Supplementary Results: Comparison of pair-fed and control groups

Amygdala

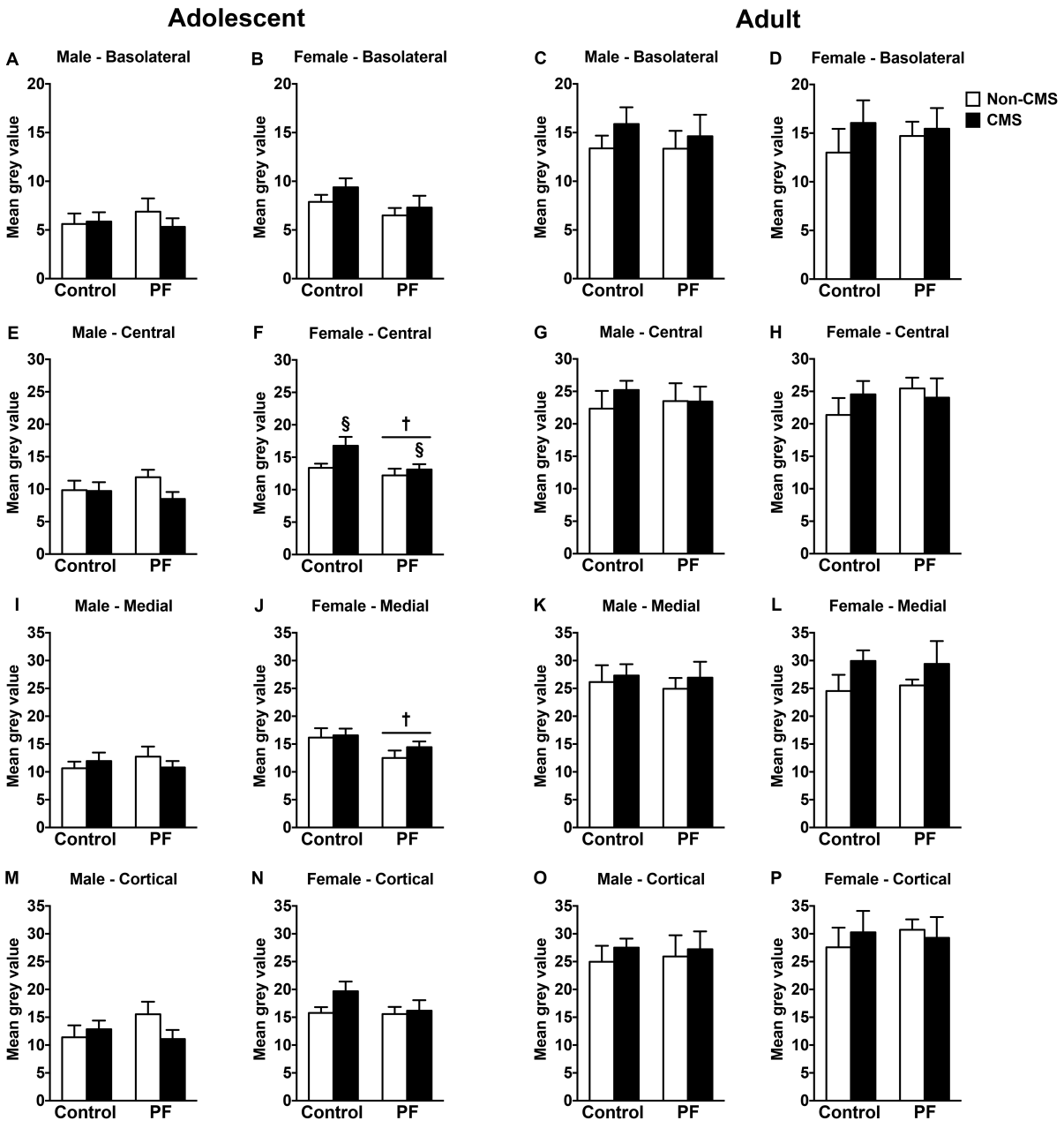
Neither pair-feeding nor adolescent CMS affected GR mRNA expression in the amygdala of adolescent males (Supplementary Figure 1A,E,I,M). By contrast, in adolescent females, pair-feeding decreased GR mRNA expression in the central and medial amygdala independently of CMS exposure [Supplementary Figure 1F, J; significant main effect of prenatal treatment for central ($F_{(1,28)}$=5.79, p=0.023) and medial ($F_{(1,28)}$=4.71, p=0.039) amygdala]. Moreover, adolescent CMS increased GR mRNA expression in the central amygdala independently of prenatal treatment [significant main effect of adolescent CMS for central amygdala ($F_{(1,28)}$=4.66, p=0.039)]. However, neither pair-feeding nor CMS affected GR mRNA expression in the basolateral and cortical amygdala (Figure 1B,N). In adulthood, neither pair-feeding nor adolescent CMS affected GR mRNA expression in the amygdala of male and female rats (Supplementary Figure 1C-D, G-H, K-L,O-P).

mPFC

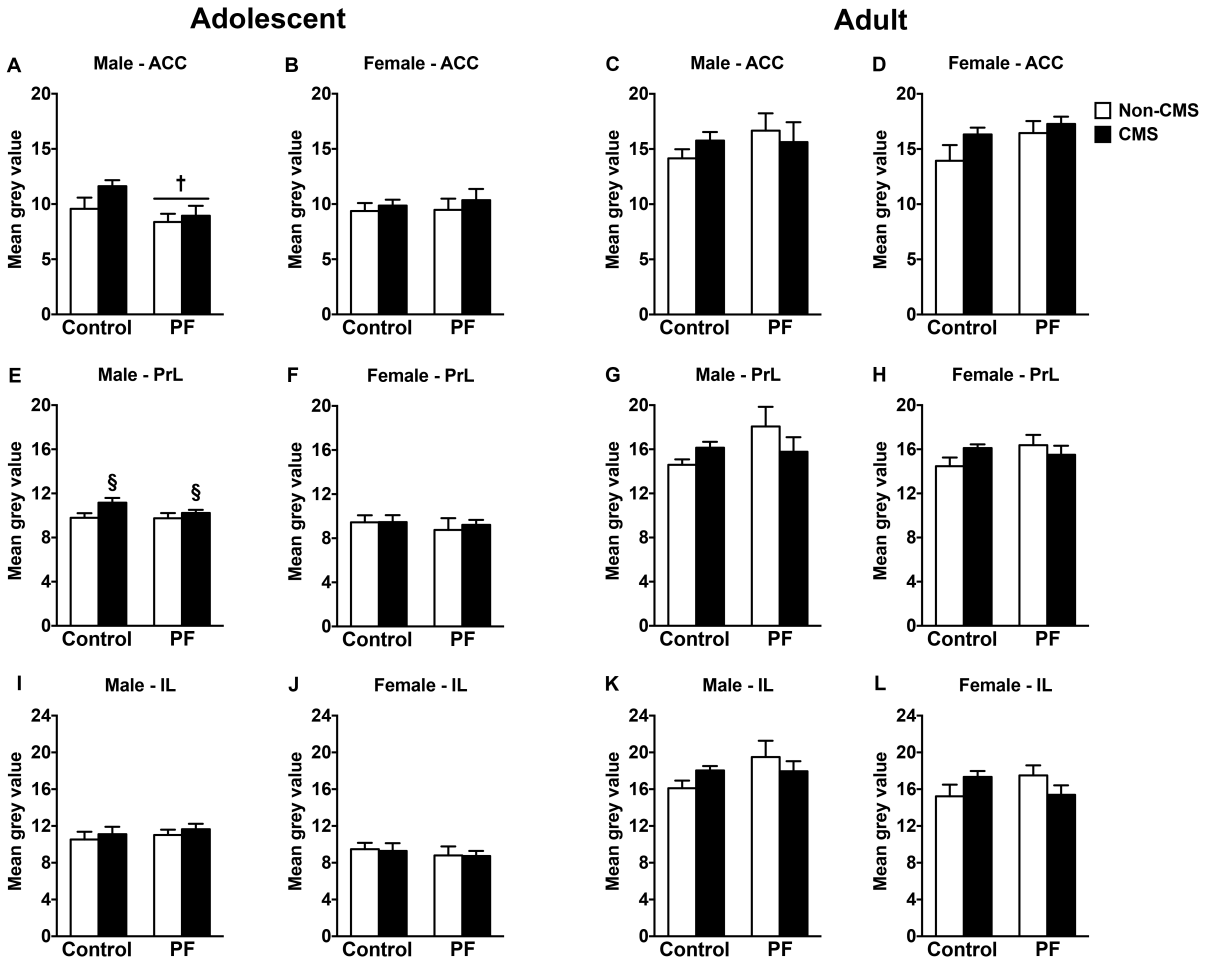
In adolescent males, pair-feeding decreased GR mRNA expression in the ACC independently of CMS exposure [Supplementary Figure 2A; significant main effect of prenatal treatment for ACC ($F_{(1,26)}$=5.56, p=0.026)]. Adolescent CMS increased GR mRNA expression in the PrL independently of prenatal treatment [Supplementary Figure 2E; significant main effect of adolescent CMS for PrL ($F_{(1,26)}$=5.13, p=0.032)]. Neither pair-feeding nor CMS affected GR mRNA expression in the IL (Supplementary Figure 2I) or in any of the mPFC subregions on adolescent female (Supplementary Figure 2B,F,J). In adulthood, neither pair-feeding nor adolescent CMS affected GR mRNA expression in any of the mPFC subregions of male and female rats (Supplementary Figure 2C-D, G-H, K-L).

PVN

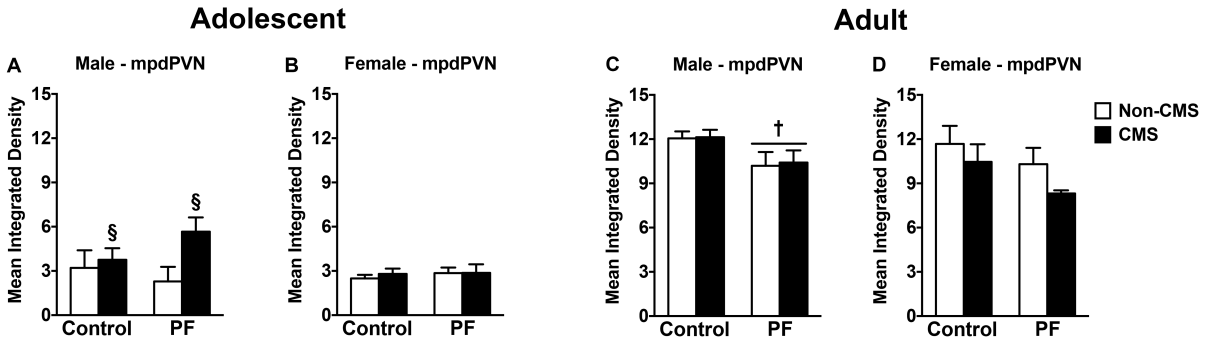
In adolescent and adult females, neither pair-feeding nor adolescent CMS affected GR mRNA expression in the mpdPVN (Supplementary Figure 3B,D). By contrast, in adolescent males, adolescent CMS increased GR mRNA expression in the mpdPVN independently of prenatal treatment [Supplementary Figure 3A; significant main effect of adolescent CMS ($F_{(1,22)}$=4.06, p=0.056)]. Moreover, in adult males, pair-feeding decreased GR mRNA expression in the mpdPVN independently of CMS exposure [Supplementary Figure 3C; significant main effects of prenatal treatment ($F_{(1,25)}$=5.98, p=0.022)].



Supplementary Figure 1. Short- and long-term effects of adolescent CMS on amygdala GR mRNA expression in control and pair-fed (PF) rats. Bars represent the mean ± SEM (mean gray value) of GR mRNA expression in the basolateral (A-D), central (E-H), medial (I-L), and cortical (M-P). † indicates a significant main effect of prenatal treatment, where all pair-fed animals are different from control animals; § indicates a significant main effect of CMS exposure, where all animals exposed to CMS are different from animals not exposed to CMS (n = 6-10 for all groups).



Supplementary Figure 2. Short- and long-term effects of adolescent CMS on mPFC GR mRNA expression in control and pair-fed (PF) rats. Bars represent the mean ± SEM (mean gray value) of GR mRNA expression in the ACC (A-D), PrL (E-H), and IL (I-L). † indicates a significant main effect of prenatal treatment, where all pair-fed animals are different from control animals; § indicates a significant main effect of CMS exposure, where all animals exposed to CMS are different from animals not exposed to CMS (n = 6-10 for all groups).



Supplementary Figure 3. Short- and long-term effects of adolescent CMS on mpdPVN GR mRNA expression in control and pair-fed (PF) rats. Bars represent the mean ± SEM (mean integrated density) of GR mRNA expression. † indicates a significant main effect of prenatal treatment, where all pair-fed animals are different from control animals; § indicates a significant main effect of CMS exposure, where all animals exposed to CMS are different from animals not exposed to CMS (n = 5-10 for all groups).

Supplementary Discussion

Pair-feeding

The pair-fed group is often used as a secondary control group in animal studies exploring the effects of maternal alcohol consumption on offspring. The pair-fed group was initially included to control for the decreased food intake that is typically observed with chronic alcohol consumption, as an attempt to separate the alcohol effects from those of possible undernutrition, and inclusion of a pair-fed group has become a customary procedure. However, this group is at best an imperfect and/or confounded control and at worst, an experimental treatment in itself. Indeed, pair-feeding can only control for the *reduced food intake* of the alcohol-consuming animals, but can never account for any of the nutritional effects associated with alcohol consumption, including alterations in absorption and utilization of nutrients (Weinberg, 1984) and increases in satiety (Lin et al., 1998). Furthermore, because pair-fed animals receive a reduced food portion – less than they would consume if allowed to eat *ad libitum* – they generally consume their entire day's ration within a few hours, and are thus essentially food deprived until their next feeding (Gallo and Weinberg, 1981; Weinberg 1984). In addition to inducing an abnormal feeding pattern, the pair-feeding procedure also introduces a mild prenatal stress component (the pregnant female is hungry for much of the day), which in itself may have long-term impacts on offspring developing neurobiological systems, including the neuroendocrine axis (Vieau et al., 2007).

Pair-feeding and GR mRNA expression in the amygdala, mPFC, and PVN

In the present study, pair-feeding resulted in few unique changes in GR mRNA expression in the amygdala, mPFC, and PVN. The pair-feeding effects in the amygdala and mPFC were transient as they were limited to the adolescent period. In adolescent females, the pair-feeding

effects were restricted to the amygdala, where pair-fed animals showed reduced GR mRNA expression in the central and medial amygdala. Conversely, in adolescent males, the pair-feeding effects were restricted to the mPFC, where pair-fed animals showed reduced GR mRNA expression in the ACC. However, PVN pair-feeding effects were only apparent in adulthood as pair-fed male rats showed reduced GR mRNA expression in the mpdPVN. Importantly, the only overlapping effects between PAE and pair-feeding were in the medial amygdala of adolescent females, where both prenatal treatments reduced the GR mRNA expression.

In summary, the current findings suggest unique effects of pair-feeding and provide further evidence that pair-feeding is not an ideal control group for models evaluating the effects of alcohol consumption during pregnancy. The effects observed in pair-fed offspring could, in themselves, lead to negative long-term effects for the neuroendocrine systems that are quantitatively and qualitatively different from those changes observed in PAE offspring.

Supplementary References

Gallo, P.V., Weinberg, J., 1981. Corticosterone rhythmicity in the rat: Interactive effects of dietary restriction and schedule feeding. J. Nutr. 111, 208-218.

Lin, H.Z., Yang, S.Q., Zeldin, G., Diehl, A.M., 1998. Chronic ethanol consumption induces the production of tumor necrosis factor-α and related cytokines in liver and adipose tissue. Alcohol. Clin. Exp. Res. 22, 231S-237S.

Vieau, D., Sebaai, N., Leonhardt, M., Dutriez-Casteloot, I., Molendi-Coste, O., Laborie, C., Breton, C., Deloof, S., Lesage, J., 2007. HPA axis programming by maternal undernutrition in the male rat offspring. Psychoneuroendocrinology 32, S16-S20.

Weinberg, J., 1984. Nutritional issues in perinatal alcohol exposure. Neurobehav. Toxicol. Teratol. 6, 261-269.