

# Do adrenal neuroendocrine and steroidal cells express the GluA2 ionotropic glutamate receptor?

Faith Kelley, Dwon Jordana, and Matthew Whim

Department of Cell Biology and Anatomy, LSU Health Sciences Center, New Orleans

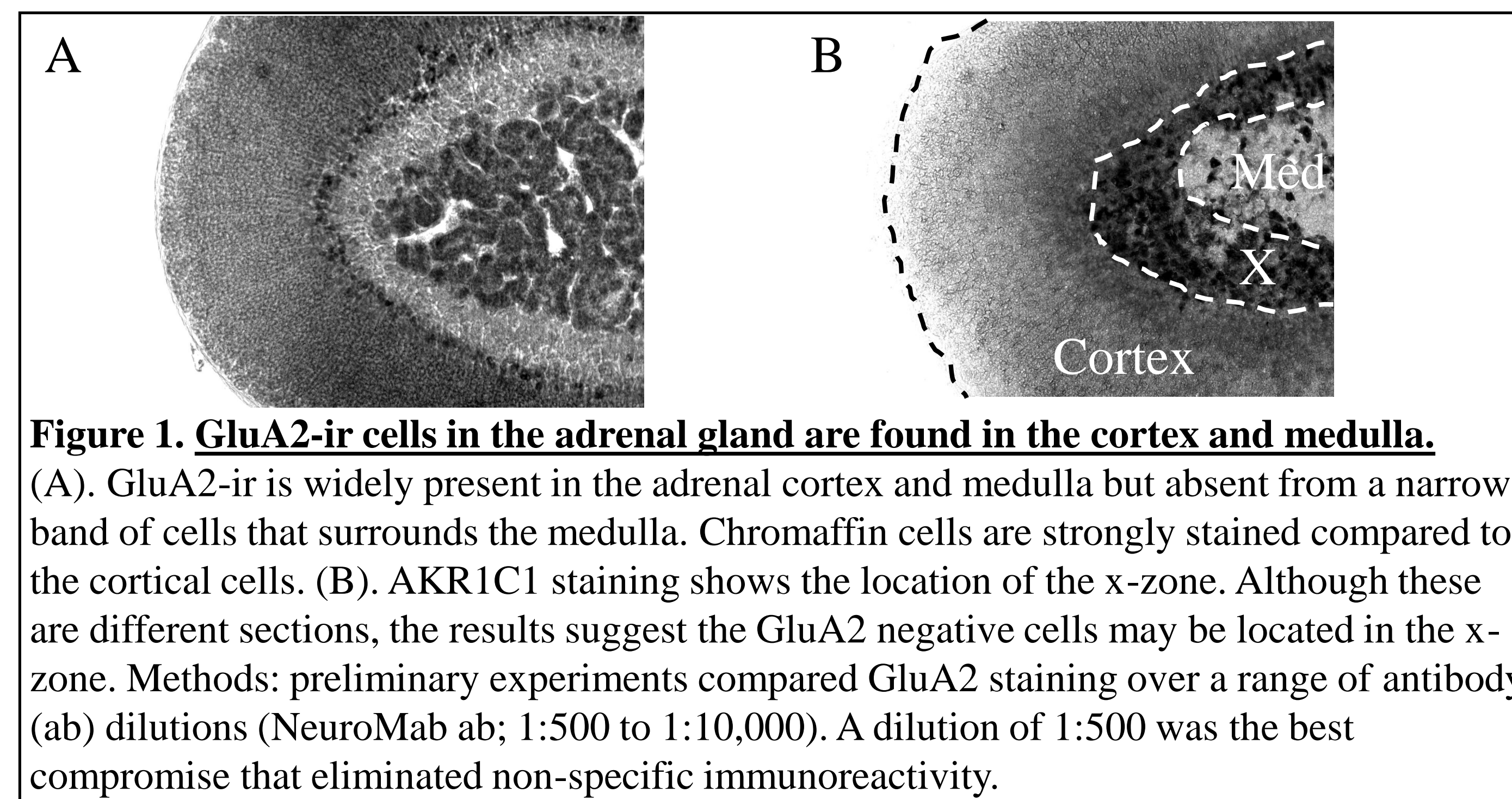
## Introduction

- The adrenal gland is a part of the endocrine system. It has 2 regions. The inner medulla contains neuroendocrine chromaffin cells that are part of the sympathetic nervous system. The outer cortex contains steroid-secreting cells (zona glomerulosa and zona fasciculata). In young male mice, and in female mice that have not been pregnant, the cortex also contains a region called the x-zone.
- GluA2 is an ionotropic receptor that is activated by glutamate, an excitatory neurotransmitter. It is widely expressed in neurons in the central nervous system where it mediates the fast component of synaptic transmission. Several studies have reported the presence of mRNA for multiple glutamate receptors (including GluA2) in the rodent adrenal gland. This is unexpected because glutamate is not thought to be a neurotransmitter in the peripheral (efferent) nervous system. However, the presence of mRNA for GluA2 does not necessarily imply the presence of the GluA2 protein. In order to confirm the presence and location of GluA2 in the adrenal gland, we used immunohistochemistry to stain for GluA2 in the mouse adrenal.
- Our hypothesis was that GluA2 is expressed by chromaffin cells in the medulla or in the preganglionic nerve that innervates the adrenal gland.
- AKR1C1 is a hydroxysteroid dehydrogenase enzyme. It is associated with synthesis of sex steroid hormones and is a specific marker of the x-zone in mice. The function of this transient cortical zone in the mouse adrenal is not known. We also stained for AKR1C1 after noticing a band of GluA2 unstained cells between the medulla and cortex.

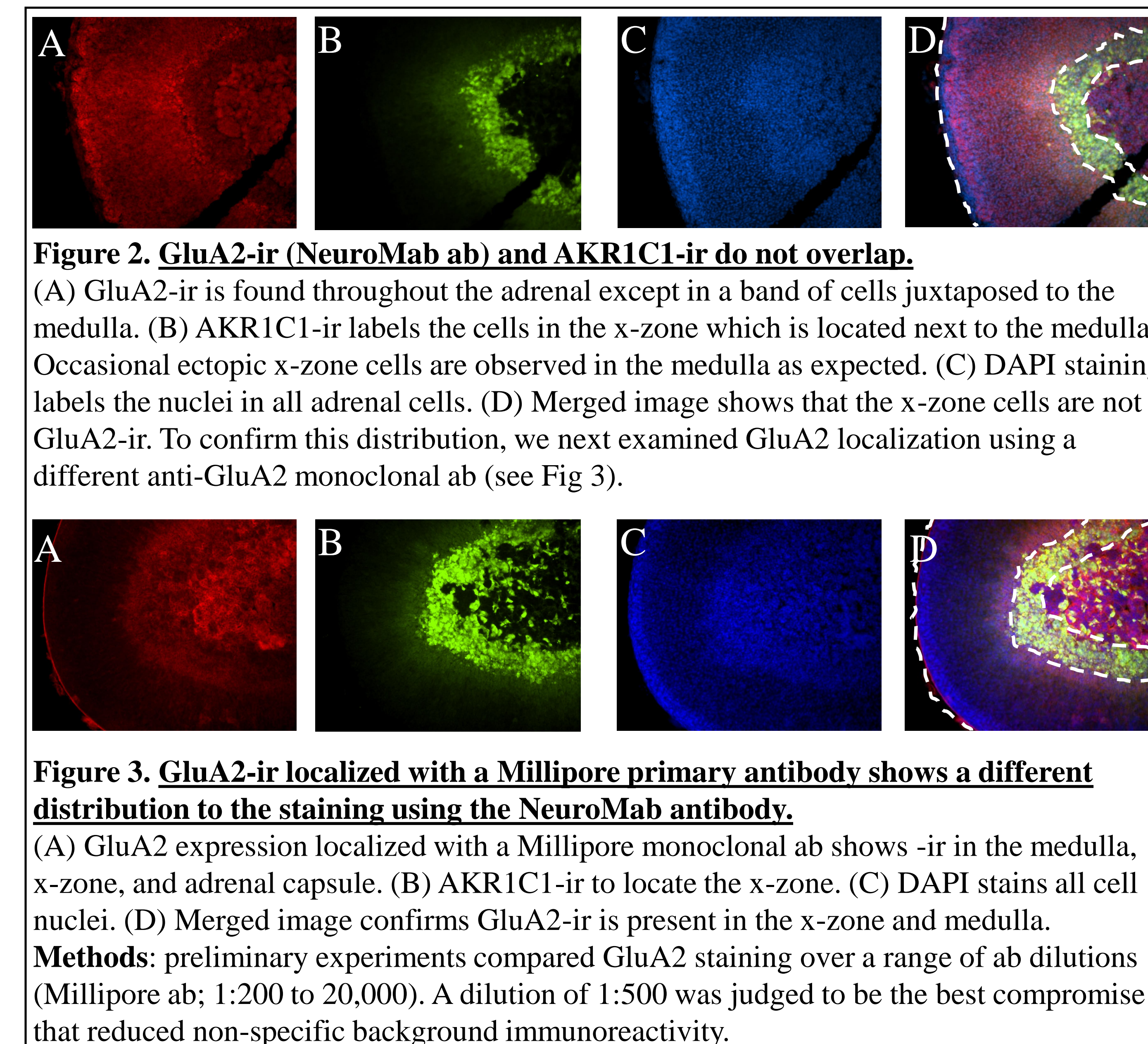
## Methods

- Female and male wild type mice were sacrificed, the adrenal glands were removed and fixed in paraformaldehyde. Cryosections (30  $\mu$ m) from these glands were then prepared for staining.
- When staining for GluA2, two different primary monoclonal antibodies were tested. Figure 1 and Figure 2 used a mouse anti-GluA2 antibody from NeuroMab (1:500). Goat anti-mouse IgG1 HRP was the secondary antibody. Figure 3 used a mouse anti-GluA2 from Millipore (1:500) as a primary antibody (ab) and donkey anti-mouse HRP as the secondary ab.
- To stain the adrenal gland cryosections in Figure 1, sections were incubated in primary antibody overnight at 4°C and then in secondary antibody labeled with HRP for 90 mins at RT. The staining was then visualized using the chromogen diaminobenzidine (DAB).
- In Figures 2 and 3, AKR1C1 expression was examined using a rabbit primary antibody (1:1000). The secondary antibody was donkey anti-rabbit Alexa 488. GluA2-ir (immunoreactivity) in these figures were detected using their respective anti-GluA2 antibodies and TSA amplification with TSA-Cy3.

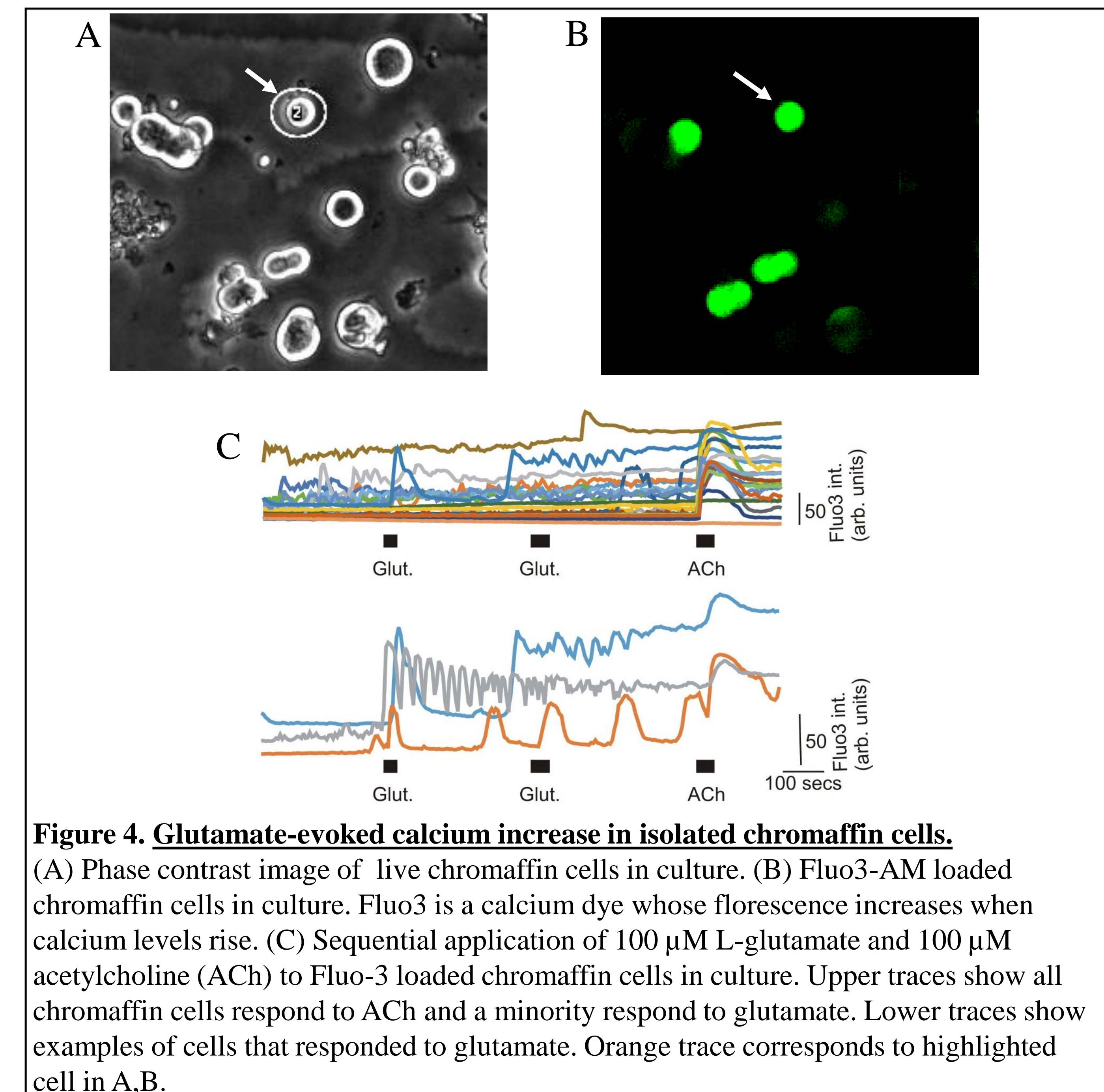
## GluA2-ir is widely distributed in the mouse adrenal gland



## Two Anti-GluA2 Antibodies Have Different Views of GluA2 Distribution



## Chromaffin Cells Respond to Glutamate



## Conclusions

- GluA2-ir was localized to chromaffin cells in the medulla in the mouse adrenal gland.
- Cortical expression of GluA2-ir was inconsistent. The NeuroMab ab showed cortical immunoreactivity *except* in the x-zone. The Millipore ab showed GluA2 expression in the x-zone but *not* in the remainder of the cortex. This illustrates a common problem when using ab's raised against membrane receptors.
- Preliminary experiments using calcium imaging indicate functional glutamate receptors are expressed in chromaffin cells. Whether these are GluA2 receptors is not known. We hypothesize that glutamate receptor activation would lead to the release of catecholamine hormones during the stress response.
- Future studies on GluA2 in the adrenal gland could involve comparing the staining of the adrenal gland with staining in a GluA2 knockout mouse to ensure GluA2 is really the protein being stained. There could also be efforts to determine the function of GluA2 in the adrenal gland.