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MATURATION OF FIRING PATTERN IN CHICK VESTIBULAR NUCLEUS NEURONS

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Abstract—The principal cells of the chick tangential nucleus are vestibular nucleus neurons participating in the vestibuloocular and vestibulocollic reflexes. In birds and mammals, spontaneous and stimulus-evoked firing of action potentials is essential for vestibular nucleus neurons to generate mature vestibular reflex activity. The emergence of spike-firing pattern and the underlying ion channels were studied in morphologically-identified principal cells using whole-cell patch-clamp recordings from brain slices of late-term embryos (embryonic day 16) and hatchling chickens (hatching day 1 and hatching day 5). Spontaneous spike activity emerged around the perinatal period, since at embryonic day 16 none of the principal cells generated spontaneous action potentials. However, at hatching day 1, 50% of the cells fired spontaneously (range, 3 to 32 spikes/s), which depended on synaptic transmission in most cells. By hatching day 5, 80% of the principal cells could fire action potentials spontaneously (range, 5 to 80 spikes/s), and this activity was independent of synaptic transmission and showed faster kinetics than at hatching day 1. Repetitive firing in response to depolarizing pulses appeared in the principal cells starting around embryonic day 16, when <20% of the neurons fired repetitively. However, almost 90% of the principal cells exhibited repetitive firing on depolarization at hatching day 1, and 100% by hatching day 5. From embryonic day 16 to hatching day 5, the gain for evoked spike firing increased almost 10-fold. At hatching day 5, a persistent sodium channel was essential for the generation of spontaneous spike activity, while a small conductance, calcium-dependent potassium current modulated both the spontaneous and evoked spike firing activity. Altogether, these *in vitro* studies showed that during the perinatal period, the principal cells switched from displaying no spontaneous spike activity at resting membrane potential and generating one spike on depolarization to the tonic firing of spontaneous and evoked action potentials.

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Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, afterhyperpolarization; AP-5, DL-2-amino-5-phosphovaleric acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CV, the coefficient of variation; E, embryonic day; EPSC, excitatory postsynaptic current; H, hatching day; I_A, transient potassium current; I_{DS}, dendrotoxin-sensitive potassium current; Ih, hyperpolarization-activated cationic current; I_k, delayed-rectifier potassium current; I_{KCa}, calcium-dependent potassium current; I_{NaP}, persistent sodium current; ISI, interspike interval; $KMeSO_4$, potassium methylsulfate; MVN, medial vestibular nucleus; OKR, optokinetic reflex; P, postnatal day; RMP, resting membrane potential; SK, small conductance, calcium-dependent potassium current; TTX, tetrodotoxin; VCR, vestibulocollic reflex; VNN, vestibular nucleus neuron; VOR, vestibuloocular reflex.

Key words: intrinsic membrane properties, spike activity, brain slice.

The vestibuloocular (VOR) and optokinetic (OKR) reflexes are involved in stabilizing visual gaze during head movements, whereas the vestibulocollic (VCR) reflex stabilizes the head position in space. To perform these functions, the VOR generates compensatory eye movements in a direction opposite to the head movements, while the OKR produces eye movements in the same direction as the image motion, and the VCR stimulates the muscles of the neck to support the head during movements. Defined as the eye speed divided by head speed, the VOR gain is an important property, which is subject to change during different types of movements. For example, during natural head rotations, the gain of compensatory eye movements is larger than 1, but when there is image motion during head turning, the gain is smaller than 1. Thus, the VOR is an adaptive response, rather than an automatic reflex (for review, see Collewijn, 1989). Compensatory eye movements require the integration of vestibular, visual, proprioceptive and other behaviorally relevant signals at the level of the vestibular nucleus neuron (VNN). Due to the adaptive capabilities of VNNs, extensive *in vitro* electrophysiological studies have been performed to understand their firing pattern in adult animals of different species (e.g. rat: Johnston et al., 1994; chick: du Lac and Lisberger, 1995; guinea-pig: Serafin et al., 1991).

So far, studies on the maturation of VNN firing pattern have focused on neurons from the medial vestibular nucleus (MVN) in rat and mouse, and also on the principal cells of the chick tangential nucleus (Peusner and Giaume, 1997; Gamkrelidze et al., 1998) (for review, see Straka et al., 2005). In rat and mouse, VNNs showed significant changes in their spontaneous and evoked firing pattern around postnatal days 14–15 (P14–P15), when the eyes opened and visual stimuli were first detected (Johnston and Dutia, 1996; Murphy and du Lac, 2001). Firing pattern in VNNs is also subject to change in response to unilateral loss of peripheral vestibular input. In humans and animals, severe symptoms of the lesion subsided within about a week, a process known as "vestibular compensation" (Aldrich and Peusner 2002). During compensation, changes in the spontaneous and evoked-spike firing patterns were detected in both the ipsi- and contralateral VNN (Darlington et al., 2002). In the developmental and compensation studies performed on mammals, the recordings were combined from different neuron classes, so that information processing by identified classes of VNNs was not determined, as done in the auditory system (e.g. Rothman and

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Manis, 2003). Accordingly, the chick tangential nucleus was selected for study here, since its principal cells offer the opportunity to study a large number of morphologically-similar neurons. In the present study, the recorded neurons were injected with biocytin to insure that the recordings were obtained only from the principal cells.

Located in the lateral medulla oblongata, the chick tangential nucleus is the interstitial nucleus of the vestibular nerve in avians. The nucleus is dominated by its principal cells, which comprise 80% of the neuron population (Peusner and Morest, 1977). The principal cells receive synaptic terminals from the large and small diameter primary vestibular fibers, cerebellum, cervical spinal cord, and contralateral vestibular nuclei (Cox and Peusner, 1990a,b). The axons of the principal cells project to four major neuronal targets: oculomotor, trochlear and abducens nuclei, and motor neurons in the high cervical spinal cord, resulting in their classification as vestibulooculocollic neurons (Cox and Peusner, 1990a). In contrast to the mammalian interstitial nucleus of the vestibular nerve (Brodal and Brodal, 1985; Langer et al., 1985), the axons of the principal cells do not project to the cerebellum. Thus, according to their afferent and efferent connections, the chick tangential nucleus most closely resembles the lateral vestibular nucleus in mammals (see Discussion).

Unlike altricial mammals, the precocial chicken undergoes rapid vestibular development during the perinatal period (Peusner and Giaume, 1997). At embryonic day 16 (E16), the embryo is confined to the eggshell, and lacks vision (Wallman et al., 1982). During the first 24 h after hatching (H0), the chickens' righting reflexes were absent (Heaton, 1975), but after 24 h (H1), the chickens could stand erect, walk, feed and drink (Decker, 1970). Perching was first observed in 4-to-5 day old hatchlings (H4–H5) (Heaton, 1975). Accordingly, the ages selected for study here were E16, H1 and H5.

Using the whole-cell patch-clamp technique, the spontaneous spike activity of the principal cells was recorded at resting membrane potential (RMP). Stimulus-evoked spike activity was induced by injecting 400 ms duration depolarizing currents at different intensities. Finally, since at H5 the spontaneous spike activity was mediated by intrinsic membrane conductances, a persistent sodium current (I_{NaP}), hyperpolarization-activated cationic current (Ih), and small conductance, calcium-dependent potassium current (SK) were investigated due to their important roles in spike activity in other neurons (e.g. de Waele et al., 1993; Johnston et al., 1994; Bennett et al., 2000; Smith et al., 2002).

EXPERIMENTAL PROCEDURES

Experimental animals

Experiments were performed on E16, and H1 and H5 chickens (*Gallus gallus*), obtained from CBT Farms (Chesterton, MD, USA). The animal protocols were approved by the Institution Animal Care and Use Committee of the George Washington University. All experiments conformed to international guidelines on the ethical treatment of animals. All efforts were made to minimize the number of animals used and their suffering. The age of the embryos was established by referring to the staging criteria of Hamburger and Hamilton (1951). The age of the hatchlings was determined by counting the day of birth as 0 days, and 24 h afterward as a 1 day old hatchling, H1.

Brain slice preparation and solutions

At E16, transverse sections (300 μm) of the medulla oblongata were cut in cold, artificial cerebrospinal fluid (ACSF) (4 °C) using a microslicer (VT1000S; Leica Instruments, Bannockburn, IL, USA) and razor blades (Feather blue blades; Ted Pella Inc., Redding, CA, USA). The embryonic slices recovered for 1 h in ACSF at room temperature (21–22 °C) in a Petri dish before they were transferred to the recording chamber. Transverse sections (240 μm) of the hatchling brainstem were cut in sucrose ACSF (1–4 °C) using a sapphire knife (Delaware Diamond Knives, Wilmington, DE, USA). After sectioning, the hatchling slices were incubated in ACSF (37 °C) for 40 min, and then maintained at 21–22 °C for at least 20 min before transferring to the recording chamber. Preheated ACSF was superfused through the recording chamber (volume, 180 μl; Warner Instruments, Hamden, CT, USA) at a rate of 2–3 ml/min, while the ACSF in the recording chamber was maintained at 30–31 °C using a temperature controller (TC324B, Warner Instruments). Sucrose ACSF contained (in mM): 210 sucrose, 2.5 KCl, 7 $MgSO_4$, 1.5 NaH_2PO_4, 1 $CaCl_2$, 26 $NaHCO_3$, and 10 D-glucose. ACSF contained (in mM): 126 NaCl, 3.5 KCl, 1.2 NaH_2PO_4, 1.3 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 D-glucose. Both sucrose ACSF and ACSF were bubbled with 95% O_2/5% CO_2 to maintain the pH at 7.2–7.4. The osmolarity was 310–320 mOsm.

All of the drugs, except CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), were dissolved in ACSF and then added to the bath ACSF to achieve the final concentration. The drugs included tetrodotoxin (TTX; 1 μM; RBI, Natick, MA, USA) to block the voltage-dependent sodium currents, including the fast sodium current, I_{Na}, and the slow, $I_{NaP;}$ DL-2-amino-5-phosphovaleric acid (AP-5; 30 μM; Sigma, St. Louis, MO, USA) to block the glutamatergic *N*-methyl-D-aspartate (NMDA) receptor-mediated currents; strychnine (1 μM; Sigma) to block the glycine receptor-mediated currents; bicuculline methochloride (10 μM; Tocris, St. Louis, MO, USA) to block the $GABA_A$ receptor-mediated currents; and apamin (100 nM; Alomone) to block the SK; cesium chloride (CsCl; 3 mM; Sigma) to block the Ih. CNQX (10 μM; RBI) was used to block the glutamatergic, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated currents by dissolving the compound in dimethyl sulfoxide (DMSO; Fisher Scientific, Fair Lawn, NJ, USA) at a concentration of 20 mM, which was added to the bath ACSF to achieve the final concentration.

Electrophysiology

Slices were viewed on a fixed-stage, upright microscope (Zeiss Axioskop, FS-1, Jena, Germany) equipped with differential interference contrast optics and a 40× water-immersion lens (NA, 0.75). Visualization of the recorded neuron and pipet was achieved using an infrared light source (filter, 770 nm), which was detected by an infrared-sensitive tube camera (Vidicon C2400-01, Hamamatsu, Hamamatsu City, Japan) and observed on a monitor (Sony). A 4× lens was inserted between the microscope and camera, and the image contrast and shading were adjusted with a camera controller (C2400, Hamamatsu).

Microelectrodes (2–5 MΩ) were pulled from borosilicate glass tube (World Precision Instruments, Sarasota, FL, USA) using a Brown/Flaming horizontal puller (P-87, Sutter Instruments, Novartis, CA, USA). KCl (n=12) and potassium methylsulfate ($KMeSO_4$) (n=12) pipet solutions were used to record from the E16 principal cells, with no differences detected in the RMPs or kinetics for the evoked action potentials with either pipet solution. Therefore, all of the data acquired at E16 were pooled for analysis. All of the