

Supplementary Materials

Although this experiment was designed to assess plasticity in neuronal function across development (examined by Age \times Condition interactions presented in the main text), data on anatomical development (VP-ir and OT-ir cell number) and sex or social condition differences in neural activity (Fos responses and VP/OT-Fos colocalization) may be of interest and are included here. We have excluded effects and interactions of Fos that do not include Condition as a variable because such effects/interactions lack context without condition information; Fos is a proxy marker of neural activity that is specifically in response to something (social condition in the present experiment).

Results: Nonapeptide neuronal distribution

PVN VP

Analysis of VP-ir at rostral and caudal levels yielded similar results and interactions, and so are combined here. We observed a main effect of Age ($F_{(2,150)} = 15.606$; $p < 0.001$; LMM; **Fig. S1A**). PND2 pups exhibited significantly fewer VP-ir neurons compared to PND9 (Mean diff. = 60.228; $p < 0.001$) and PND21 pups (Mean diff. = 58.938; $p < 0.001$).

We found no main effect of Sex ($F_{(1,150)} = 0.012$; $p = 0.912$; LMM) or Condition ($F_{(2,150)} = 1.170$; $p = 0.393$; LMM). In addition, we found no significant interactions for Sex \times Condition ($F_{(2,150)} = 0.731$; $p = 0.483$; LMM) or Sex \times Age ($F_{(2,150)} = 0.025$; $p = 0.975$; LMM).

PVN OT

Analysis of OT-ir at rostral and caudal levels yielded similar results and interactions, and so are combined here. We observed a main effect of Age ($F_{(2,150)} = 81.612$; $p < 0.001$; LMM; **Fig. S1B**). PND2 pups exhibited significantly fewer OT-ir neurons compared to PND9 (Mean diff. = 119.214; $p < 0.001$) and PND21 pups (Mean diff. = 118.607; $p < 0.001$).

We found no main effect of Sex ($F_{(1,150)} = 0.882$; $p = 0.349$; LMM) or Condition ($F_{(2,150)} = 2.248$; $p = 0.092$; LMM). In addition, we found no significant interactions for Sex \times Condition ($F_{(2,150)} = 0.165$; $p = 0.848$; LMM) or Sex \times Age ($F_{(2,150)} = 1.855$; $p = 0.160$; LMM).

SON VP

We observed a main effect of Age ($F_{(2,152)} = 10.031$; $p < 0.001$; LMM; **Fig. S1C**). Interestingly, PND21 pups exhibited significantly fewer VP-ir neurons compared to PND2 (Mean diff. = 27.348; $p = 0.002$) and PND9 pups (Mean diff. = 32.963; $p < 0.001$), suggesting neuronal pruning or downregulation of peptide production at an age after PND9 and prior to weaning (PND21).

We found no main effect of Sex ($F_{(1,152)} = 0.001$; $p = 0.971$; LMM) or Condition ($F_{(2,152)} = 0.201$; $p = 0.818$; LMM). In addition, we found no significant interactions for Sex \times Condition ($F_{(2,152)} = 2.591$; $p = 0.078$; LMM) or Sex \times Age ($F_{(2,152)} = 0.611$; $p = 0.544$; LMM).

SON OT

We found a main effect of Age ($F_{(2,152)} = 15.955$; $p < 0.001$; LMM; **Fig. S1D**). Posthoc analyses revealed that PND2 pups exhibited significantly fewer OT-ir neurons compared to PND9 (Mean diff. = 30.804; $p < 0.001$) and PND21 pups (Mean diff. = 25.772; $p < 0.001$).

We observed no main effect of Sex ($F_{(1,152)} = 0.382$; $p = 0.538$; LMM) or Condition ($F_{(2,152)} = 0.703$; $p = 0.497$; LMM). In addition, we found no significant interactions for Sex \times Condition ($F_{(2,152)} = 0.910$; $p = 0.405$; LMM) or Sex \times Age ($F_{(2,152)} = 0.018$; $p = 0.982$; LMM).

AH VP

Analyses yielded no main effects of Age ($F_{(1,147)} = 0.478$; $p = 0.621$; LMM; **Fig. S1E**), Sex ($F_{(1,147)} = 1.141$; $p = 0.287$; LMM) or Condition ($F_{(2,147)} = 1.814$; $p = 0.167$; LMM). Furthermore, we did not observe significant interactions for Sex \times Condition ($F_{(2,147)} = 0.472$; $p = 0.625$; LMM) or Sex \times Age ($F_{(2,147)} = 0.510$; $p = 0.602$; LMM).

SCN VP

Analyses yielded no main effects of Age ($F_{(1,148)} = 1.147$; $p = 0.321$; LMM; **Fig. S1G**), Sex ($F_{(1,148)} = 0.728$; $p = 0.395$; LMM) or Condition ($F_{(2,148)} = 3.033$; $p = 0.086$; LMM). Furthermore, we did not observe significant interactions for Sex \times Condition ($F_{(2,148)} = 1.013$; $p = 0.366$; LMM) or Sex \times Age ($F_{(2,148)} = 2.299$; $p = 0.104$; LMM).

MPO OT

We observed a main effect of Age ($F_{(2,154)} = 27.468$; $p < 0.001$; LMM; **Fig. S1F**). PND2 pups exhibited significantly fewer OT-ir neurons compared to PND9 (Mean diff. = 3.972; $p = 0.015$) and PND21 pups (Mean diff. = 10.873; $p < 0.001$). In addition, PND9 exhibited significantly fewer OT-ir neurons compared to PND21 pups (Mean diff. = 6.900; $p < 0.001$).

We found no main effect of Sex ($F_{(1,154)} = 0.627$; $p = 0.430$; LMM) or Condition ($F_{(2,154)} = 2.200$; $p = 0.073$; LMM). In addition, we observed no significant interaction for Sex \times Condition ($F_{(2,154)} = 0.191$; $p = 0.827$; LMM). However, we observed a trending interaction of Sex \times Age ($F_{(2,154)} = 2.950$; $p = 0.055$; LMM). Posthoc analyses revealed that, similar to the main effect of Age above, that female PND2 pups have significantly fewer OT-ir neurons compared to PND9 pups (Mean diff. = 6.861; $p = 0.002$), but that PND2 and PND9 male pups do not have significantly different OT-ir neuron numbers (Mean diff. = 1.083; $p = 1.000$).

BST OT

We found a main effect of Age ($F_{(2,150)} = 17.144$; $p < 0.001$; LMM; **Fig. S1H**). Posthoc analyses revealed that PND2 pups exhibited significantly fewer OT-ir neurons compared to PND9 (Mean diff. = 7.852; $p < 0.001$) and PND21 pups (Mean diff. = 7.708; $p < 0.001$).

We observed no main effect of Sex ($F_{(1,150)} = 2.080$; $p = 0.151$; LMM) or Condition ($F_{(2,150)} = 0.359$; $p = 0.699$; LMM). In addition, we found no significant interactions for Sex \times Condition ($F_{(2,150)} = 0.650$; $p = 0.524$; LMM) or Sex \times Age ($F_{(2,150)} = 1.123$; $p = 0.328$; LMM).

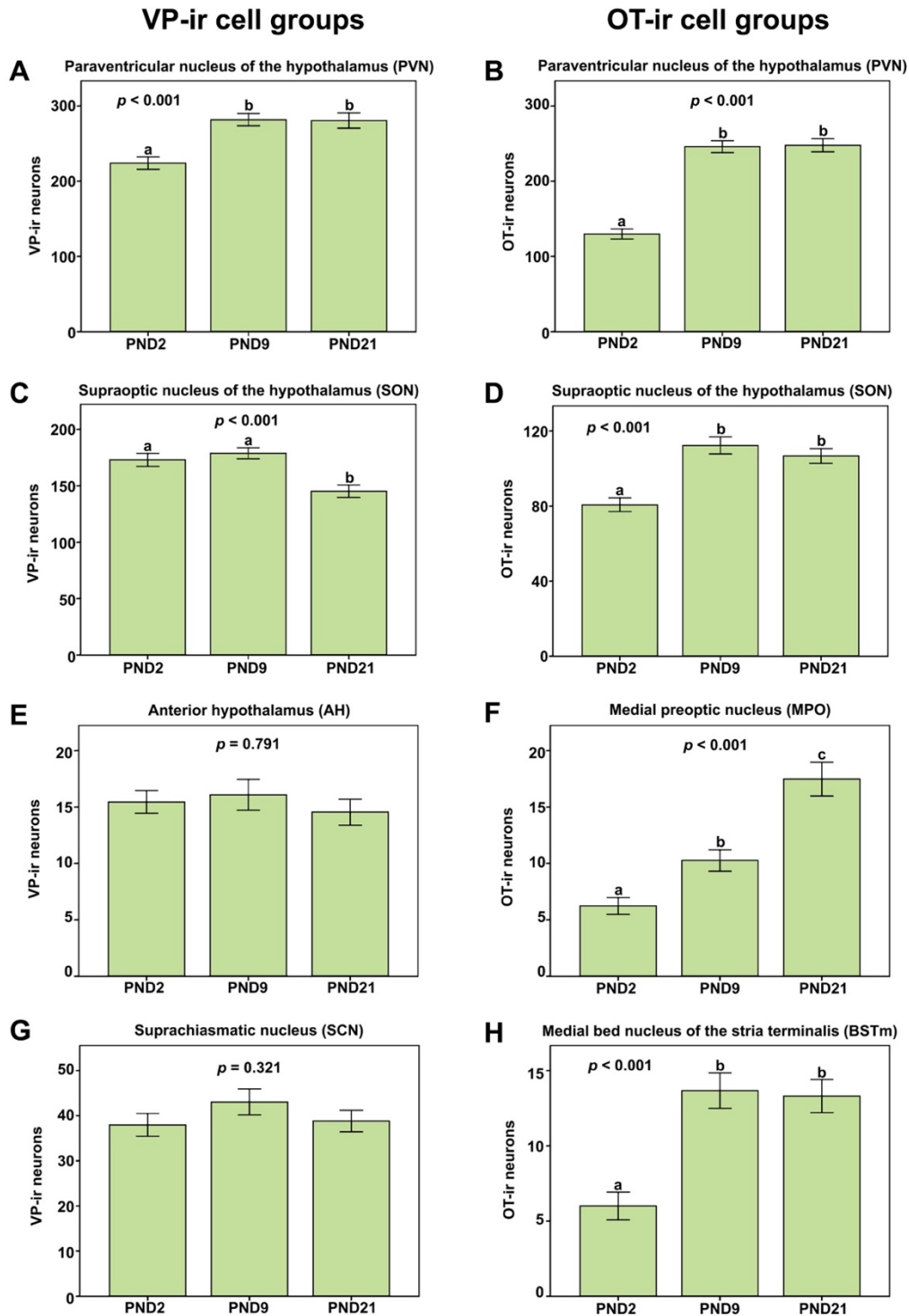


Fig. S1. Development of nonapeptide neuronal cell groups. Mean (\pm SEM) number of vasopressin (VP) and oxytocin (OT) immunoreactive (ir) neurons at different developmental stages (PND2, PND9, and PND21) in the **(A,B)** paraventricular nucleus of the hypothalamus (PVN), **(C,D)** supraoptic nucleus of the hypothalamus (SON), **(E)** anterior hypothalamus (AH), **(F)** medial preoptic nucleus (MPO), **(G)** suprachiasmatic nucleus (SCN), and **(H)** the bed nucleus of the stria terminalis (BST). Letters above graphs (a, b, c) indicate statistical similarity.

Results: Nonapeptide neural activity (Fos colocalization)

Surprisingly, we did not observe a main effect of Condition or a significant Sex × Condition interaction for VP-Fos or OT-Fos co-labeling for any of the VP and OT cell groups examined. These findings mirror the lack of significance for the Age × Condition interaction in the main text. These data suggest that nonapeptide-dependent neural activity, as assessed by Fos co-expression, does not differ based on our social manipulations.

Results: Fos responses

PVN Fos

Analysis of total (rostral and caudal levels combined) PVN Fos-ir yielded a main effect of Condition ($F_{(2,150)} = 3.946$; $p = 0.041$; LMM). However, posthoc analyses did not yield significant differences between groups. In addition, we did not find a Sex × Condition ($F_{(2,150)} = 0.252$; $p = 0.778$; LMM) interaction.

Separate analysis of rostral PVN Fos-ir also yielded a main effect of Condition ($F_{(2,150)} = 3.323$; $p = 0.039$; LMM), with Isolated pups exhibiting a trend to have more Fos-ir than subjects in the Together (Mean diff. = 41.326; $p = 0.058$) condition. We did not observe a Sex × Condition ($F_{(2,150)} = 0.345$; $p = 0.709$; LMM) interaction.

Analysis of caudal PVN Fos-ir did not yield a main effect of Condition ($F_{(2,150)} = 2.759$; $p = 0.066$; LMM) or a significant Sex × Condition interaction ($F_{(2,150)} = 0.157$; $p = 0.855$; LMM).

SON Fos

We did not observe a main effect of Condition ($F_{(2,152)} = 1.418$; $p = 0.245$; LMM) or a significant Sex × Condition interaction ($F_{(2,152)} = 0.087$; $p = 0.917$; LMM) for SON Fos-ir.

AH Fos

Analyses did not yield a main effect of Condition ($F_{(2,147)} = 0.043$; $p = 0.958$; LMM) or a significant Sex × Condition interaction ($F_{(2,147)} = 0.585$; $p = 0.558$; LMM) for AH Fos-ir.

SCN Fos

We did not find a main effect of Condition ($F_{(2,148)} = 0.895$; $p = 0.411$; LMM) or a significant Sex × Condition interaction ($F_{(2,148)} = 0.161$; $p = 0.851$; LMM) for SCN Fos-ir.

MPO Fos

Analyses of MPO Fos-ir revealed a main effect of Condition ($F_{(2,154)} = 6.241$; $p = 0.002$; LMM). Posthoc analyses revealed that subjects in the Together condition exhibited significantly less Fos-ir compared to subjects that were Isolated from (Mean diff. = 25.893; $p = 0.001$) or Reunited with (Mean diff. = 20.511; $p = 0.009$) their families. The Age × Condition interaction presented in the main text suggests that this effect is driven by PND21 pups.

We did not observe a significant Sex × Condition interaction ($F_{(2,154)} = 0.381$; $p = 0.684$; LMM).

BST Fos

We did not find a main effect of Condition ($F_{(2,150)} = 0.369$; $p = 0.692$; LMM) or a significant Sex × Condition interaction ($F_{(2,150)} = 0.412$; $p = 0.663$; LMM) for BST Fos-ir.