum of 1 week before recovery surgery. If surgeries took place on the same day, rats were placed with their former cage-mate immediately after recovery. If surgeries were on consecutive days, rats were housed singly for up to 18 hours to recover from the procedure and then reintroduced to their former cage mate. A heating blanket was provided under half the cage for 18 hours post-surgery. While during continuous

pair-housing of rats with a cement cap caused little to no damage to the cement cap, gnaw marks on the headstages penetrating the protective cover were found after the light-off period of one rat that had a headstage attached. Therefore, to prevent damage to the headstage, rats in the headstage group (5 cages) were separated in the same cage during the lights-off period by a grid partition in the middle of the cage(22). This allowed olfactory, visual and auditory cues. Rats were housed together during the lights-on period.

## Statistics

Descriptive statistics and graph creation were performed in Prism 5 (GraphPad, San Diego, USA). The mean and standard deviation for both groups were calculated based on individual rats.

## Results and Discussion

Examples of the pair-housing with separation during the light-off period and enrichment are presented in Figure 1 D-G. The rat grimace score was under 0.5 after 3 days post-surgery and the welfare score was under 2 from 3 days post-surgery, Figure 1 A and B, indicating mild pain and good recovery post-operatively with little to no physiological changes. Rats weight loss after surgery was  $5.8\% \pm 3.9\%$  7 days post-surgery and returned to the weight at surgery 12-14 days post-surgery, continuing to increase thereafter, Figure 1 C. When comparing between male and female rats, welfare scores developed similarly post-operatively, but weight loss was less in female rats (-0.7%, -2.8% at 7 days post-operatively) while weight gain was minimal at 8 weeks (+13.6%, +6.8%). While it is not possible to draw conclusions regarding outcomes compared to singly housed animals due to the lack of a control group, Pinnell *et al.* reported



lower weight gain in pair housed rats compared to singly housed rats in a related cranial study with headstages(17). It is widely accepted that group-housing provides animal welfare benefits, especially in regards to behaviour and stress(6, 7, 11-13) and is therefore recommended by Norecopa(19). The addition of hammocks to the environmental enrichment did not result in headstage loss in any of the rats housed in those cages (Figure 1G).

Only mild adverse events occurred as a result of pair-housing, Table 1. These included minor headstage damage and two incidents of biting. ~~Pair housing represents a refinement in the husbandry of rats with cranial implants with mild adverse events that resolved within a maximum of 2 days occurring in <10% of rats, Table 1. These are milder than the effects of individual housing on animal behaviour, stress, and physiological status(6, 7, 11-13).~~ No animals were excluded as a result of pair-housing which meets the principle of reduction. In all three animals that lost the headstage softening of the bone around at least one screw was observed post-mortem linking the loss to local inflammation but no cracked bones that would indicate mechanical dislodgement. The number of animals (4/36) that lost the headstage in this cohort was in line with previous outcomes in the facility (1/5 - 1/8 with single housing). In summary, observed side effects were mild and headstage loss was not attributed to pair-housing and thus pair-housing represents a refinement in the husbandry of rats with cranial implants.

In the case of fragile headstages, the protocol was further refined to allow temporary pair-housing while protecting the equipment from detrimental damage. ~~Environmental enrichment, which has been shown to be beneficial to animal welfare(6), was included without adverse events. Neither bedding material, in form of paper shreds which have been proven safe for animals with cranial implants, nor the provision of hammocks caused damage~~

~~to the cranial implants (Figure 1 G).~~ For long-term continuous housing of animals in pairs, however, sturdy headstages or devices that can withstand gnawing are needed (17, 18).

Finally, with paired or group housing, behavioural data from rats that are housed together may not be statistically independent as behaviour is directly impacted by the presence of a conspecific. This can be controlled for through the use of mixed models or similar methods to model the effect of cage or group.

## **Conclusions**

We report our experience with a protocol for pair-housing of rats with cranial implants including modifications for fragile devices. Rats showed good recovery after surgery and no additional moderate or severe adverse events were observed that could be attributed to pair-housing. In conclusion, to increase animal welfare, rats with cranial implants can be housed in pairs and safe enrichment can be provided which would counteract behavioural and physiological changes associated with individual housing.

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**Declaration of conflicting interests:** The Authors declare that there is no conflict of interest

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**Data availability statement:** Data presented in this short report is available on reasonable request from Dr. Judith Evers ([Judith.evers@ucd.ie](mailto:Judith.evers@ucd.ie), Room 149, Engineering Building, Belfield, Dublin 4, Ireland).

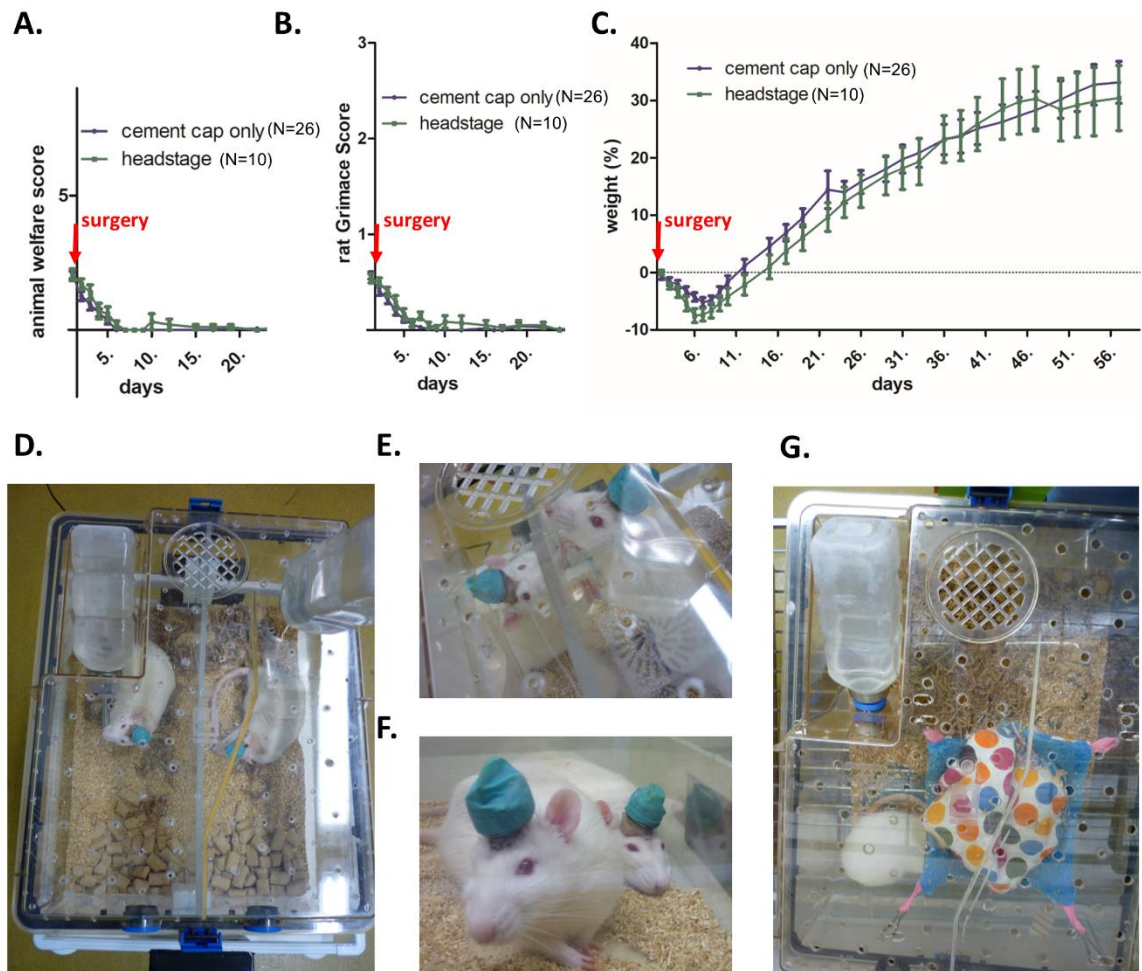


Figure 1: Mean  $\pm$ SEM of rat welfare score, rat grimace score and weight gain/loss in relation to weight at surgery post cranial surgery; cement cap only (n=26); headstage (n=10) (A-C.). separation (D.). Interaction during temporary separation (E.). Pair of rats during unseparated period (F.). Housing with enrichment and hammock for 2 rats (one visible only in picture, G.)

<b>Severity</b>	<b>Specific to pair-housing</b>	<b>Specific to headstage surgery</b>
Mild (resolved itself)	Bite (x2)  Headstage damage (x1) but headstage still functional	Scratching (x6)  Chromodacryorrhea (red eye) (x2)
Moderate (treatment needed)	none	Scratching that needed trimming of the cement cap
Severe (humane endpoint)	none	Headstage loss (x3)  Infection (x1)

*Table 1: Summary of adverse events in pair-housed rats with cranial implants.*

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