Dabbling with Piezo2 for mechanosensation

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Nature has devised various strategies for sensing food so species can coexist and exploit different types of prey. Sharks can detect amino acids in blood as low as 1 ppb, while hawks and buzzards scan the Earth from a height of ∼10,000 feet looking for rodents. However, not all birds rely on their keen sense of sight to acquire food. Albatross hover above the water to smell floating food and foraging ducks utilize sense of touch to capture insects beneath the water. In PNAS, Schneider et al. (1) examine the molecular alterations in vertebrates that lead to diverse types of feeding behavior. They study foraging birds that rely on distinct sensory input: visual (chickens) vs. tactile (ducks and geese). Dabbling ducks utilize their soft bills/beaks to sense food without visual or olfactory cues just like primates can feel touch through fingertips.

Sense of touch is primitive and crucial for survival but still the least-understood sensory modality in the vertebrates. Significant efforts are underway to understand mechanosensation at a cellular and molecular level (2). The hairless skin of mammals detects touch and vibration via specialized corpuscles (Meissner and Pacinian) innervated by rapidly adapting mechanoreceptors. In ducks, the tactile information is carried out by Herbst and Grandry corpuscles located in the glabrous skin of bills, tongue, and oral cavity. In a previous study, the authors established that adult duck's trigeminal neurons produce robust mechanically activated electrical responses in vitro. In addition, trigeminal ganglia from several species of adult tactile foraging birds contain significantly large-diameter cells, which suggested but did not prove the presence of large mechanoreceptor populations (3).

The focus of the current study by Schneider et al. is to build on previous work by characterizing molecular and cellular mechanisms in the bill and trigeminal system of ducks. Another aspect is to decipher molecular alterations between tactile and visual foraging birds. The authors picked late-stage duck embryos as a model system since their development is largely complete in ovo and easily accessible to experimental manipulation. They performed ex vivo techniques to stimulate the bill and record electrical activity from the intact neurons within trigeminal ganglia. Immunostaining of duck's embryo and electrophysiological analysis confirmed that rapidly adapting mechanoreceptors in duck bill are functional well before hatching.

It has been shown that the neurotrophic factor receptor TrkA underlies the development of most thermoreceptors and nociceptors, while TrkB is responsible for mechanoreceptors (4). Schneider et al. analyzed the expression of TrkA and TrkB in the trigeminal of ducks and chicken and compared it to the reported quantifications in chickens (5) mice and rats (6, 7). Both embryonic and adult duck neurons express significantly higher TrkB (67%) compared with TrkA (7%) (Table 1). Therefore, unlike mice or chickens that do not require tactile methods to find food, duck neurons are programmed to develop more mechanoreceptors than nociceptors and thermoreceptors.

To complement the results of TrkB abundance and mechanoreceptors, the authors focused on expression of mechanotransduction channel Piezo2 (8). Since its discovery in 2010, Piezo2 has been established to detect light touch (9–11), proprioception (12), and lung inflation (13) in mouse models partly based on its robust expression in sensory neurons. Indeed, mechanically gated Piezo2 channels are found in 69% of duck trigeminal neurons, while nociceptors and thermoreceptors TRPV1 and TRPM8 were expressed in 16% and 2% of the cells (Table 1). On the contrary, in chicken trigeminal neurons, Piezo2, TRPV1, and TRPM8 were present in 35%, 37%, and 10% of the cells, respectively, distributions similar to those found in mice (14).

What Are the Key Differences in Mechanoreceptors of Tactile vs. Visual Foraging Birds?

Using electrophysiological approaches, Schneider et al. recorded activity of dissociated trigeminal neurons in response to direct stimulation of the cellular somata with a glass probe. Based on the inactivation property of ion channels—where channels become dormant despite the applied stimulus—mechanically stimulated

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Table 1: Comparison of duck and chicken trigeminal neurons

Foraging birds	Feeding behavior	Mechanosensitive neurons, %	Piezo2 expression, %	TrkB expression, %
Ducks	Tactile	66	69	67
Chickens	Visual	20	35	27

The summary of various factors leading to higher mechanoreceptor population in ducks than in chickens. Higher percentage of mechanosensitive trigeminal neurons along with the increased expression of mechanically activated channel Piezo2 and neurotrophic factor receptor TrkB specify that ducks are predisposed to sense-of-touch–driven feeding behavior.

currents are classified as fast, intermediately, and slowly inactivating (15, 16). Both species exhibit all three types of current responses with distinct features: (*i*) 66% of duck neurons produced mechanically activated currents and only 20% of chicken were mechanosensitive; (*ii*) duck neurons respond to lower thresholds of cell indentation compared with chicken and exhibit elevated current density in cells; and (*iii*) the larger fraction of mechanosensitive currents in ducks fall under the category of slowly inactivating (47%) where the decay time constant is >30 ms. These results suggest that a higher percent of mechanoreceptors in duck are capable of transmitting light touch into electrical responses, where slow inactivation current can convert touch into excitation.

Does Piezo2 Contribute to the Mechanosensitivity of Duck Trigeminal Neurons?

To address this question, authors used fluorescently labeled siRNA designed to target a specific region in Piezo2 (17). Dissociated trigeminal neurons treated with siRNA against Piezo2 decreased mRNA and protein production and increased the fraction of nonresponder (i.e., neurons that do not generate electrical signals upon mechanical stimulation). These mechano-insensitive neurons are more likely to have Piezo2 as the major mechanosensitive channel. Despite Piezo2's knockdown, some cells exhibited mechanically induced currents. There are two distinct possibilities: either inefficient Piezo2 knockdown in these cells or another channel capable of carrying out mechanically induced currents in Piezo2-deficient neurons. Hence, the duck embryo could serve as the model system in the quest for additional mechanically activated channels which carry out the conversion of tactile forces into electrical signals.

Molecular Basis of Touch Sensation: Comparison of Duck and Mouse Piezo2

In mouse somatosensory neurons, Piezo2 reduction by siRNA (8, 17) or via conditional knockout (12) eliminates only fast inactivating currents where the decay constant is <10 ms, suggesting that the other two types of currents are Piezo2 independent. However, in duck trigeminal neurons, Piezo2 largely contributes to the generation of intermediate and slowly inactivating current with prolonged decay constant of >10 ms. These interesting results led the authors to compare the functional properties of mouse and duck Piezo2 in a heterologous expression system. Mouse and duck Piezo2 exhibit similar characteristics when transfected in HEK293T cells, but one key difference stands out: duck Piezo2 has slower inactivation kinetics ($\tau_{inactivation}$ = 5 ms) than mouse channels ($\tau_{inactivation}$ = 3 ms). This difference becomes larger at depolarizing potentials, where the inactivation rate of duck Piezo2 converts from fast ($\tau_{inactivation}$ = 5 ms) to slow ($\tau_{inactivation}$ > 30 ms). These findings suggest that duck Piezo2 can serve multiple purposes in somatosensory neurons based on voltage-dependent inactivation kinetics.

How could Piezo2 contribute to more than one type of mechanosensitive current in the same cell? In principle, any factor that can change the inactivation kinetics of Piezo2 can convert the channel from fast to slow inactivating gating modes. Various components of dynamic cell membrane such as lipid rafts, incorporation of signaling lipids, amphipathic molecules, or endogenous modulators of Piezos can affect inactivation kinetics. Unlike other cationic channels such as K^+, Na^+, Ca^{2+}, TRP, or two pore domain potassium channels, there are only two-family members of mechanically activated Piezos. It is not surprising that various cellular components can exploit the kinetics of Piezo channels to exhibit more than one type of response in the same cell. The inactivation of Piezo2 and its homolog Piezo1 is also of immense interest in human pathophysiology (18, 19). Most documented Piezo-related disorders are gain-of-function mutations that largely affect inactivation kinetics, raising the possibility that structural and functional domains or simple point mutations in Piezos exist to produce a spectrum of mechanosensitive molecules.

The findings by Schneider et al. extend our mechanistic understanding of tactile forces and feeding behavior. The high density of the corpuscles in the bill skin combined with histological and electrophysiological data argues that the majority of duck trigeminal neurons are mechanoreceptors. This study advances the fact that mechanoreceptor expansion in trigeminal ganglia is not a universal feature in birds, but specially designed for the ones that rely on touch sensation to find food. The results strongly suggest that Piezo2 stands out as a key evolutionary mediator in vertebrate neuronal mechanosensation.

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