**Regulation of hypothalamic CRH expression by augmented maternal care**

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**Abstract**

**Rationale:** Augmented maternal care early in life results in a modified neuroendocrine response to stress, improved cognitive performance, and resilience to depressive symptoms later in life. Corticotropin-releasing hormone (CRH), an important component of the neuroendocrine stress-response, is stably downregulated in the paraventricular nucleus (PVN) of the hypothalamus after augmented maternal care, which might contribute to the resulting phenotype, but the mechanisms of CRH repression are unclear. We previously found reduced glutamatergic input onto PVN CRH neurons, and a stable increase of the transcriptional repressor, neuron restrictive silencing factor (NRSF), following augmented maternal care. Here, we aimed to employ controlled in vitro systems to examine if the decrease in glutamatergic input directly causes the downregulation of CRH mRNA and protein levels, and if NRSF contributes to this repression.

**Methods:** Hypothalamic PVN sections from postnatal day (P) 8-12 rats were cultured in vitro, and treated with ionotropic glutamate receptor antagonists (CNQX and MK-801) or vehicle (H₂O). Oligonucleotides coding for neuron restrictive silencing element (Nrsf) or scrambled sequence were used to disrupt NRSF binding in vitro. CRH mRNA and protein levels were measured using qRT-PCR and immunohistochemistry. NRSF occupancy at CRH gene was determined using chromatin immunoprecipitation (ChIP).

**Results:** Decreased CRH mRNA and protein, and increased binding of NRSF to the Crh gene, were observed in CNQX/MK-801 treated samples relative to controls. Co-treatment with Nrsf largely reversed the decline in Crh expression.

**Conclusion:** The reduction in CRH mRNA and protein upon ionotropic glutamate receptor blockade in vitro supports the idea that the transient decline in glutamatergic input to CRH neurons mediates downregulation of CRH levels following augmented maternal care. NRSF is a direct mediator of this molecular cascade in vitro.

**Summary**

1. Blockade of excitatory neurotransmission is sufficient to decrease CRH mRNA and protein levels.
2. A persistent increase in NRSF protein level is observed after handling in vivo.
3. NRSF directly mediates this reduction in CRH expression in vitro and in vivo.

**Future Directions**

1. Perform ChIP-seq for NRSF to examine the repertoire of genes that are coordinately regulated by augmented maternal care.
2. Determine if increasing NRSF levels in the PVN in vivo improve cognitive performance and resilience to stress of control rats.

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**References**


