

Chronic migraine via nitroglycerin treatment progresses cognitive decline and amyloid pathology in the 3xTG mouse model



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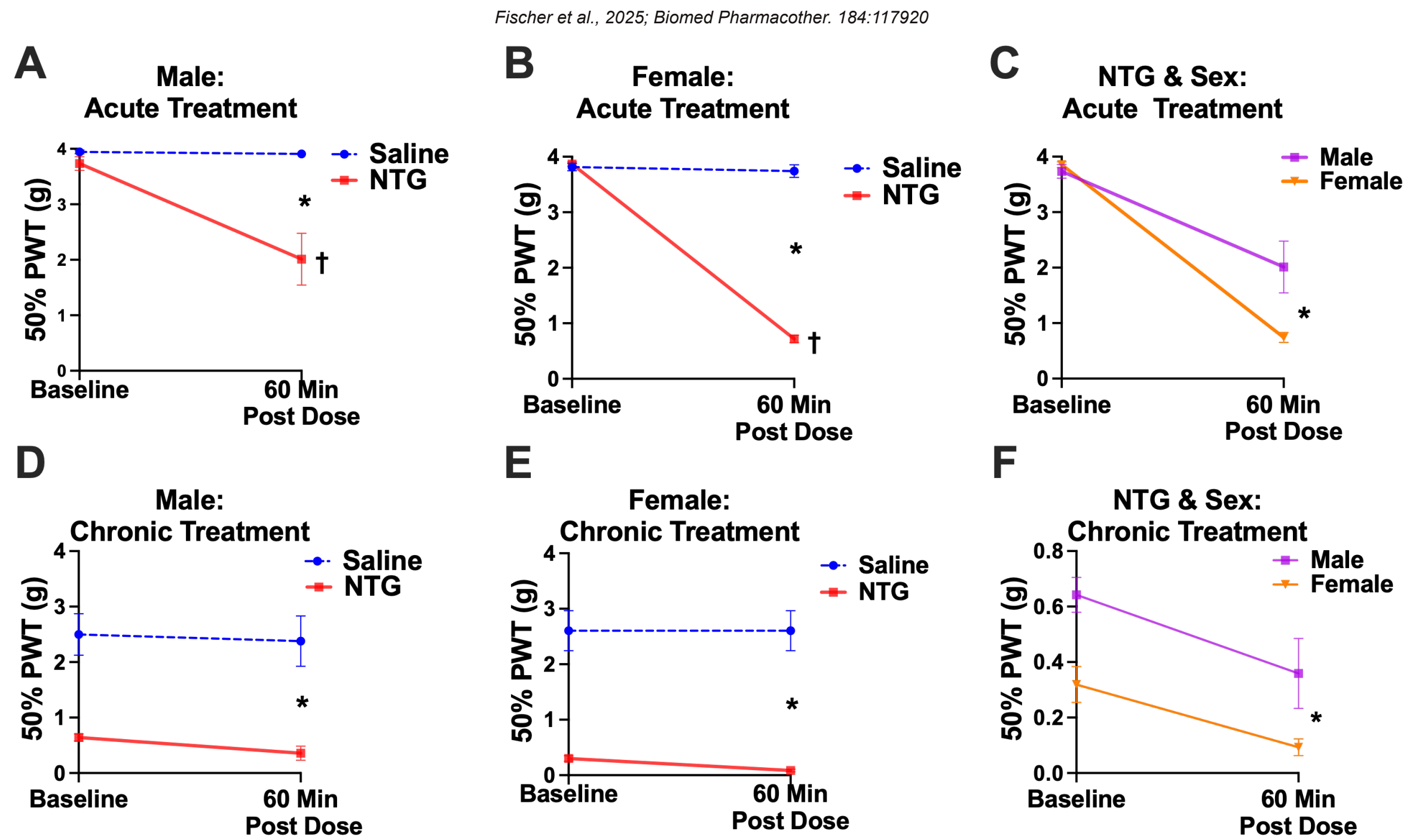


Abstract

Alzheimer's Disease (AD) is commonly associated with other health conditions that increase the risk of developing AD later in life. An understanding of these health conditions that contribute to the onset of AD will promote disease prevention strategies. Migraine disorder is a disabling chronic disease condition that affects over 15% of the general population. Clinical reports show that AD is prevalent in individuals with a history of migraine, and that the incidence of AD is significantly higher in those that suffer from migraine at a younger age (1,2). More specifically, females with a history of migraine have a 37% higher risk of developing AD compared to same-age males with a history of migraine. To date, it remains unclear how to address sex-associated migraine disorder in patients with AD due to our limited knowledge of the molecular crosstalk behind these two CNS disorders in animal models. **Methods:** We combined an AD and migraine mouse model by using the transgenic AD mouse containing three familial AD mutations (3xTG) exposed to the migraine causing agent, Nitroglycerin (NTG). This combined migraine-AD mouse model allowed us to monitor the effect of NTG on the pain threshold, behavioral changes, and pathogenic amyloidogenesis in males and females. We tested the hypothesis that chronic migraine disorder induced by NTG accelerates the development of cognitive decline and amyloid pathology in 3xTG mice. **Results:** Using the von Frey test, we show that 3xTG female and male mice have accelerated pain responses to single acute and chronic NTG injections (delivered IP) compared to vehicle treated 3xTG mice. However, only 3xTG female mice exposed to systemic NTG injections displayed deficits in spatial learning and memory and significant accumulation of amyloid beta (A β) in histological sections. **Conclusions:** Collectively, our findings suggest that the migraine causing agent, NTG, accelerates AD-related cognitive decline and A β accumulation in 3xTG mice, with more pronounced effects in female 3xTG mice specifically. This area of research is highly novel to the AD field in that we are the first to reverse translate the increased human AD incidence in chronic migraine sufferers to preclinical animal models.

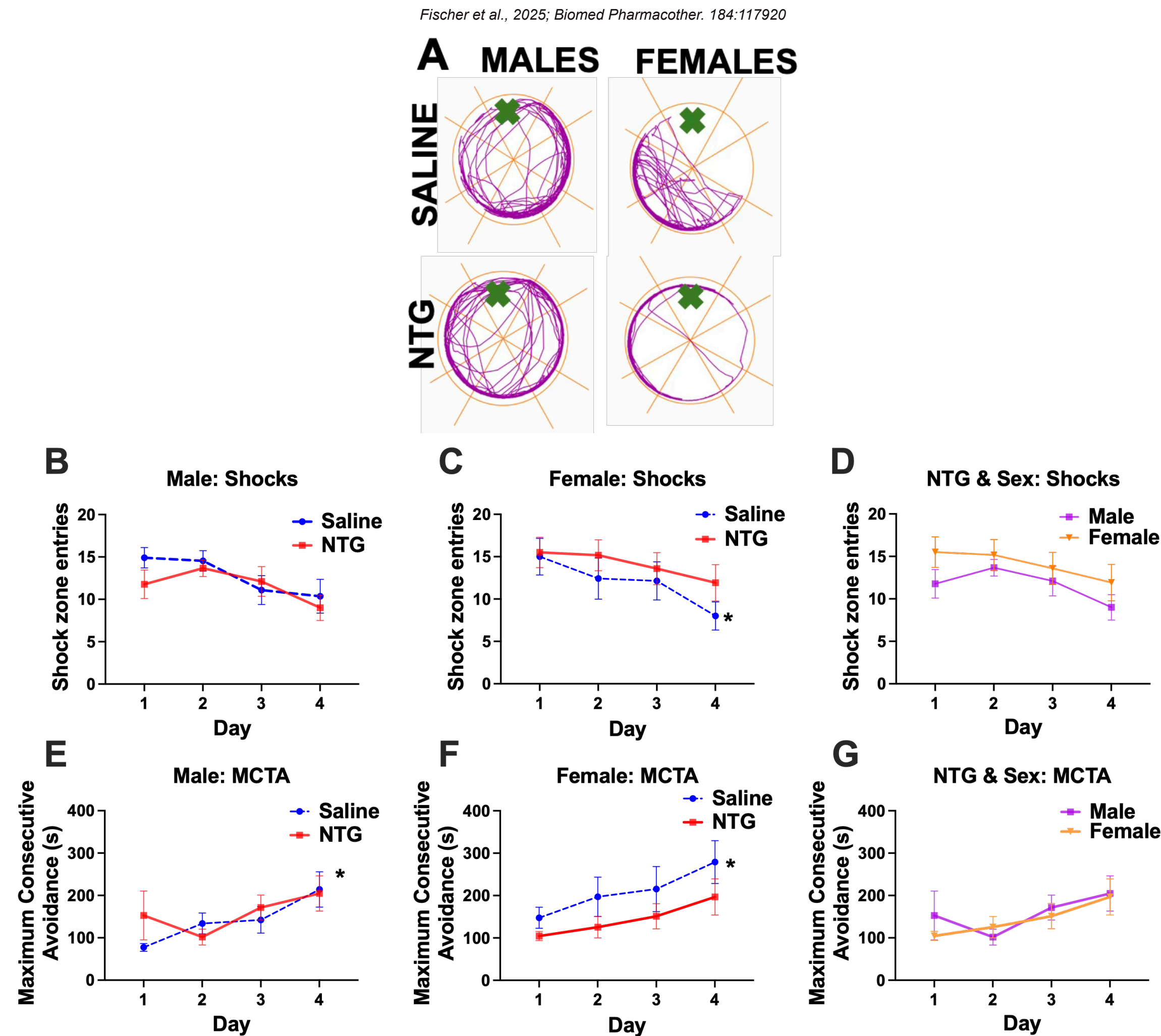
AD is prevalent in individuals with a history of migraine, and the incidence of AD is significantly higher in those that suffer from migraine at a younger age. Here, we determined whether this finding in the clinic reverse translates to the AD-like 3xTG mouse model.

1 The migraine causing agent, NTG, induced greater pain responses in female 3xTG mice



IP injection of NTG results in reduced pain thresholds in AD-like 3xTG mice. Von Frey filaments were applied to the left and right hind paws, and the mechanical paw-withdrawal threshold (PWT) allodynia was measured at a baseline level, 60 min after the first injection (acute treatment), and 3 days after the last chronic injection with NTG or saline control (chronic treatment). The PWT response to the acute exposure to NTG and saline control in males (A) and females (B). The PWT was significantly reduced in females compared to males in response to acute NTG single dose IP injection (C). The PWT response to the chronic exposure to NTG and saline control in males (D) and females (E). The PWT was significantly reduced in females compared to males in response to chronic NTG treatment (F). Chronic exposure to NTG reduces pain threshold in male and female mice. N (saline) = 11 males and 14 females; N (NTG) = 9 males and 12 females. The * symbol indicates significant differences between treatment groups. The † symbol indicates the significant difference between Baseline and 60 min post-exposure measured in NTG-treated groups. Statistical significance: $p < 0.05$.

2 Chronic NTG treatment impaired spatial learning in 3xTG female mice



IP injection of NTG affects spatial memory dynamics in 3xTG mice. The NTG and saline treated mice were evaluated by the active place avoidance (APA) test consisting of 4 consecutive days of testing (Day 1 – 4) beginning 3 days from the last NTG treatment. A) Representative track plots of male and female 3xTG mice treated IP with NTG and saline measured on Day 7 after the last NTG injection. B & C) The total number of shocks received by an individual male and female mouse from the NTG and saline-treated groups. D) The impact of sex on the total number of shocks received in mice treated with NTG. E & F) The maximum consequence time avoidance (MCTA) between shocks observed in an individual male and female mouse from the NTG and saline-treated groups. G) The impact of sex on the MCTA in mice treated with NTG. The X symbol indicates the initial position of the mouse in the APA apparatus. $p < 0.05$ was considered a statistically significant difference. N (saline) = 11 males and 14 females; N (NTG) = 9 males and 12 females. The * symbol indicates significant differences between mice from the saline treated group observed between Day 1 and Day 4 ($p < 0.05$). No significant differences were observed in the NTG treated groups.

Does chronic treatment with NTG worsen AD-like pathology in 3xTG mice?

2 Chronic NTG treatment enhanced the expression of amyloid precursor protein (APP) in 3xTG female mice

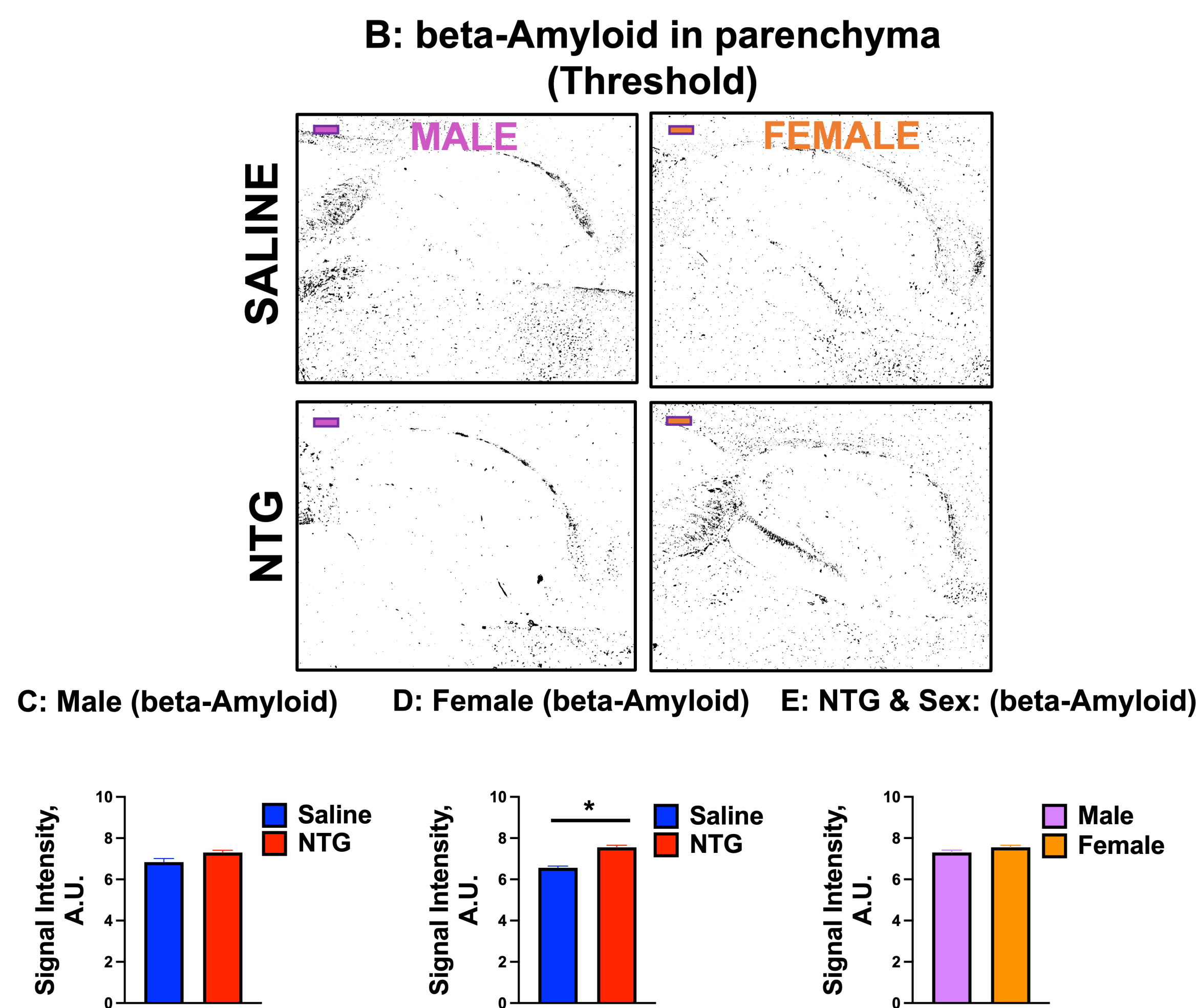
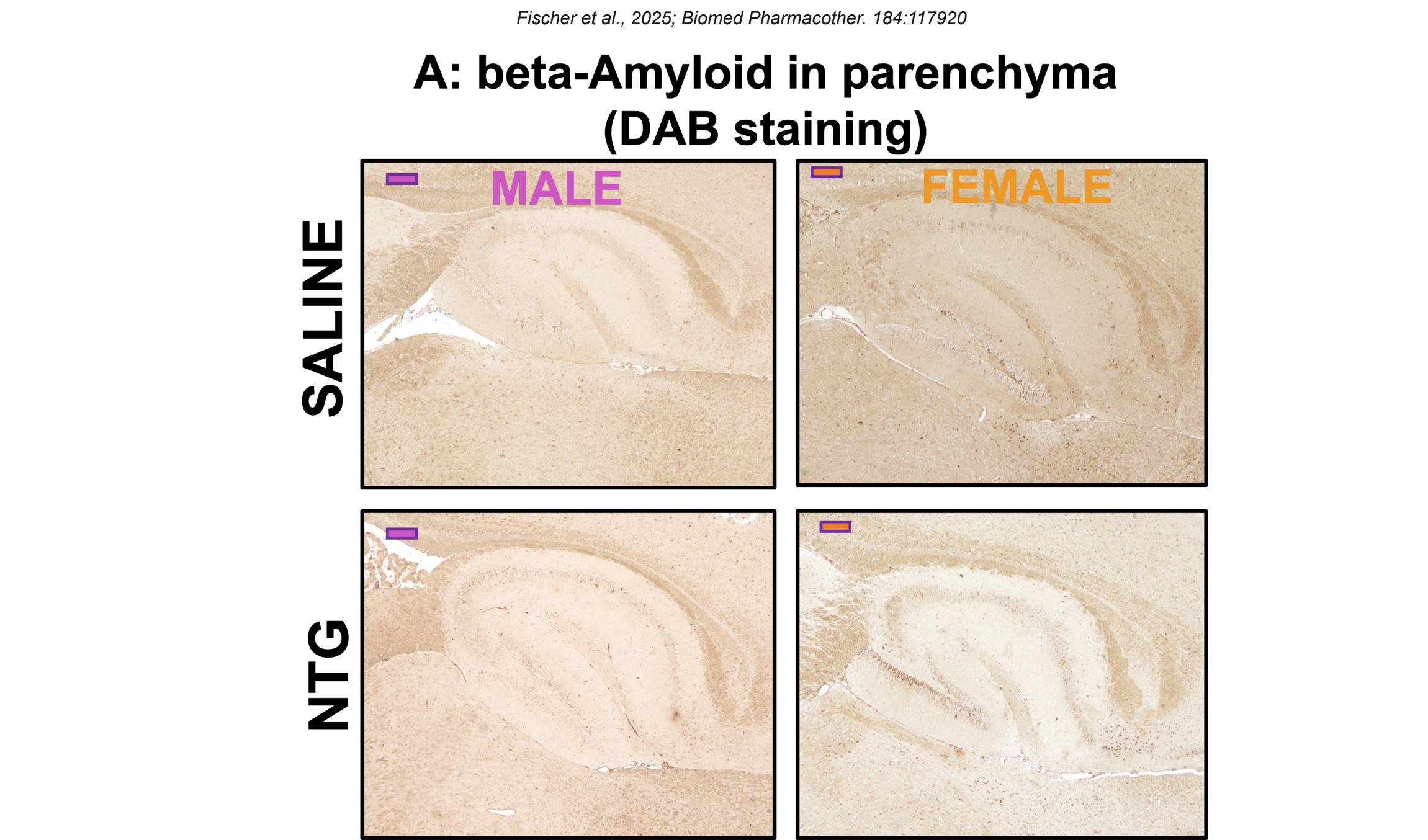


Figure legend 3: IP injection of NTG accelerates accumulation of the C-terminal APP in the brain parenchyma of 3x-Tg mice. A) Representative images of beta-Amyloid in the brain parenchyma of male and female mice. B) Images threshold created with the Otsu protocol using representative images. C) C-terminal APP signal intensity observed in males; D) C-terminal APP signal intensity observed in females; E) Sex-associated effect of NTG on C-terminal APP expression in the brain parenchyma. Scale bar – 200 μ m. N = 5 mice/group. * $p < 0.05$.

Conclusions

Here, we established a promising novel experimental model to study the effects of migraine on AD-related cognitive decline and pathology. Specifically, we found:

- The migraine causing agent, NTG, increases pain responses to a greater degree in 3xTG females relative to males.
- Chronic NTG treatment resulted in impaired performance in 3xTG female mice during the spatial learning and memory test, active place avoidance.
- 3xTG female mice showed accelerated amyloid precursor protein accumulation in the brain parenchyma following chronic NTG treatment.

Ultimately, these data suggest that chronic migraine induced by repeated NTG injections may enhance pain levels, cognitive decline, and AD-like pathology in the 3xTG mouse model, with the greatest impact on the female population.

References

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