



Impact of topical lidocaine-prilocaine analgesia on grooming behavior after ear notching of mice

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Introduction

Finnish animal welfare legislation requires establishments to provide “appropriate pain relief” when performing ear notching for the identification of laboratory rodents¹. However, scientific evidence supporting the behavioral benefits of specific pain management methods remains limited. Our study aimed to determine whether topical application of lidocaine-prilocaine ointment modifies grooming behavior of the treated body area.

Methods

A total of 103 group-housed, 12 – 19 weeks old male and female C57BL/6JRj mice were randomized to the following 4 groups (n = 25 – 26):

1. topical analgesic ointment + ear notch;
2. topical analgesic ointment + handling;
3. vehicle ointment + ear notch;
4. vehicle ointment + handling.

Treatments were applied 30-60 minutes prior to notching or handling only. At specific timepoints (30, 60, 120, 240, 360 minutes and 24, 48, 72 hours) Grooming Transfer Test² was performed under ultraviolet-A light conditions, with fluorescent oil applied to the ear during notching-associated handling or handling only. The grooming behavior of the animals was scored by a blinded observer.

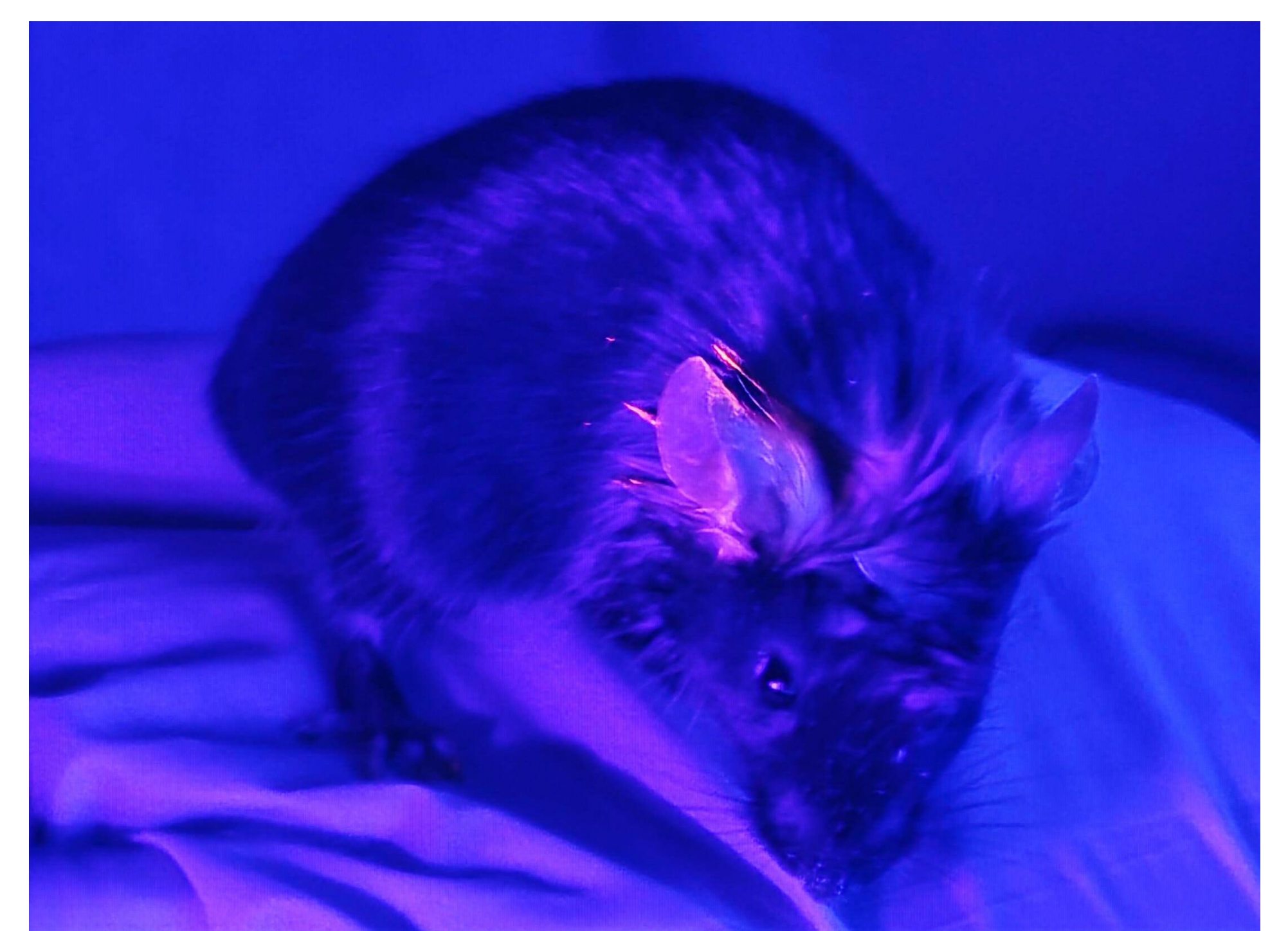


Figure 1. Representative image of the Grooming Transfer Test. Fluorescent oil applied to the ear of a mouse is visualized under ultraviolet-A light to assess removal of the fluorescent marker during the test.

Results

In pairwise comparisons of the vehicle- and analgesic-treated notched groups, mice treated with analgesic ointment showed a shift toward higher Grooming Transfer Test scores, reflecting faster removal of the fluorescent marker, compared with vehicle-treated controls (Figure 2). Differences were most pronounced at 240- and 360-minute timepoints, with less consistent differences observed at earlier and later timepoints. This pattern was consistent with the time-to-event analysis (Figure 3) of complete removal of fluorescent oil (Grooming Transfer Test score 5), which showed that mice treated with lidocaine–prilocaine ointment reached the behavioral endpoint earlier than vehicle-treated controls, indicating a shorter duration of grooming suppression.

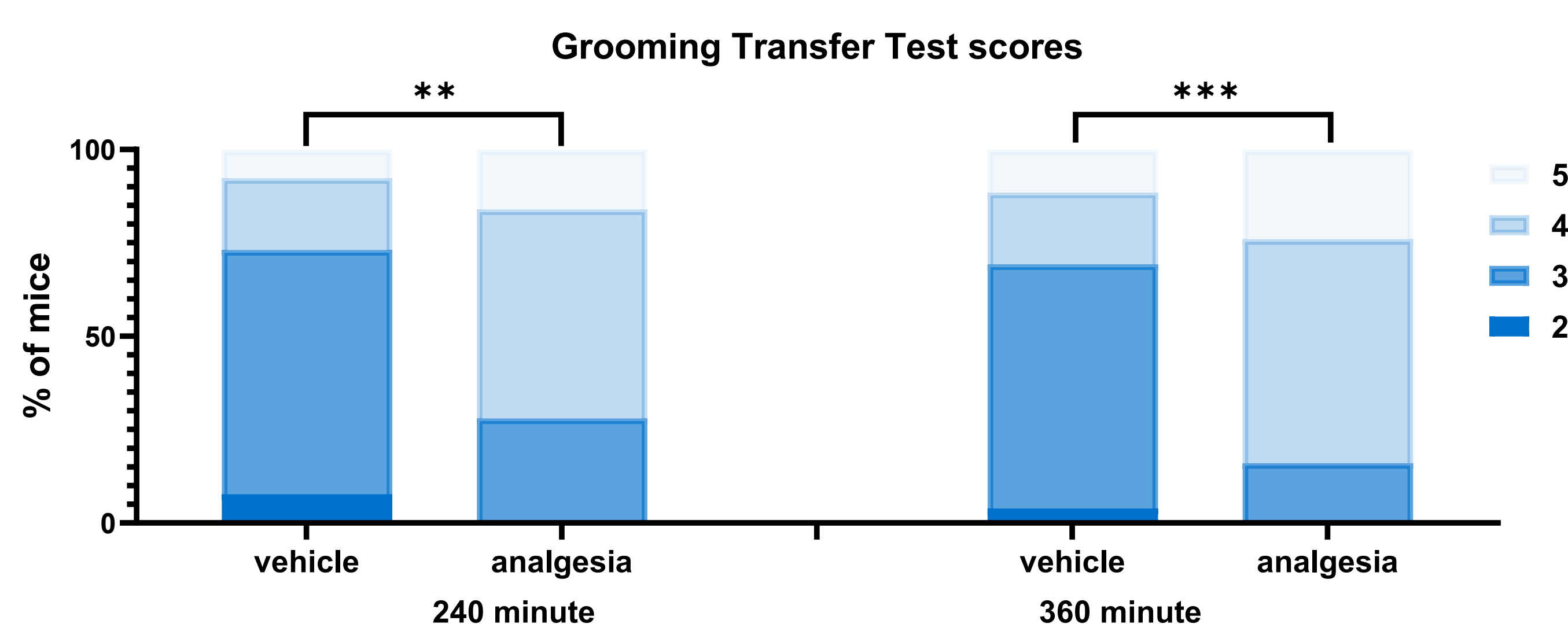


Figure 2. Grooming Transfer Test score distribution of vehicle- and analgesic-treated groups following ear notching as percentage (%) of mice. The distribution of scores differed significantly between groups at 240- and 360-minute timepoints (Fisher’s exact test, $P = 0.0071$ and $P = 0.0006$, respectively; $n = 25$ – 26 animals/group), with a shift toward higher scores in analgesic-treated mice.

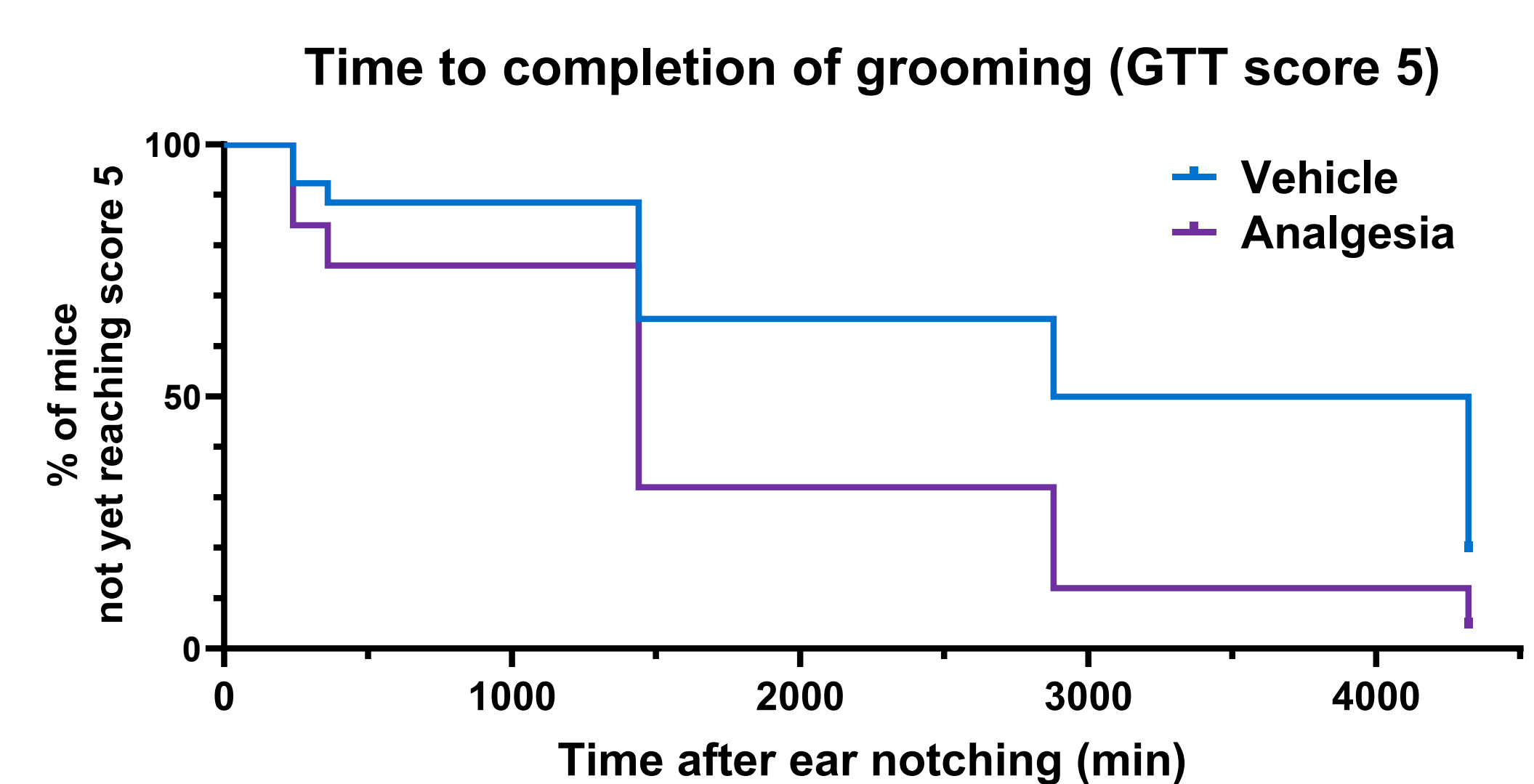


Figure 3. Kaplan–Meier curves showing the percentage of mice not yet reaching the behavioral endpoint (Grooming Transfer Test score 5) following ear notching. Analgesic-treated mice reached the endpoint earlier than vehicle-treated controls (log-rank [Mantel–Cox] test, $p = 0.0048$), indicating a shorter duration of grooming suppression. Tick marks indicate censored observations.

Conclusions

Topical lidocaine–prilocaine analgesia resulted in higher Grooming Transfer Test scores and earlier completion of the grooming task following ear notching, indicating a reduced disruptive effect of ear notching on grooming behavior. The most pronounced differences between groups were observed at 240–360 minutes, corresponding to the period of greatest treatment effect. These data is supporting the use of this analgesic method as a refinement measure under current animal welfare legislation.

References

¹Valtioneuvoston asetus eläimille tehtävistä toimenpiteistä ja keinollisen lisäämisen menetelmistä (1165/2023), Section 3(13) [Government Decree on procedures performed on animals and methods of artificial reproduction]. Available from: www.finlex.fi

²Oliver, V. L., Thurston, S. E., & Lofgren, J. L. Using Cageside Measures to Evaluate Analgesic Efficacy in Mice (*Mus musculus*) after Surgery. *J Am Assoc Lab Anim Sci*. 2018;57(2):186-201. PMID: PMC5868385

Disclosures

¹HN, AT, ST, A-MK, PP: employees of Experimentica Ltd., where the study was conducted.

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