Metabotropic Glutamate Receptor and Fragile X Signaling in a Female Model of Escalated Aggression

Supplemental Information

To begin to assess potential commonalities in the neural regulation of escalated aggression, behavioral plasticity following aggressive experience was assessed in a separate group of male subjects. In addition, the expression of *Psd-95* and *Sapap-3* mRNA were quantified following aggressive experience in males. Adult male Syrian hamsters were purchased from Charles River Laboratories (Wilmington, MA, USA) at approximately 60 days of age. Male subjects (n = 6) were housed individually in large cages (50.8 x 40.6 x 20.3 cm), whereas male intruder stimulus animals (n = 6) were pair-housed in smaller cages (43.2 x 22.9 x 20.3 cm). For male-male behavior tests, a male stimulus hamster was placed into a male subject's home cage for 10 min on 5 consecutive days. Male test times were longer than in female experiments because male hamsters are typically less aggressive than females (1) and 5 min was not sufficient to elicit high levels of aggressive behavior. As in females, each session was video recorded and later scored for the latency to the subject's first attack and the total number of attacks. The subject was paired with a different intruder male to minimize the likelihood that an intruder male would show submission to a familiar dominant subject. A **c**ontrol group of individually housed males remained in their home cages for the duration of the experiment and did not receive aggressive experience.

To assess whether aggressive experience increased the expression of *Psd-95* and *Sapap-3*, male subjects were given 5 daily aggressive experiences and then sacrificed by rapid decapitation 1 wk following the last aggressive experience. As in females, bilateral tissue punches (1-mm diameter) were immediately collected from the NAc and CP. For each brain

area, punches from one hemisphere were stored in RNA-later (Quiagen, Valencia, CA, USA) for qPCR analysis of *PSD-95* and *SAPAP*-3. Tissue samples were homogenized and RNA and cDNA were synthesized as detailed in the Methods of the main text. Significant differences in group means were detected using one-way ANOVAS or *t*-tests with Bonferroni corrections, $p < 0.05$.

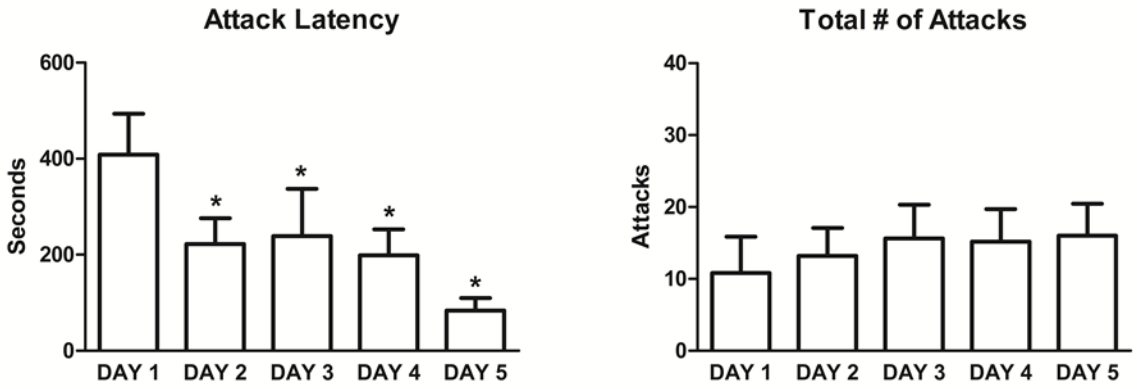

Figure S1. Aggressive experience results in escalated aggression in male hamsters. Left) Like in females, repeated aggressive experience resulted in a significant decrease in attack latency across time in male subjects ($F_{(4,16)} = 5.677$, $p < 0.01$). Newman-Keuls post-hoc tests indicated a significant decrease in attack latency on day 2 ($p < 0.05$), day 3($p < 0.05$), day 4 ($p < 0.05$), and day 5 ($p < 0.01$) when compared to day 1. Right) Also similar to females, the total number of attacks did not differ across testing days in males.

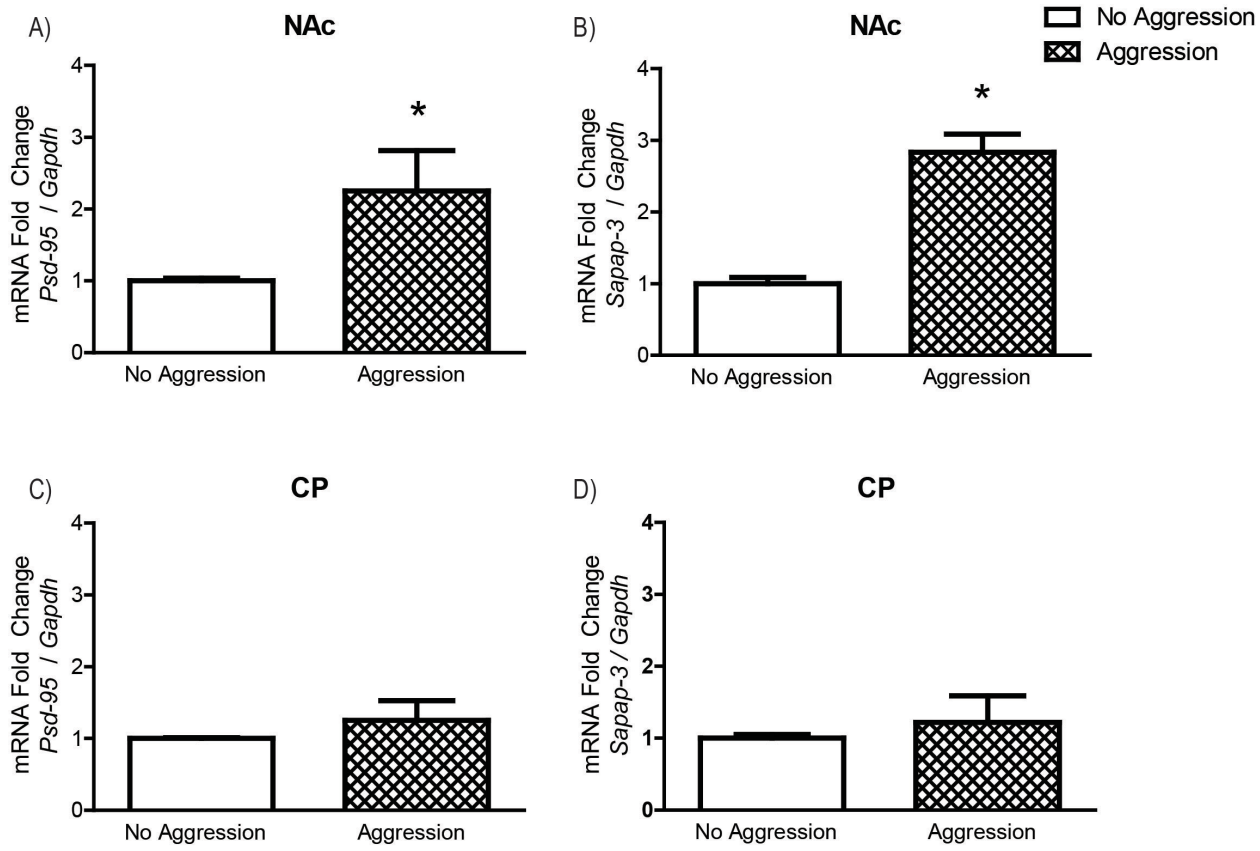

Figure S2. Aggressive experience increases the expression of PSD-95 and SAPAP-3 mRNA in male hamsters. (**A**) Like in females, male subjects sacrificed one week after the last aggressive experience had a significant increase in *Psd-95* mRNA in the NAc compared to control males who did not have aggressive experience ($t_{(10)} = 2.239$, $p < 0.05$). (**B**) Aggressive experience also resulted in large increase in *Sapap-3* mRNA in the NAc compared to control males ($t_{(10)} = 6.737$, $p < 0.001$). (**C, D**) These changes appear to be specific to the NAc, as aggressive experience did not increase *Psd-95* ($t_{(10)} = 0.791$) or *Sapap-3* ($t_{(10)} = 0.596$) mRNA in the CP.

Supplemental Reference

1. Payne, A.P. and H.H. Swanson, (1970): Agonistic behaviour between pairs of hamsters of the same and opposite sex in a neutral observation area. *Behaviour* 36 (4): 260-9.