

Specific hypothalamic activation pattern by mGlu5 receptor blockade *in vivo* during pubertal development in female mice

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Background

Puberty is characterized by important changes in brain networks. The glutamate system plays a main role in modulating the onset of puberty as shown for NMDA receptor agonists and antagonists (Bettendorf, et al. 1999; Meijs-Roelofs, et al. 1991). However, the underlying mechanisms are poorly understood. Metabotropic mGlu5 receptors (mGluR5) are tightly linked to NMDA receptors (Perroy, et al. 2008). One subpopulation of GnRH-positive neurons is specifically activated by agonists of class I metabotropic receptors and are kisspeptin-insensitive (Fig.1; Dumalska, et al. 2008). The effect of mGluR5 blockade on neurohormonal mechanisms controlling puberty initiation were not studied yet.

Figure 1

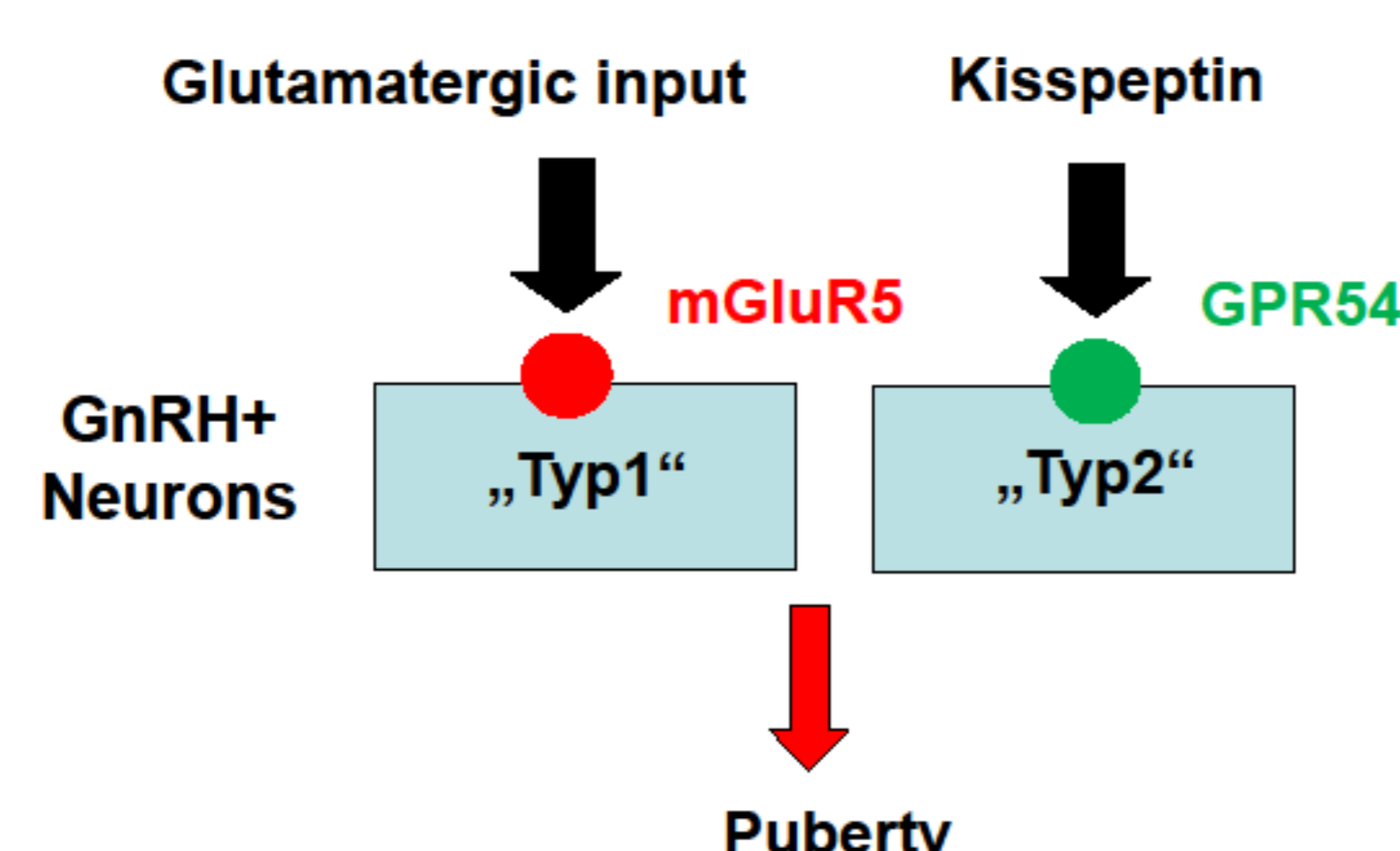


Fig. 1. Proposed model for the mGluR5-mediated action on GnRH-neurons, independent from Kisspeptin activation, based on the two different GnRH-expressing neuron subpopulations („Typ 1“ and „Typ 2“), identified by Dumalska et al. 2008.

Objectives

To determine the effect of pharmacological blockade of mGluR5 on neuronal activation during puberty and changes associated with the initiation of puberty.

Methods

We used profiling of the expression of the immediate early gene *c-fos*, as marker of neuronal activity triggered by the selective mGluR5 antagonist 2-Methyl-6-(phenylethynyl)pyridine (MPEP). Female mice at postnatal day (P) 16 to 40 (n=6) were treated intra peritoneal (i.p.) with 30 mg/kg MPEP. Coronal brain sections (50 μm) were obtained and DAB immunohistochemical analysis of *c-Fos* expression was performed using a rabbit anti-*c-Fos* primary antibody (1:10.000, Calbiochem). Serum levels of LH and FSH were measured in P26 and P30 female mice (n=5) after 7 days of MPEP (Fig. 2) or normal saline treatment by non-magnetic bead immunoassay. Additionally body growth, weight and uterus weight were measured.

Figure 2

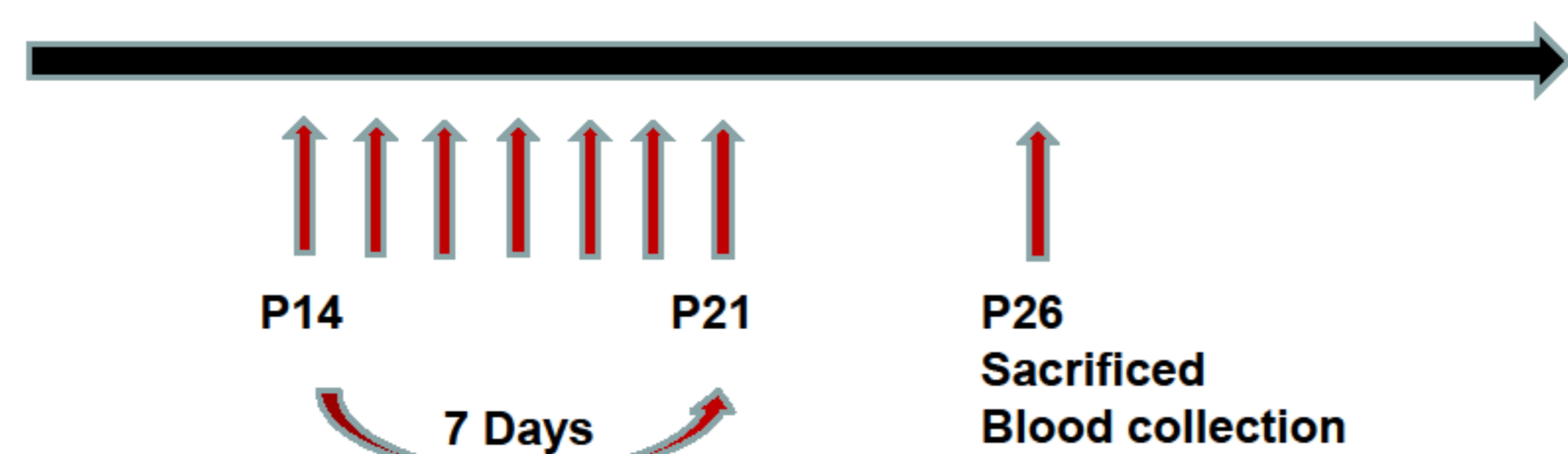


Fig.2 Protocol of daily i.p. treatments with NaCl 0,9 % and MPEP.

Conclusions

1. Our data provide new insights into the role of mGluR5 in pubertal development of female mice. Chronic MPEP treatment reduced LH and FSH levels at P26, suggesting a role of mGluR5 in the glutamatergic control of the hypothalamic-pituitary-gonadal (HPG) axis.
2. MPEP administration activated *c-fos* robustly in the PVNH, a key regulator of the hypothalamic-pituitary-adrenal (HPA) axis. Future studies may further clarify the underlying neurobiological mechanisms and their functional consequences.

The authors declare no conflict of interests.

Results

1. MPEP activates paraventricular nucleus of the hypothalamus (PVNH) We found a remarkably specific activation of the paraventricular nucleus of the hypothalamus (PVNH) by MPEP, starting at P16 and continuing throughout puberty (P16 - P40), (Fig.3a-3e).

Figure 3

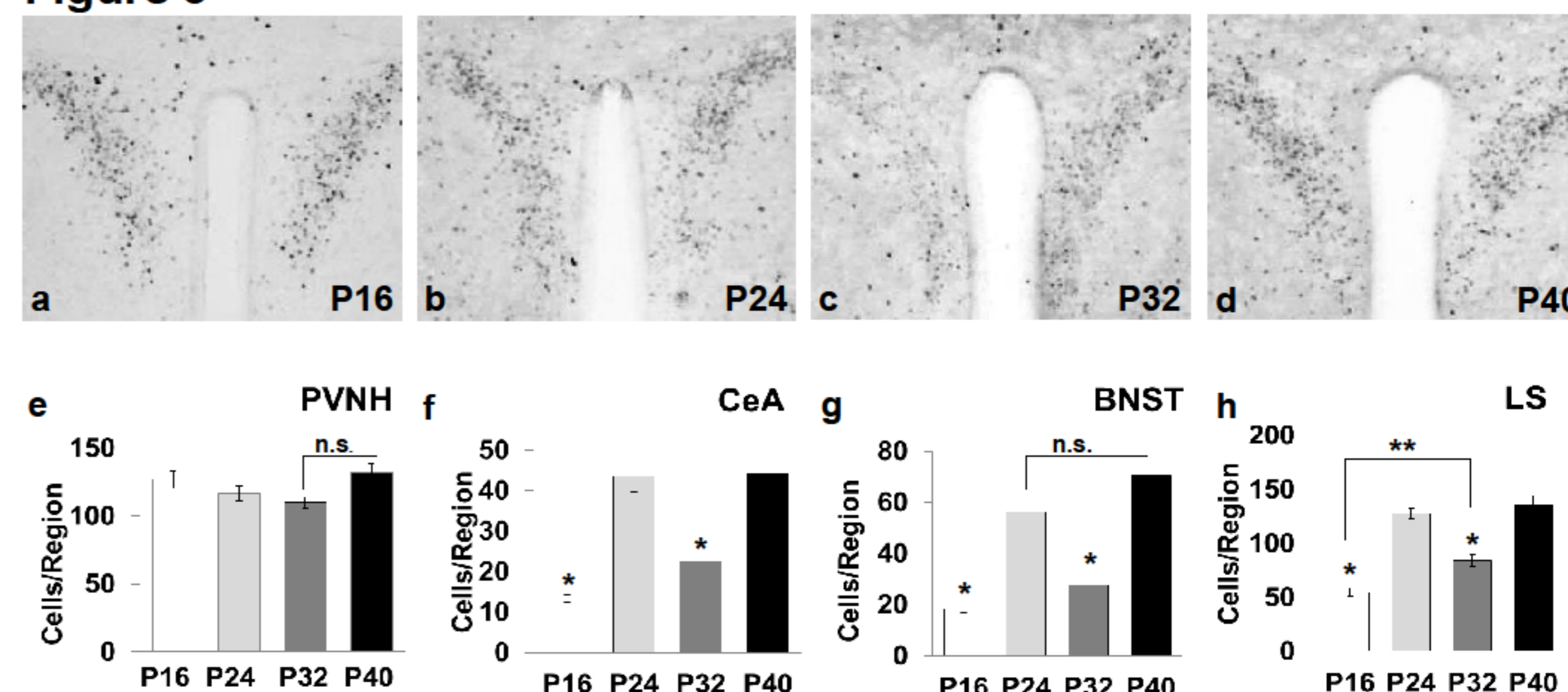


Fig. 3 Neuronal activation by MPEP in stress-related brain regions during peri-pubertal development. MPEP (30mg/kg) induced conserved high *c-Fos* expression in the PVNH at P16 (a), P24 (b), P32 (c) and P40 (d), quantification in (e). In contrast, lower variable *c-Fos* expression at different time points was visible in the in the CeA (f), BNST (g) and LS (h). CeA, central amygdala; PVNH, paraventricular nucleus of the hypothalamus; PVT, paraventricular nucleus of the thalamus. Statistical differences determined by ANOVA (* $p < 0.001$, ** $p < 0.05$, n.s. not significant). All data are means \pm SEM.

Low levels of MPEP-induced *c-Fos* expression were determined also in other stress-related areas, with increasing expression during development from P16 to P40 as quantified in the central amygdala (CeA) (Fig. 3f), bed nucleus of stria terminalis (BNST) (Fig. 3g) and lateral septum (LS) (Fig. 3h).

2. MPEP reduced LH and FSH levels at P26, but not at P30

MPEP reduced LH and FSH levels (pg/ml) as compared to normal saline (LH 38.43 \pm 7.65 to 26.68 \pm 4.18, for FSH 119.08 \pm 45.66 to 86.38 \pm 17.45, n.s.) at P26 (Fig. 4a). Absolute LH and FSH levels increased to P30 (LH 81,77 \pm 14,75, respective 91,46 \pm 7,89 saline/MPEP; FSH 163,46 \pm 52,95, respective 159,69 \pm 22,06 saline/ MPEP n.s.) (Fig. 4b).

Figure 4

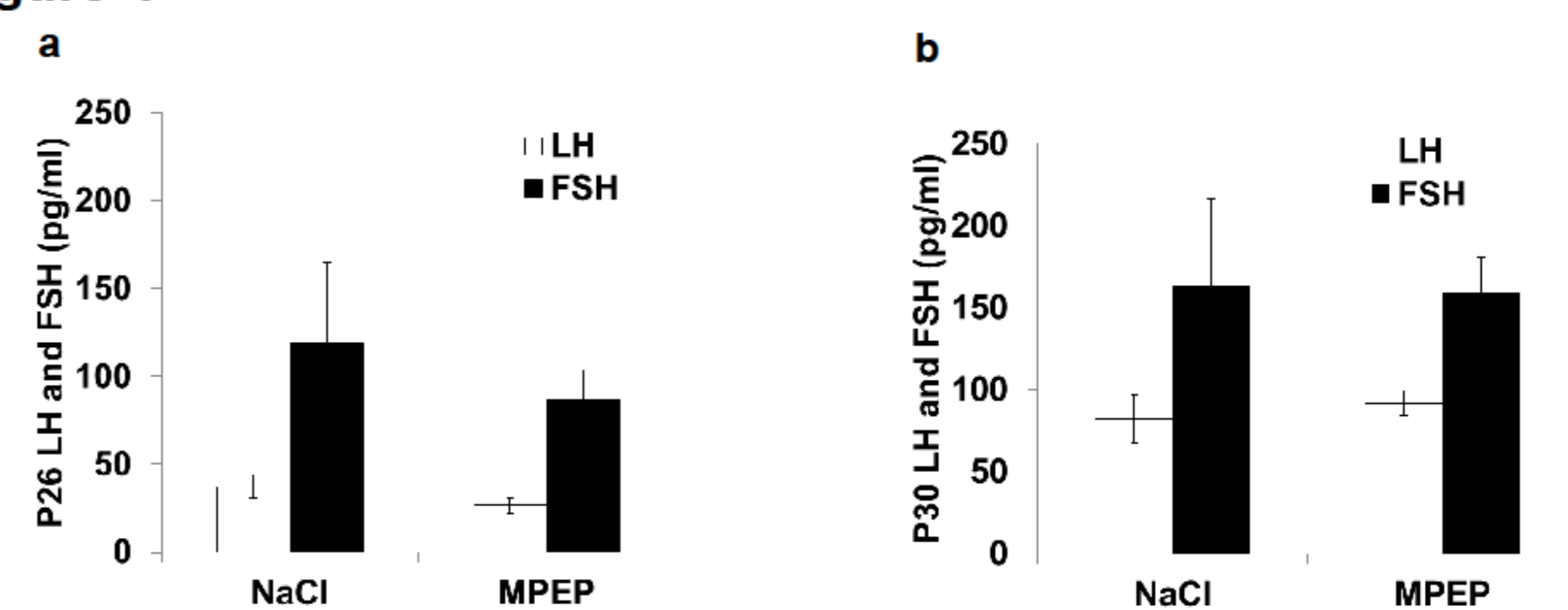


Fig.4 LH and FSH levels in pg/ml, at P26 (a) and P30 (b) after 7 days of treatment with MPEP or saline.

3. MPEP treatment does not affect growth, body weight or uterine development at P26 (Fig 5a) and P30 (Fig 5b)

Figure 5

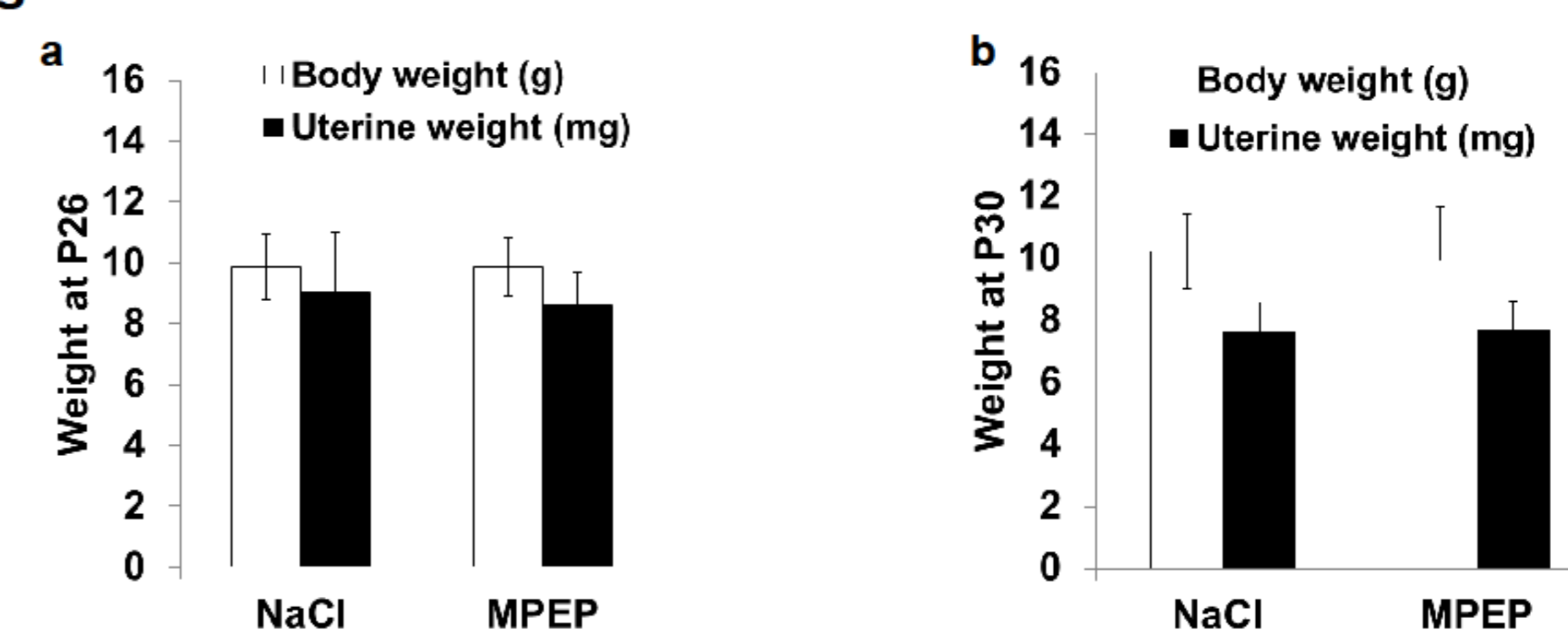


Fig.5 Body weight (g) and uterine weight (mg) at P26 (a) and P30 (b) after 7 days of treatment with MPEP or saline, n.s.

References

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