Preparation of adult mouse brain slices of the hypothalamus and amygdala to investigate the neurophysiological properties of kisspeptin neurons.

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Introduction

Kiss1 neurons in the mammalian brain release the neuropeptide kisspeptin, stimulating gonadotropin-releasing hormone (GnRH) release and activating the hypothalamic–pituitary–gonadal (HPG) axis, which controls fertility and reproduction: pulsatile release of GnRH from the hypothalamus is required for gametogenesis and the production of sex steroid hormones (1). We have recently published that *Kiss1* neurons in the arcuate nucleus from female mice show neuronal plasticity just before the onset of puberty, showing differences between 3 weeks and 7-8 weeks of age in action potential firing and ion channel expression (2). We are also investigating sex differences in the firing properties of amygdala *Kiss1* neurons from adult mice (19 weeks), which are implicated in sexual behaviour. For all of these experiments we use mice bred from heterozygous *Kiss1-Cre* (3) and homozygous tdTomato mice to visualize *Kiss1* neurons. In female mice, estrous cycle stage is determined by cytological examination of vaginal lavages over several days (4).

Procedures

Brains are rapidly removed and submerged in ice-cold sucrose-based solution bubbled with 95% O_2/5% CO_2. We use an ice-cream maker to prepare a slushy sucrose mix, enough to keep topping up the chamber during slicing; we also pre-chill the chamber in the freezer to help keep the slicing solution colder for longer. We trim the brain to remove a lot of surrounding tissue to increase speed at reaching the areas of interest while making slices. The brain tissue block is then glued onto the stainless steel platform of a Campden 7000smz Vibrating Microtome (Campden Instruments, UK) that is held very tightly in the slicing chamber by a magnet. We cut sections through the parts of the brain we don't need relatively quickly (0.2mm/sec) but through the regions of interest at much slower speeds (0.05mm/sec). We use the standard steel Campden blades, which we change every 1-2 weeks depending on usage, and re-calibrate each time: a particularly nice feature of this vibratome is that you can follow the guide to minimise the degree of up/down movement of the vibrating blade, reducing the amount of tissue damage as it moves through the brain tissue. Our coronal slices through the hypothalamus of adult male or female mice are 250-300 microns thick; for adult mouse amygdala kisspeptin neurons we use 180 micron slices because of the sparsity of neurons, particularly in female mice. Slices are transferred to an incubation chamber containing artificial cerebrospinal fluid at 30 °C, saturated with 95% O_2 and 5% CO_2, and

left undisturbed for at least 60 minutes prior to experiments.

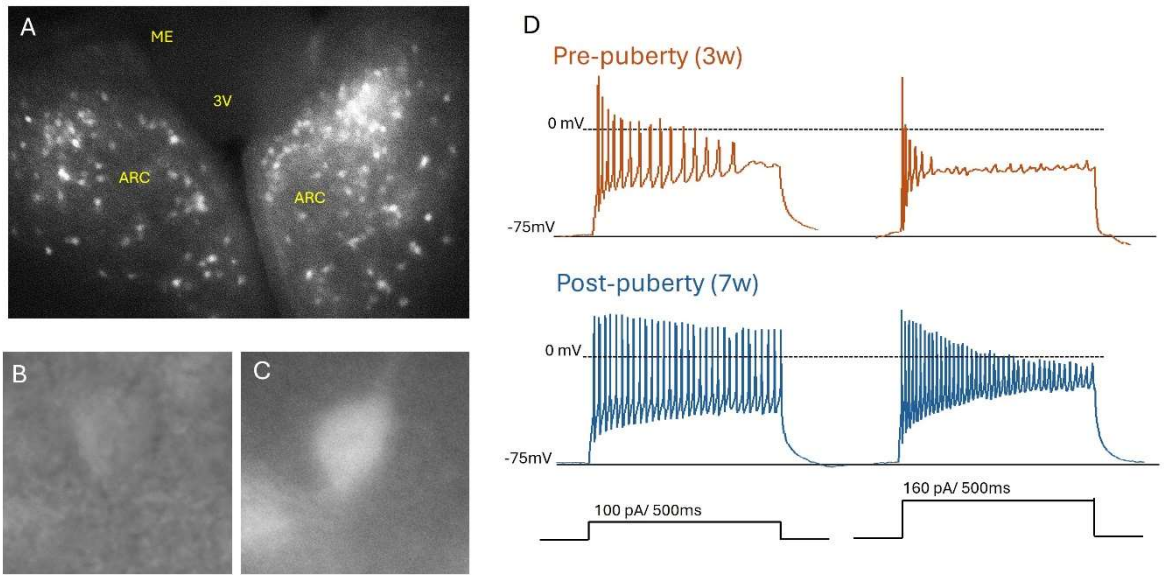

Figure 1 A. Brain slice of adult mouse hypothalamus through the arcuate nucleus (250 microns) with a low power (4x lens) and green light to reveal tdTomato-expressing *Kiss1* neurons. B. High power (40x lens) light image of an arcuate neuron. C. Same cell as B in green light. D. Changes in evoked action potential firing in arcuate *Kiss1* neurons of female mice at puberty (reported by Zhang *et al.*, 2025).

Specific examples

We are able to obtain brain slices from adult mice that have a good number of healthy tdTomato-positive neurons for whole-cell patch-clamp recordings both in the arcuate nucleus (Figure 1A-C) and in the amygdala. For whole-cell patch-clamp recordings of action potential firing (Figure 1D), we use a K^+-Gluconate-based intracellular pipette solution (2). We determine resting membrane potential at the start of each experiment and use a 5mV/ 10ms test pulse throughout the experiment to monitor the stability of recordings by measuring series resistance and membrane resistance. We also use freshly prepared slices made this way for isolating *Kiss1* neurons to generate pools of cells for qPCR (2)

References

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