

Grimace scale as a robust metric for nociception in a nitroglycerin (NTG)-induced mouse migraine model.

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BACKGROUND

Migraine, with its associated headache and neurological symptoms, is a severely impairing condition affecting more than a billion people worldwide. Although migraine is one of the most common neurologic disorders, the neurophysiological causes and thereby potential intervention avenues, remain mostly unknown, highlighting the need for a fast turnaround model to study this condition with consistent and robust readouts.

MATERIALS and METHODS

C57BL6 RccHsd mice (3 months) were treated with NTG or vehicle every second day after the first for a total of 5 injections. As a control, Sumatriptan or vehicle was co-injected during testing days at day 1, day 9, or after a week of no NTG treatment at day 16. On each testing day, the mice were injected, handled after 30 min and 60 min, behaviorally evaluated after at 120min, and sampled after 240min post injection. Behavioral characterization was done with grimace scale, body and facial grooming, light/dark-box, and hot plate testing. Biochemical and histological evaluation is ongoing.

RESULTS

In acute and chronically NTG-treated mice, the grimace scale analysis showed a strong increase in nociceptive responses that can be alleviated by co-injection of sumatriptan, similarly to what has been observed in humans. Interestingly, this effect could only be observed if mice were kept awake between the injection and the behavioral test through repeated handling. Additional assessment of behavior only showed mild and sporadic differences between NTG- and vehicle-treated mice, primarily due to male mice. Hot plate sensitivity testing did not show any differences

SUMMARY and CONCLUSION

Biochemical and histological analysis of are currently ongoing to complete the characterization, but our results show that the NTG-induced mouse migraine model robustly reveals core features of acute *in-vivo* migraine symptoms with a strong focus on gender differences and thus, represent a valuable animal model that can be successfully employed to test novel drug agents and therapies to tackle this prevalent disease.

RESULTS

Grimace Scale

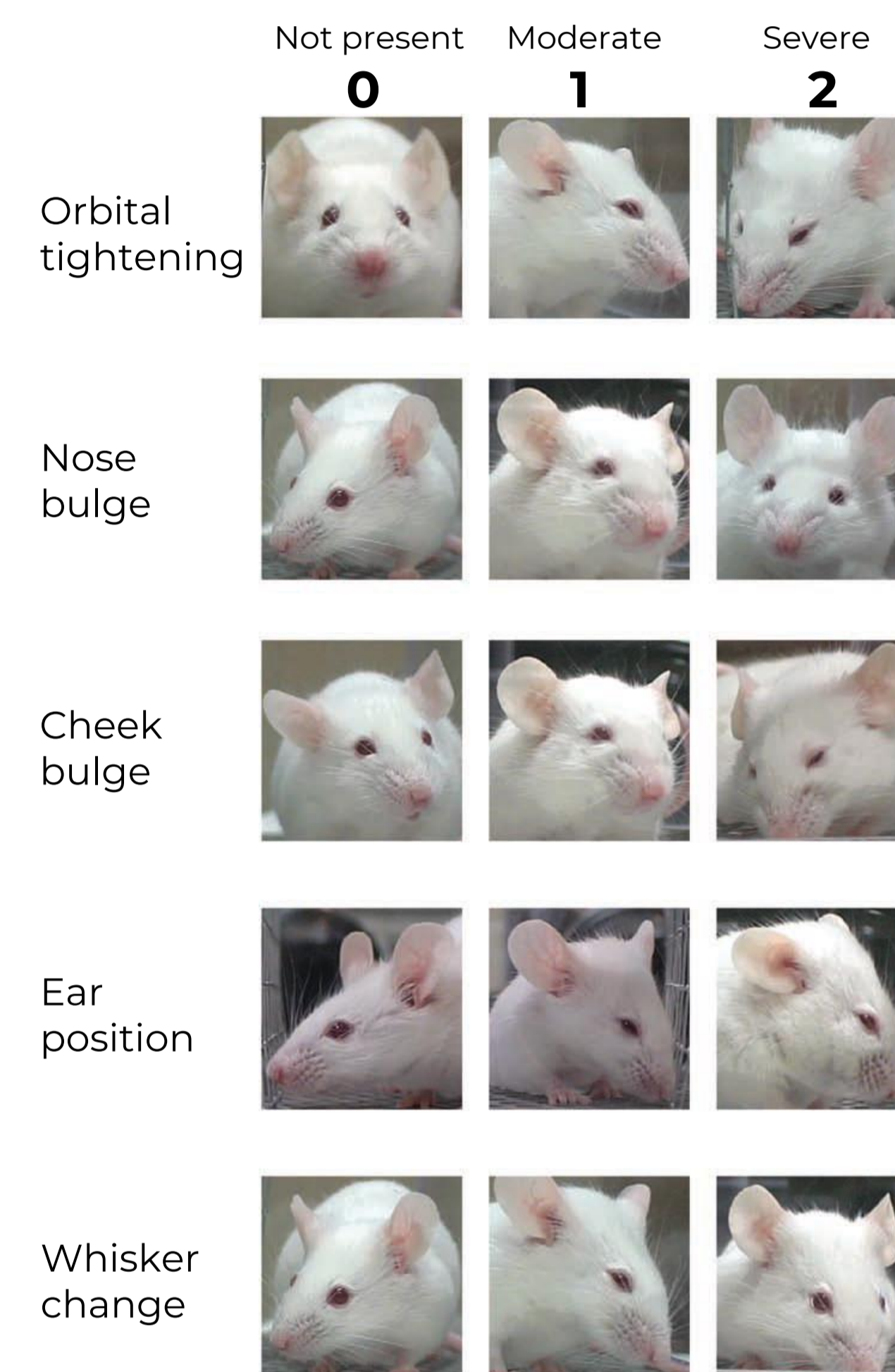


Figure 1 Grimace Scale scoring example. Representative images of the 5 facial features indicating pain, evaluated on a scale from 0-2. Images taken from *Langford et. al., 2010*.

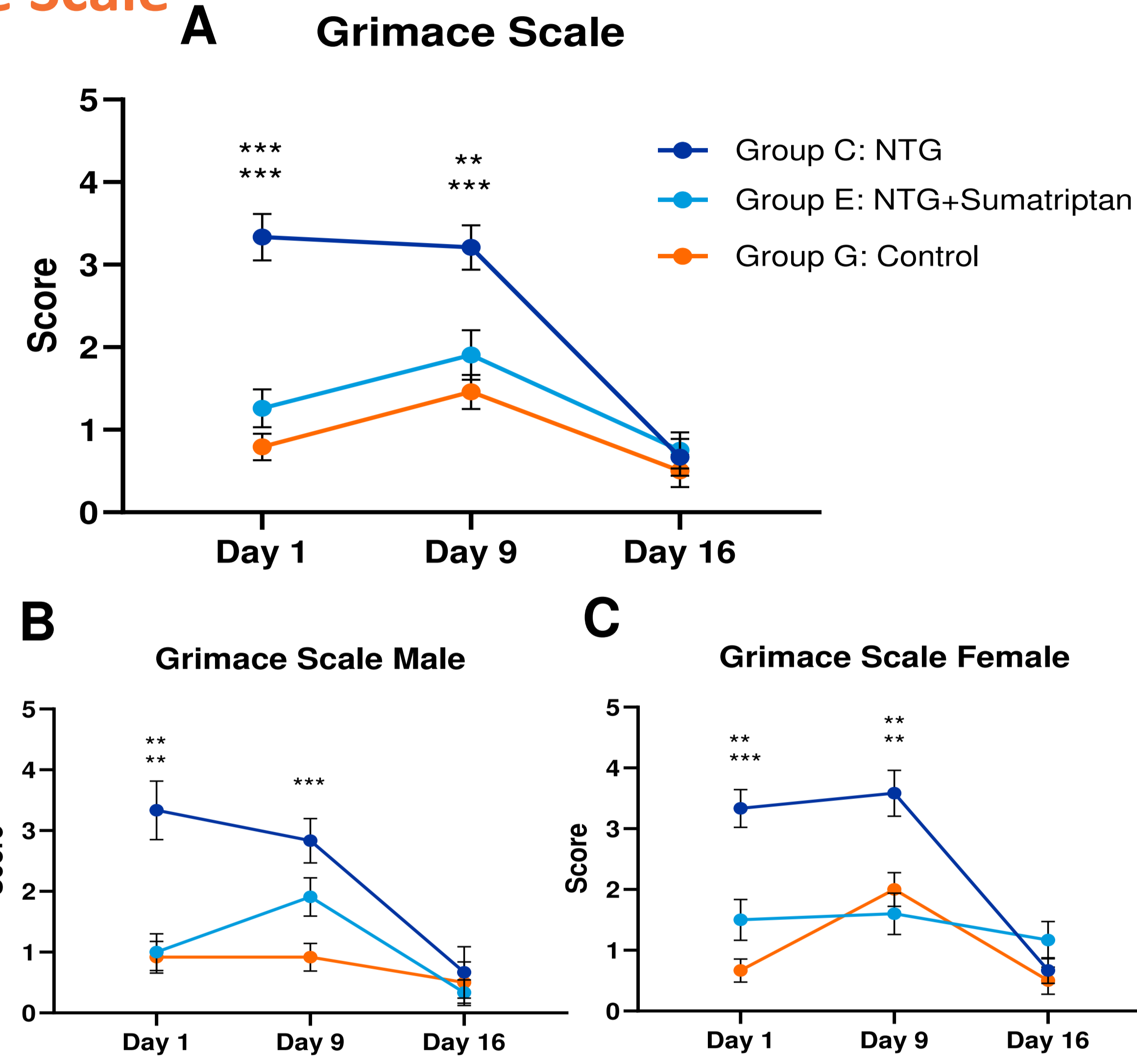


Figure 2 NTG injection leads to significantly higher Grimace Scale scores, counteracted by Sumatriptan co-injection. Grimace Scale scores of NTG, NTG+Sumatriptan, or vehicle injected mixed (A), male (B), or female (C) mice after 2h. Mean ± SEM. Mixed model ANOVA + Bonferroni post hoc test. **p<0.01, ***p<0.001.

Grooming

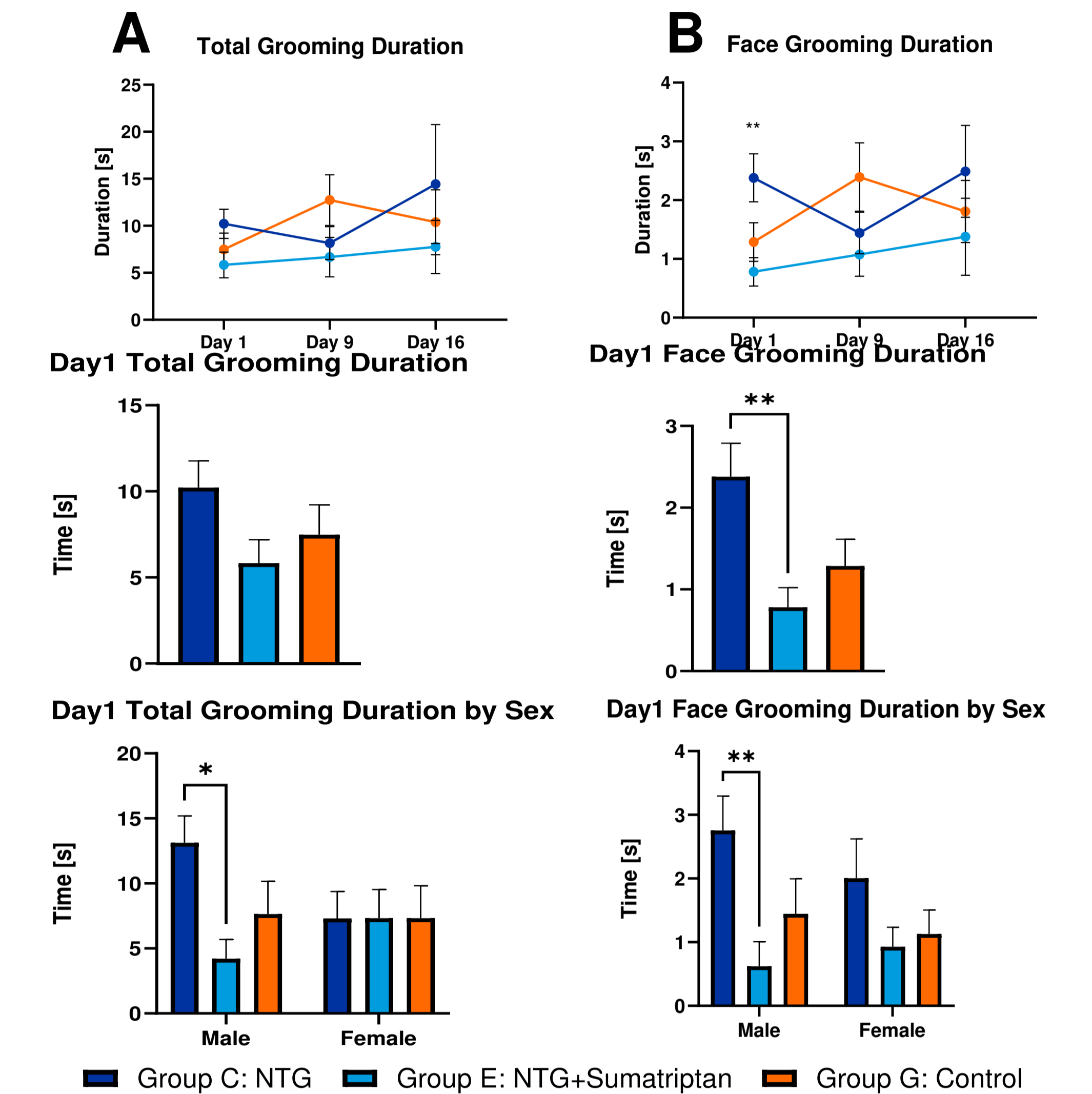


Figure 3 Only acute treatment with NTG leads to increased face grooming behavior, mostly seen in male mice. Total grooming and face grooming duration of NTG, NTG+Sumatriptan, or vehicle injected mice. Data is depicted as longitudinal data (A), only day 1 (B), and only day 1 separated by sex (C). Mean ± SEM. Mixed model ANOVA + Bonferroni post hoc test. *p<0.05, **p<0.01, ***p<0.001.

Light/Dark Box

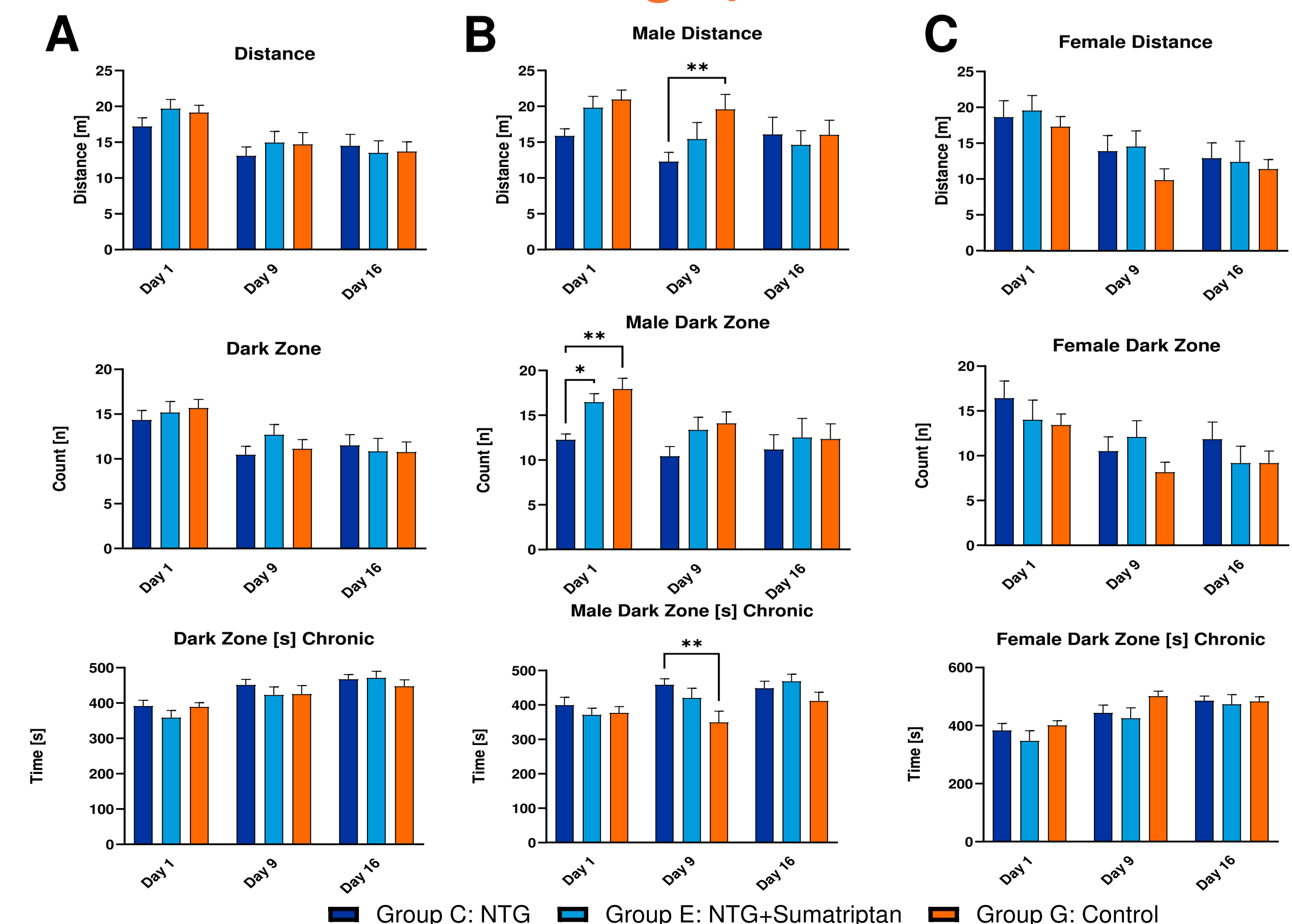


Figure 4 Only male mice show significant differences after NTG injection in the light/dark box. Distance travelled, dark zone entries, and dark zone time of NTG, NTG+Sumatriptan, or vehicle injected mixed (A), male (B), or female (C) mice in the light/dark box. Mean ± SEM. Mixed model ANOVA + Bonferroni post hoc test. *p<0.01, ***p<0.001.

Figure 4 Experimental overview of induced migraine model. Mice were tested after single (day 1) or repeated (day 9) injection of NTG and assessed for increased allodynia by grimace scale, grooming and hotplate tests as well as light sensitivity in the light/dark box. Sumatriptan was used as a control and was only injected on treatments days (1, 9, 16). Recovery from treatment regime was tested 1 week after last NTG injection (day 16).

