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Research paper

Developmental expression of Kv1 voltage-gated potassium channels in the avian hypothalamus

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HIGHLIGHTS

- Avian hypothalamic neurons demonstrate a developmental increase in outward current.
- E12 hypothalamic neurons exhibit robust phasic action potentials compared to E8.
- Kv1.2, Kv1.3, and Kv1.5 mRNA is present in embryonic avian hypothalamic neurons.
- Increase in Kv1.2 and Kv1.5 likely contributes to electrophysiological changes.

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ABSTRACT

Specialized hypothalamic neurons integrate the homeostatic balance between food intake and energy expenditure, processes that may become dysregulated during the development of diabetes, obesity, and other metabolic disorders. Shaker family voltage-gated potassium channels (Kv1) contribute to the maintenance of resting membrane potential, action potential characteristics, and neurotransmitter release in many populations of neurons, although hypothalamic Kv1 channel expression has been largely unexplored. Whole-cell patch clamp recordings from avian hypothalamic brain slices demonstrate a developmental shift in the electrophysiological properties of avian arcuate nucleus neurons, identifying an increase in outward ionic current that corresponds with action potential maturation. Additionally, RT-PCR experiments identified the early expression of Kv1.2, Kv1.3, and Kv1.5 mRNA in the embryonic avian hypothalamus, suggesting that these channels may underlie the electrophysiological changes observed in these neurons. Real-time quantitative PCR analysis on intact microdissections of embryonic hypothalamic tissue revealed a concomitant increase in Kv1.2 and Kv1.5 gene expression at key electrophysiological time points during development. This study is the first to demonstrate hypothalamic mRNA expression of Kv1 channels in developing avian embryos and may suggest a role for voltage-gated ion channel regulation in the physiological patterning of embryonic hypothalamic circuits governing energy homeostasis.

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1. Introduction

Shaker family voltage-gated potassium channels (Kv1) are major determinants of membrane excitability in many types of neurons [1,2], contributing to the maintenance of resting membrane potential [3], action potential repolarization [4], interspike interval [5], and neurotransmitter release [6]. Variable expression of Kv1 channels in the plasma membrane of neurons can alter neuronal excitability [7–10], thus altering the activity of certain brain regions. Aberrant neuronal excitability during the developmental programming of neuronal circuits may alter the long-term physiological behavior of an organism [11]. Thus, it is important to understand whether Kv1 channels contribute to the electrophysiological events that occur during gestational development.

The hypothalamus is a brain region that mediates the regulation of critical metabolic processes throughout the body [12,13]. For example, prenatal temperatures have been shown to influence postnatal hypothalamic thermosensitivity in birds [14], underscoring the importance of the gestational environment on the long

Abbreviations: Kv1, Shaker family voltage-gated potassium channel; E8, embryonic day 8; E12, embryonic day 12; aCSF, artificial cerebrospinal fluid; Opt, optic tract; 3v, third ventricle; ARC, arcuate nucleus; VMH, ventromedial hypothalamus; ME, median eminence; Elec, electrode.

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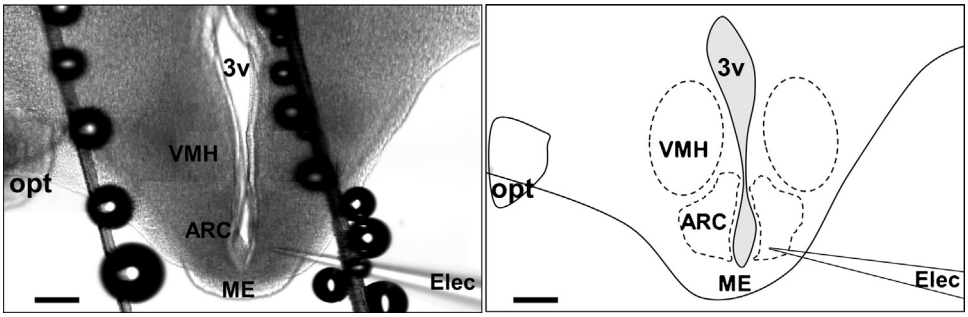

Fig. 1. Embryonic avian hypothalamic slice cultures for electrophysiological recordings.
Representative photomicrograph of an E12 chicken coronal brain slice affixed to the electrophysiological recording chamber (left). Corresponding regions of interest as adapted from an avian neuroanatomy atlas of a 2-week old chicken brain (right) [34]. Opt, optic tract; 3 v, third ventricle; ARC, arcuate nucleus; VMH, ventromedial hypothalamus; ME, median eminence; Elec, electrode. (Scale bar = 200 μm).

Table 1
Forward and reverse qPCR primer sequences.

mRNA	Forward primer sequence	Reverse primer sequence	Annealing temp.	Amplicon size
Kv1.2	5′-GGA AGA AGC CAT GGA GAT GT-3′	5′-GCA ACC AGA CCT GTC TCT GA-3′	59 °C	98 bp
Kv1.3	5′-CAG GGA TGA CCA CGA CTA TG-3′	5′-AAC AGC AGA GGA CGA GGA AT-3′	59 °C	100 bp
Kv1.5	5′-CAG GTC TGG CTC ATC TTT GA-3′	5′-GCA GAA GGT GAT GAT GGA GA-3′	59 °C	102 bp
β-actin	5′-GCG CAA GTA TTC TGT CTG GA-3′	5′-GGG GCC AGA CTC ATC ATA CT-3′	59 °C	100 bp

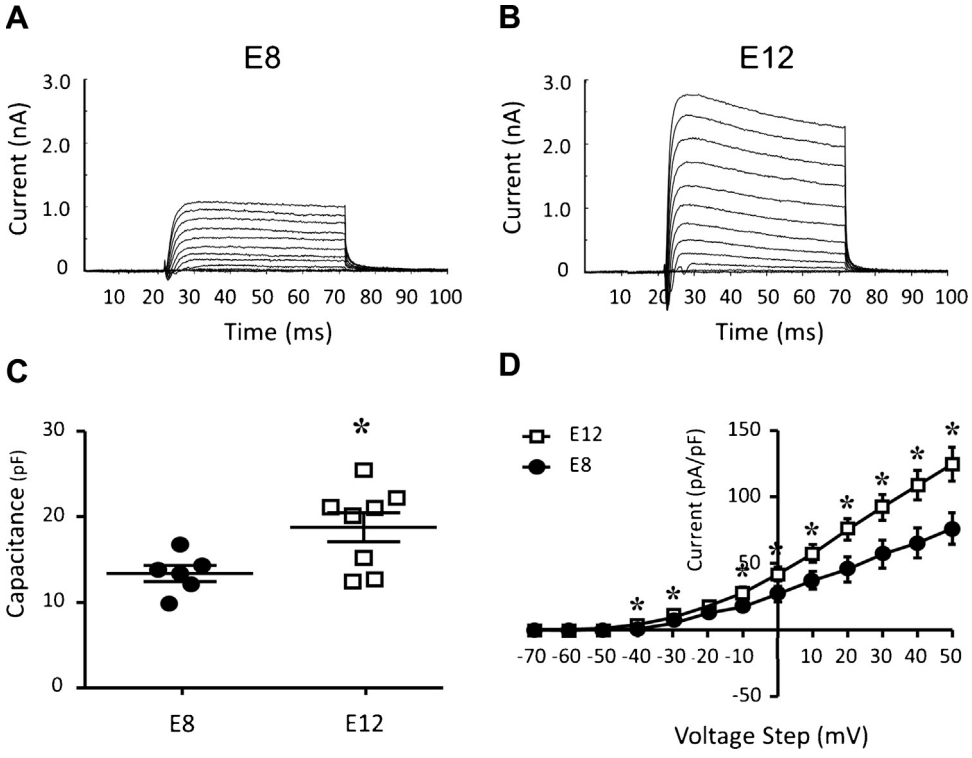

Fig. 2. Developmental changes in outward current recorded from avian hypothalamic neurons.
Average voltage-clamp recordings from neurons of E8 (A; n = 6) and E12 (B; n = 8) hypothalamic brain slices. Membrane capacitance was estimated by calculating the integral of the initial capacitance transient in response to a 10 mV hyperpolarizing pulse (C). Capacitance compensated steady state current-voltage relationships (D) demonstrate a significant developmental increase in outward currents recorded from hypothalamic neurons (*p < 0.05).

term physiology of the organism. Despite recent interest in the influence of prenatal factors on hypothalamic development and consequent adult metabolism, little remains known about the electrophysiological development of hypothalamic neurons [15–17]. Specialized neurons in the arcuate nucleus (ARC) of the hypothalamus can integrate the homeostatic balance between food intake and energy expenditure via peripheral signaling, mechanisms that are well conserved between mammals and birds [18,19]. Therefore, understanding the developmental expression and function of Kv1 channels in the arcuate nucleus of the avian hypothalamus may provide insight into the early patterning of electrophysiological activity in these neurons.

The Kv1 family of voltage-gated potassium channels is composed of eight distinct genes that code for separate alpha subunit proteins, Kv1.1 through Kv1.8 [20]. Homomultimeric or heteromultimeric assembly of these subunits through a T1 tetramerization

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