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## **Title**

Sex Difference in Calbindin Cell Number in the Mouse Preoptic Area: Effects of Neonatal Estradiol and Bax Gene Deletion

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## Abstract

The sexually dimorphic nucleus of the preoptic area (SDN-POA) was first discovered in rats and is one of the most famous and best studied sex differences in the field of neuroscience. Though well documented in rats (larger in males than females), this sex difference was only recently able to be observed in mice due to the discovery of the protein calbindin-D28k as a marker. Recent studies have shown a larger, more distinct calbindin-immunoreactive (ir) cell cluster in male mice compared to females. However, the exact location of the cluster and whether the sex difference is one of total cell number or cell distribution remains unclear. In this study, we use defined contours to demonstrate that male mice have more calbindin-ir cells than females both in the central cell cluster and areas surrounding the cluster. We also report a full masculinization of these characteristics in females given a single injection of estradiol benzoate (EB) on the day of birth. The potential role of cell death in the development of this sex difference was tested using mice with a deletion of the *bax* gene, which codes for a pro death factor required for the establishment of other sex differences in the mouse brain. We demonstrate that *bax* knockout (KO) mice have more cells in the POA region in general, but eliminating cell death does not affect the development of the sex difference in calbindin-ir cell number, nor does it affect calbindin-ir cell spread. Taken together, this suggests that cell death is not a significant underlying mechanism in the establishment of the sex difference in the calbindin-ir cell cluster in the mouse POA.

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