Molecular basis of tactile specialization in the duck bill

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Edited by Joseph S. Takahashi, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX, and approved October 10, 2017 (received for review May 26, 2017)

Tactile-foraging ducks are specialist birds known for their touch-dependent feeding behavior. They use dabbling, straining, and filtering to find edible matter in murky water, relying on the sense of touch in their bill. Here, we present the molecular characterization of embryonic duck bill, which we show contains a high density of mechanosensory corpuscles innervated by functional rapidly adapting trigeminal afferents. In contrast to chicken, a visually foraging bird, the majority of duck trigeminal neurons are mechanoreceptors that express the Piezo2 ion channel and produce slowly inactivating mechano-current before hatching. Furthermore, duck neurons have a significantly reduced mechano-activation threshold and elevated mechano-current amplitude. Cloning and electrophysiological characterization of duck Piezo2 in a heterologous expression system shows that duck Piezo2 is functionally similar to the mouse ortholog but with prolonged inactivation kinetics, particularly at positive potentials. Knockdown of Piezo2 in duck trigeminal neurons attenuates mechano current with intermediate and slow inactivation kinetics. This suggests that Piezo2 is capable of contributing to a larger range of mechano-activated currents in duck trigeminal ganglia than in mouse trigeminal ganglia. Our results provide insights into the molecular basis of mechanotransduction in a tactile-specialist vertebrate.

mechanosensitivity | mechanoreception | trigeminal ganglia | Piezo2 | mechano-gating

Of all of the sensory modalities possessed by vertebrates, mechanosensation remains the least understood at the cellular and molecular level. Rodents, the standard laboratory model for mechanosensation, mostly use whiskers for tactile discrimination, whereas other vertebrates rely on organs covered with glabrous (hairless) skin, such as fingertips and palms in primates, the star organ in the star-nosed mole, or the bill of tactile-foraging waterfowl (1–5). In the glabrous skin, many aspects of mechanical stimulation are sensed by Meissner and Pacinian corpuscles, the detectors of transient touch and vibration. The corpuscles are innervated by rapidly adapting neuronal mechanoreceptors, which function by a poorly understood molecular mechanism (6–8). With this in mind, we turned our attention to the domestic duck (*Anas platyrhynchos domesticus*), a tactile-specialist bird known for its sophisticated feeding behavior (9).

In contrast to visually foraging birds, such as chicken (*Gallus gallus domesticus*), ducks can find food in muddy water relying primarily on the sense of touch. In ducks and other tactile-foraging waterfowl, such as geese, the acquisition of tactile information is carried out by Herbst and Grandry corpuscles, the analogs of the mammalian Meissner and Pacinian corpuscles, respectively, which are located below the epidermis of the glabrous skin covering the bill, tongue, and oral cavity. In adult birds, the corpuscles are innervated by rapidly adapting mechanosensory afferents from trigeminal ganglia (TG) and relay tactile information from the periphery to the principal trigeminal nucleus (PrV) in the brainstem (1, 5, 10–12). In tactile foragers, the relative size of PrV is enlarged compared with visual foragers, suggesting the presence of an expanded population of mechanoreceptors in TG (13). Accordingly, we showed that the majority of neurons in TG of several species of tactile-foraging birds are large-diameter cells, consistent with the idea of mechanoreceptor expansion. We also demonstrated that duck TG neurons produce robust mechano-activated (MA) current in vitro (14).

In this study, we sought to investigate previously uncharacterized cellular and molecular adaptations for mechanoreception in the bill and trigeminal system of the domestic duck and to directly compare the mechanosensitivity of trigeminal neurons in tactile- and visually foraging birds. We also aimed to examine the molecular basis of the neuronal MA currents. Taking advantage of the fact that ducks are precocial birds, whose development largely completes *in ovo*, we focused on studying late-stage duck embryos, whose cells are also more amenable to experimental manipulations than those of adults. Here, we show that the embryonic duck bill contains mechanosensory corpuscles at a density comparable to that in the fingertips and palms of primates. Ex vivo electrophysiological experiments reveal that the molecular machinery that controls rapid adaptation of mechanically evoked firing is fully developed in the duck bill before hatching. We show the majority of neurons in duck, but not chicken, TG are low-threshold mechanoreceptors and that knockdown of the mechano-gated ion

Significance

Tactile-specialist birds of the Anatidae family possess unique mechanosensory abilities with which they efficiently select edible matter in muddy water without visual or olfactory cues. Mechanical stimuli are transmitted by trigeminal mechanoreceptors innervating the bill, a highly specialized tactile organ. We show mechanosensory specialization in ducks involves the formation of functional rapidly adapting mechanoreceptors prior to hatching. Unlike in visually foraging chicken, most trigeminal neurons in ducks are touch receptors, which develop following a unique pattern of neurotrophic factor receptor expression and produce robust mechano-current via the Piezo2 channel with novel properties. Our results uncover possible evolutionary adaptations contributing to potentiation of mechanoreception in an organ-specific manner and reveal the molecular identity of a neuronal mechanotransducer with prolonged inactivation kinetics.

Author contributions: E.R.S., E.O.A., E.O.G., and S.N.B. designed research; E.R.S., E.O.A., M.M., J.D.M., E.O.G., and S.N.B. performed research; E.R.S., E.O.A., V.P.S., P.G.G., R.H.L., E.O.G., and S.N.B. analyzed data; and E.R.S., E.O.A., P.G.G., R.H.L., E.O.G., and S.N.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. KY012733).

See Commentary on page 12853.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1708793114/-/DCSupplemental.

channel Piezo2 suppresses intermediately and slowly inactivating mechano-current in these cells, contrasting with its role in mice as the mediator of only fast mechano-current. Our studies reveal a pattern of specialization in TG and uncover anatomical, neuronal, and molecular adaptations that subserve mechanoreception in a tactile-specialist vertebrate.

Results

Rapidly Adapting Mechanoreceptors in Duck Bill Are Functional Before Hatching. We performed histochemical analysis of the skin covering the dorsal bill of late-stage duck embryos (days E24–E26) using antibodies against the neuronal β-tubulin (Tuj1), also known to be expressed in the lamellar sheaths of Pacinian corpuscles (15). The staining revealed a network of nerve endings terminating in Herbst and Grandry corpuscles 100–500 μm below the surface of the skin (Fig. 1 *A*–*C* and Fig. S1). Using light-sheet and confocal microscopy combined with whole-mount immunostaining, we found the cumulative density of total corpuscles reaches 173 ± 6/mm^2 of dorsal bill skin (mean ± SD, n = 3) (Fig. 1*D* and Movie S1), rivaling the density in glabrous skin of other tactile specialists, such as in the fingertips of primates and the nose of the star-nosed mole (2, 5, 16, 17). Studies from adult ducks and geese showed that Grandry and Herbst corpuscles are innervated by trigeminal mechanoreceptors with rapidly adapting firing (10–12). These mechanoreceptors generate action potentials during the onset and offset of the mechanical stimulus but not during the static phase. Rapid adaptation stems from a poorly understood mechanism, which involves a contribution from the nerve afferent terminus and surrounding somatic components, such as the granular and lamellar cells in Grandry and Herbst corpuscles, respectively. To test if the mechanism of rapid adaptation is functional in embryonic corpuscles, we developed an ex vivo technique to extracellularly record electrical activity from the bodies of intact neurons in TG in response to mechanical stimulation of the bill (Fig. 1*E*). Remarkably, 10 of the 10 neurons that responded to a mechanical step indentation of the bill produced rapidly adapting discharges recorded in TG (Fig. 1 *F* and *G*). These data agree with the high density of mechanosensory corpuscles in the bill and demonstrate that the molecular machinery controlling rapid adaptation of mechanically evoked firing is fully developed before hatching. The accessibility of the duck embryos, rather than adult birds, to experimental manipulations allowed us to further explore the cellular and molecular basis of tactile specialization in duck TG.

Up-Regulation of Mechanoreceptors in TG of Tactile-Foraging Duck and Goose. Since afferent endings are essential for the development of mechanosensory corpuscles (3), the presence of an exceptionally dense corpuscle population in the duck bill strongly suggests the existence of a large number of mechanoreceptive neurons in TG. We previously demonstrated that TGs of several species of adult tactile-foraging birds from the *Anatidae* family mostly contain large-diameter cells, implying the presence of an unusually large population of mechanoreceptors (14). Here, we sought to directly test this hypothesis. First, we analyzed the expression of neurotrophic factor receptors TrkA and TrkB in TG of a tactile bird (duck) and a visually foraging bird (chicken). Along with other important factors, TrkA underlies the development of most nociceptors and thermoreceptors, and TrkB underlies the development of most mechanoreceptors (18). In mature ganglia of mice and rats, most somatosensory neurons express TrkA, whereas TrkB is limited to 10–30% of the cells (19–25). In the TG of a late-stage (E19–E20) domestic chicken embryo, a precocial visually foraging bird without tactile specialization in the beak, TrkA and TrkB were present in 36% and 27% of neurons, respectively, (Fig. 2 *A* and *B* and Fig. S2*A*), agreeing with data reported earlier (26). In striking contrast, we found that TrkA was expressed in only 7% of embryonic duck

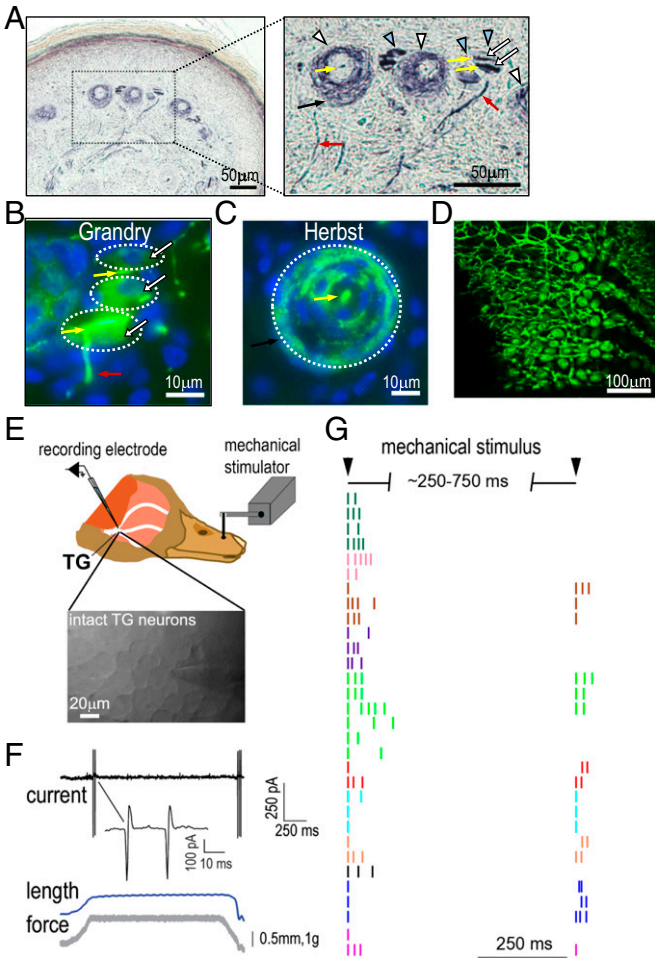

Fig. 1. Rapidly adapting mechanoreceptors in duck bill are functional before hatching. (*A*–*C*) Immunostaining of the skin from the lateral edge of the dorsal bill of a duck embryo with Tuj1 antibody reveals Herbst (white arrowhead) and Grandry (blue arrowhead) corpuscles innervated by trigeminal mechanoreceptors (red arrows). Mechanoreceptors terminate in the core of the corpuscles (yellow arrows), sandwiched between Tuj1-reactive satellite cells (white arrows; the cells are outlined with a dashed line in *B*) of Grandry corpuscles or surrounded by several layers of lamellar cells in Herbst corpuscles (the black arrow points at the outer layer of lamellar cells, indicated by a dashed line in *C*). Nuclear staining: DAPI. (*D*) Light-sheet microscopy and Tuj1 immunostaining of a whole-mount preparation of embryonic duck bill skin. Shown is a still image from Movie S1. (*E*) Ex vivo extracellular recordings from intact TG neurons in response to mechanical stimulation of the bill. (*Inset*) Exposed TG with an electrode attached to a neuron. (*F*) A representative rapidly adapting discharge recorded from a TG neuron in response to force- and indentation-controlled stimulation of the bill. (*G*) Raster plot demonstrates the prevalence of rapidly adapting mechanoreceptors among mechanosensitive duck TG neurons. Individual neurons are denoted by colors. Each tic mark indicates an action potential.

TG neurons, whereas the majority expressed TrkB (67%) (Fig. 2 *A* and *B* and Fig. S2*A*). Importantly, TrkA and TrkB were present in nearly identical proportions in TG neurons from late-embryonic and adult ducks (Fig. S2*B*), arguing against incomplete development as the cause for the observed neuronal distribution (27). These results show that, unlike other vertebrates such as mice or chickens, duck TG are predisposed to develop more mechanoreceptors than nociceptors and thermoreceptors. These data agree with the finding that TrkB is a critical factor for the normal development of mechanosensory corpuscles (28, 29).

Next, we analyzed the expression of the somatosensory ion channels TRPV1 and TRPM8, which together encompass most

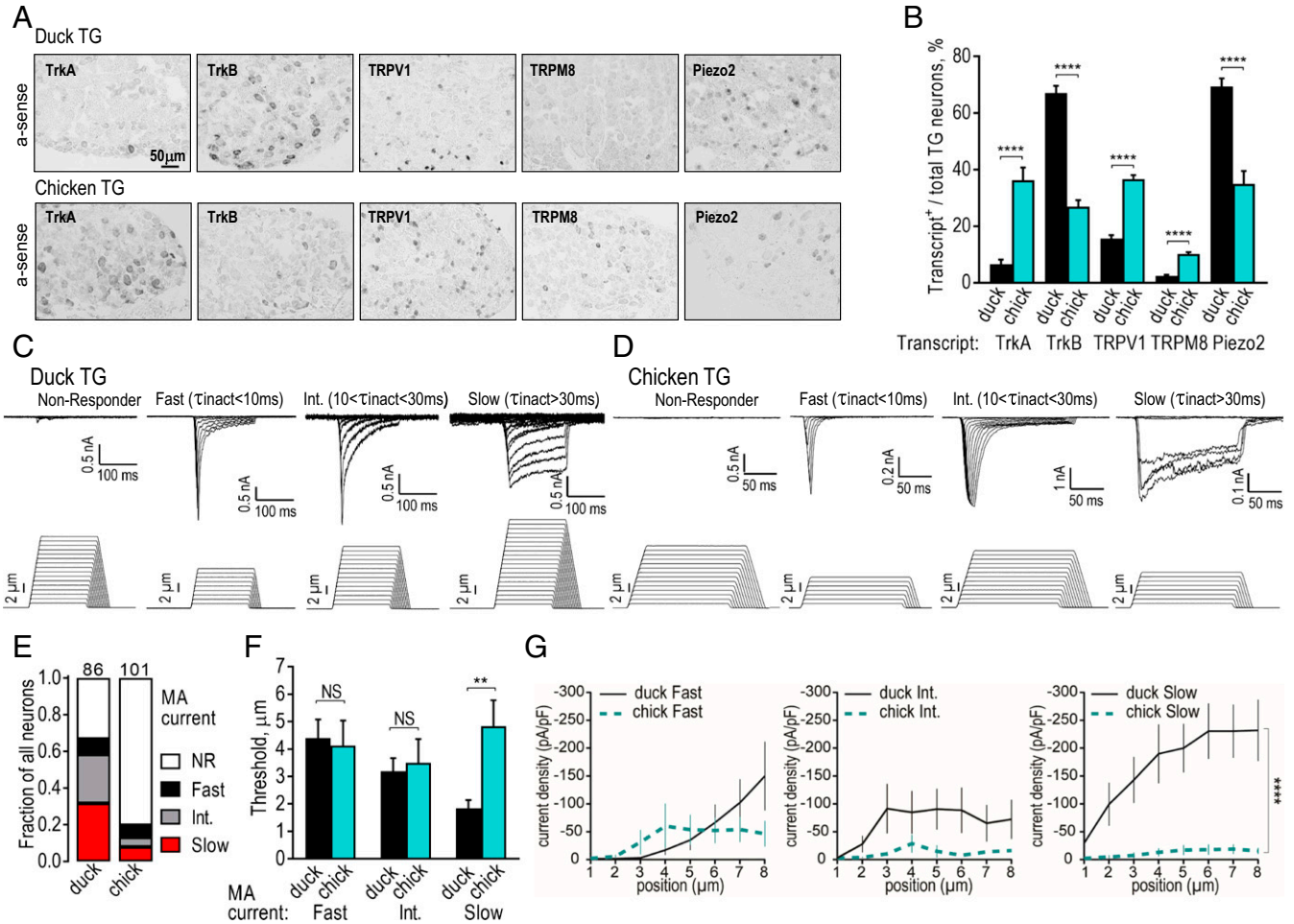

Fig. 2. Mechanoreceptor expansion is specific to duck TG. (*A*) Representative images of RNA in situ hybridization for the indicated targets in embryonic duck and chicken TG. (*B*) Quantification of the abundance of neurons expressing the indicated targets in duck and chicken TG (mean ± SE from ≥1,700 neurons obtained from at least seven TG sections from two or more animals for each target; ****$P \leq 0.0001$, two-tailed *t* test). (*C* and *D*) Exemplar whole-cell MA current traces recorded in dissociated TG neurons in the voltage-clamp mode at −74.6 mV holding potential in response to mechanical stimulation of the soma with a glass probe to the indicated depth. MA currents are classified based on the MA current inactivation rate (τ_{inact}). (*E*) Quantification of the prevalence of fast, intermediate, and slow MA current among mechanosensitive duck and chicken TG neurons. NR, nonresponder. (*F*) Quantification of the MA current activation threshold (mean ± SE) from 40 duck and 18 chicken neurons (**$P \leq 0.01$, NS, not significant, $P > 0.05$; Kruskal–Wallis test with Dunn's multiple comparisons). (*G*) Peak MA current density measured at different indentation depths in mechanosensitive TG neurons (mean ± SE from 40 duck and 18 chicken neurons; ****$P \leq 0.0001$, ordinary two-way ANOVA with Bonferroni correction for multiple comparisons).

nociceptors and thermoreceptors (30, 31). In accordance with the paucity of TrkA$^+$ neurons, TRPV1 and TRPM8 were expressed in 16% and 2% of duck TG neurons, respectively. In contrast, the mechano-gated ion channel Piezo2 was present in 69% of cells (Fig. 2 *A* and *B* and Fig. S2*A*). On the other hand, in chicken TG TRPV1, TRPM8, and Piezo2 were present in 37%, 10%, and 35% of neurons, respectively (Fig. 2 *A* and *B* and Fig. S2*A*), a distribution similar to that found in mice (32–35). To investigate the prevalence of mechanoreceptors in other tactile-foraging species, we analyzed TG from the Canada goose (*Branta canadensis*), a precocial tactile-foraging bird from the *Anserinae* subfamily (10). In adult goose, the majority of TG neurons expressed Piezo2 (53%), and far fewer expressed TRPV1 (20%) and TRPM8 (4%), a distribution similar to that in duck TG (Fig. S3). Taken together, our histological analyses suggest that mechanoreceptor expansion in TG is not a general avian feature but may be specific to tactile-foraging ducks and geese.

To complement our histological analysis, we investigated the mechanosensitivity of duck and chicken TG neurons directly by recording MA current in response to a stimulation of the cellular soma with a glass probe. Based on the rate of exponential decay, MA currents are classified as fast, intermediately, and slowly inactivating (14, 36–39). The three types of current were present in neurons from both species (Fig. 2 *C* and *D*). However, whereas 57 of 86 (66%) duck TG neurons produced MA current in response to mechanical stimulation, only 20 of 101 (20%) chicken neurons were mechanosensitive (Fig. 2*E*). In comparison with chicken, duck neurons had a significantly reduced mechano-activation threshold and elevated MA current density in cells with slow MA current (Fig. 2 *F* and *G*). Thus, not only are mechanosensitive neurons more numerous in duck than in chicken TG, but neurons with slow MA current exhibit an elevated ability to convert touch into excitation.

It is interesting that neurons with fast MA current are the least numerous group in ducks (12% of all mechanosensitive cells) and are the most numerous group in chicken (44%) (Fig. 2*E*). The distribution of MA current types among chicken neurons is similar to that found in mice and rats, where fast MA current is present in 30–60% of mechanosensitive neurons (14, 27, 34, 40). The prevalence of neurons with slow inactivation kinetics in ducks (47%) together with a low threshold of activation and high MA current density could reflect somatosensory specialization

toward the detection of light touch via increased charge influx upon mechanical stimulation. Taken together, our histological and functional data show an up-regulation of the mechanoreceptor population in duck TG, consistent with the pattern of TrkB and TrkA expression. The expansion of mechanoreceptors in duck TG reveals a pattern of somatosensory ganglia specialization sufficient to innervate the exceptionally dense population of Grandry and Herbst corpuscles in duckbill skin.

Piezo2 Mediates a Subset of Mechano-Current with Prolonged Inactivation in Duck TG. We sought to investigate the molecular basis of touch detection in duck neuronal mechanoreceptors, taking advantage of our finding that this is the most abundant neuronal group in duck TG. MA current is essential for neuronal touch sensitivity. Piezo2 is known to contribute exclusively to the generation of fast MA current in mouse somatosensory neurons (34, 35, 41–43), whereas MA currents with intermediate and slow inactivation kinetics are thought to be Piezo2-independent (44–46). Since the percentage of duck neurons expressing Piezo2 far exceeds the percentage of neurons with fast MA current, which are the least numerous group of mechanosensitive neurons in duck TG, we hypothesized that Piezo2 also contributes to the MA currents with prolonged inactivation. To test this, we first cloned Piezo2 from duck TG and analyzed its functional properties in HEK293T cells in comparison with mouse Piezo2 (mPiezo2), a well-characterized ortholog. Immunoblot showed that duck Piezo2 protein (dPiezo2) is expressed in transfected HEK293T cells in duck TG and bill skin (Fig. 3*A* and Fig. S4). dPiezo2 produced nonselective, Gd^{3+}-sensitive MA current with an activation threshold identical to the mouse ortholog, mPiezo2 (Fig. 3 *B*, *C*, *F*, and *G*). The similarly high amplitude of mouse and dPiezo2 MA current (Fig. 3*D*) eliminates the potentially confounding effect of endogenous Piezo1 in these cells (44). dPiezo2 demonstrated significantly slower inactivation kinetics than mPiezo2 at −80 mV (mPiezo2 τ_{inact} = 3.1 ± 0.17 ms, dPiezo2 τ_{inact} = 5.1 ± 0.47 ms, mean ± SE, n = 26–29) (Fig. 3*E*). This difference becomes larger at depolarized potentials, where the rate of dPiezo2 inactivation converts from fast to slow (τ_{inact} >30 ms) (Fig. 3 *G* and *H*). Although the increase in inactivation rate is small at physiologically relevant voltages, these data show that the inactivation rate of dPiezo2 can be reversibly converted from fast to slow in the same cell.

To test the role of Piezo2 in the generation of MA current in duck TG neurons, we used fluorescently labeled siRNA designed against a region in Piezo2 previously reported to be susceptible to siRNA-mediated knockdown (34, 41). Treatment of dissociated TG neurons with siRNA against Piezo2 decreased Piezo2 mRNA and protein by ∼40%, while no decrease was detected in control (Fig. 4 *A*–*C*). siRNA treatment had no noticeable effect on neuronal fitness, as revealed by unchanged input resistance, resting membrane potential, and cell capacitance in fluorescent siRNA-containing neurons (Fig. S5). At the same time, the down-regulation of Piezo2 was accompanied by an ∼50% increase in the fraction of neurons without MA current (Fig. 4*D*). These additional mechano-insensitive neurons are probably those in which Piezo2 was the major mechanotransducer and in which expression knockdown was the most efficient. Notably, while Piezo2 knockdown did not change the apparent threshold of mechanical activation (Fig. S6), it led to a significant decrease in MA current density in neurons with intermediate and slow MA current (Fig. 4 *E* and *F*). The remaining MA current in these cells could be due to the presence of other mechanotransducers in addition to Piezo2 or to incomplete knockdown. Taken together, our data reveal that Piezo2 contributes to the generation of intermediate and slow MA current in duck trigeminal neurons.

Discussion

Feeding in ducks relies on the precise acquisition of tactile information from transient touch and vibration. Rapidly adapting mechanoreceptors are ideal for encoding the fast-acting stimuli

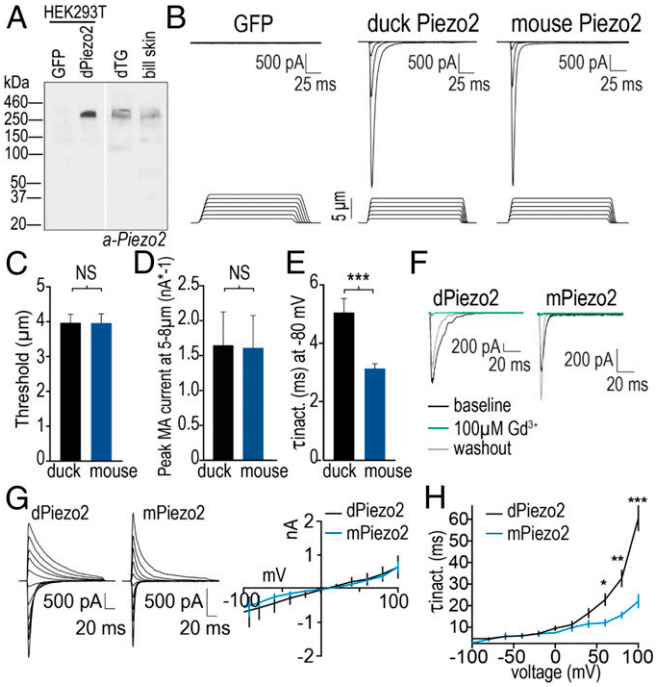

Fig. 3. dPiezo2 has slower kinetics of inactivation than the mouse channel. (*A*) Western blot shows Piezo2 expression in duck TG (dTG), bill skin, and HEK293T cells transfected with dPiezo2. (*B*) Representative whole-cell MA current traces recorded in the voltage-clamp mode (V_{hold} −80 mV) in HEK293T cells expressing the indicated constructs, in response to mechanical stimulation of the cellular soma with a glass probe. (*C*–*E*) Quantification of the Piezo2 MA current activation threshold (*C*), amplitude (*D*), and inactivation rate τ_{inact} (*E*) in HEK293T cells at −80 mV (data are the mean ± SE from 29 dPiezo2- and 26 mPiezo2-expressing cells). Tau values averaged across traces with −0.1 to −2 nA MA current; ***$P \leq 0.001$; NS, not significant (Welch's t test). (*F*) Exemplar MA current traces showing reversible inhibition of dPiezo2 MA current by Gd^{3+} in HEK293T cells (V_{hold} −80 mV). (*G*) Representative traces and current–voltage plots of Piezo2 MA currents in HEK293T cells evoked at different voltages in response to a mechanical indentation of 6–8 µm (mean ± SE, n = 2–6 for each voltage). (*H*) Quantification of MA current inactivation from *E* (mean ± SE, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, two-way ANOVA with Bonferroni-corrected paired comparisons; n = 2–6 for each voltage).

because they fire only during the dynamic phase of the stimulus, providing high temporal resolution. Here we show that the mechanism that produces the rapid adaptation of afferent firing is fully developed *in ovo*, providing insight on the observation that ducklings can forage side-by-side with adults shortly after hatching.

Consistent with the high density of the corpuscles in the bill skin, our histological and electrophysiological data show that the majority of duck trigeminal neurons are mechanoreceptors. The expansion of mechanoreceptors is unusual for somatosensory ganglia of rats and mice, where most neurons are nociceptors and thermoreceptors (32). Studies in rodents and chickens showed that while $TrkB^+$ mechanoreceptors arise as the dominant group early in development, they are later outnumbered by $TrkA^+$ nociceptors and thermoreceptors (18, 27). We find that the majority of late-embryonic and adult duck TG neurons express TrkB, whereas TrkA expression is limited to a small population of cells. This suggests that mechanoreceptor expansion in tactile-foraging ducks is driven by a developmental program different from that found in chicken, a visual forager, or in rodents.

Neuronal mechanoreceptors are essential for touch physiology. Even though the somatic components of the mechanosensory end-organs play important roles in detecting physical stimuli, the neurons are innately mechanosensitive, i.e., they can covert touch into excitatory MA current in the absence of other tissue

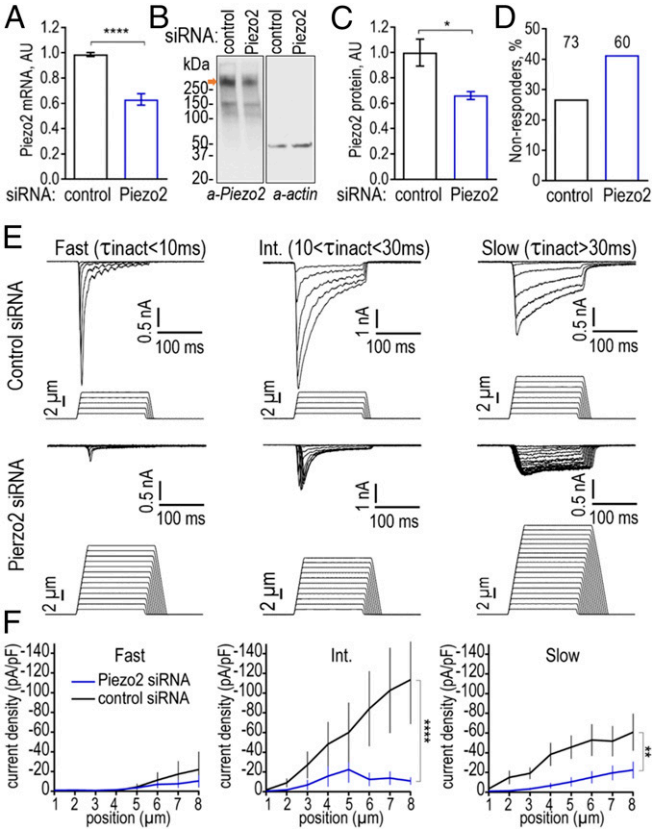

Fig. 4. Knockdown of Piezo2 suppresses intermediate and slow mechanocurrent in duck TG. (*A*) Quantification of siRNA-mediated Piezo2 mRNA knockdown in dissociated duck TG neurons relative to untreated cells by qPCR (mean ± SE from six independent transfections; $^{****}P \leq 0.0001$, two-tailed *t* test). (*B* and *C*) Immunoblot analysis (*B*) and quantification (*C*) of the knockdown of Piezo2 protein expression (red arrow) in dissociated duck TG after control or Piezo2 siRNA treatment, normalized to actin in each sample (mean ± SE from 12 transfections from three independent TG preparations; $^{*}P \leq 0.05$, two-tailed *t* test). (*D*) Quantification of the proportion of mechano-insensitive TG neurons after control or Piezo2 siRNA treatment. Recordings were made only from neurons that received siRNA, as determined by fluorescence. (*E* and *F*) Exemplar whole-cell MA current traces (*E*) and quantification of MA current density (*F*) in dissociated TG neurons after control or Piezo2 siRNA treatment (mean ± SE, $^{****}P \leq 0.0001$, $^{**}P \leq 0.005$, ordinary two-way ANOVA with Bonferroni correction for multiple comparisons). Fast MA current: $n = 4$ control siRNA, $n = 5$ Piezo2 siRNA; intermediate (Int.) MA current: $n = 15$ control siRNA, $n = 9$ Piezo2 siRNA; slow MA current: $n = 31$ control siRNA, $n = 21$ Piezo2 siRNA. E_{hold}, −74.6 mV.

components (47). Mechanical stimulation evokes three major types of MA current in somatosensory neurons: fast, intermediately, and slowly inactivating (14, 36–39). In mouse somatosensory neurons, Piezo2 depletion by siRNA (34, 41) or via conditional knockout (35, 42) eliminates only fast MA current, suggesting that the other MA current types are Piezo2-independent. We show that neurons with fast MA current represent the smallest group of mechanosensitive cells in duck TG, whereas many more neurons express MA current with prolonged inactivation. Knockdown of Piezo2 expression by siRNA significantly suppresses intermediate and slow MA current density, suggesting that Piezo2 contributes to the generation of these types of current. We did not detect an effect of Piezo2 knockdown on fast MA current, possibly due to the very low abundance of this group of neurons. Our results do not rule out the existence of other mechanotransducers with slow or intermediate inactivation kinetics, which may or may not coexpress with Piezo2 in the same neuron. The contribution of such mechanotransducers could be more prominent in mouse neurons than in duck cells, explaining the absence of an effect of Piezo2 knockdown on these MA currents. Overall, our results show that Piezo2 is an evolutionarily conserved mediator of neuronal mechanosensation in vertebrates and that the channel can contribute to more than one type of MA current in somatosensory neurons.

The rate of Piezo2 inactivation controls the amount of excitatory charge entering the cell upon mechanical stimulation. Inactivation of Piezo2 and its homolog Piezo1 can be prolonged at positive potentials (34, 48), by mutations (49–55), pharmacologically (56, 57), or by the destruction of the cytoskeleton or after repeated stimulation (58), fluid shear stress (59), or osmotic swelling (60). These data establish that Piezo channel kinetics is modulated by intracellular factors. Interestingly, single-channel recordings from Piezo1 in a lipid bilayer revealed the absence of inactivation (61), suggesting that Piezo channels could be intrinsically non- or slowly inactivating even at potentials close to physiological, whereas the fast inactivation observed in cells requires additional components. We therefore hypothesize that the differences in dPiezo2 kinetics of inactivation in HEK293T cells and duck TG neurons are due to posttranslational modifications, properties of the plasma membrane, cytoskeleton, auxiliary subunits, or gating modifiers (57, 60, 62–66), which are present (or absent) in such neurons and remain to be identified.

It remains to be determined which type of MA current is present in the rapidly adapting mechanoreceptors that innervate Grandry and Herbst corpuscles. Studies in cat Pacinian corpuscles showed that mechanical stimulation of the inner core triggers slowly inactivating receptor potential, indicating the presence of a mechanotransducer with slowly inactivating MA current (67). It is therefore possible that the mechanoreceptors with rapidly adapting firing that innervate the vibration-sensitive Herbst or Pacinian corpuscles express slowly inactivating MA current, which, as we show, is partially mediated by Piezo2. Consistently, Piezo2 mutants with prolonged inactivation transduce high-frequency stimulation much more efficiently than the fast-inactivating wild-type channel (68). The importance of Piezo2 for rapidly adapting mechanoreceptors could be general: a recent report documented that humans without functional Piezo2 exhibit deficits in the perception of vibratory stimuli in their hairless skin in the frequency range detectable by Pacinian corpuscles (69).

The high density of Grandry and Herbst corpuscles in the bill together with the expansion of mechanoreceptors in TG suggest duck embryos as a potential model to study functional properties and molecular organization of rapidly adapting mechanoreceptors innervating glabrous skin. Genome editing-based approaches and cell type-specific control of activation in duck cells will be needed to reveal other mechanotransducers and to delineate the contribution of somatic and neuronal components to the fine-tuning of the mechanosensory corpuscles.

Materials and Methods

Animal procedures were approved by and performed in compliance with the Institutional Animal Care and Use Committee of Yale University. Immunohistochemistry, biochemical analysis, microscopy, cloning, and in situ hybridization were performed using standard procedures. For detailed description of ex vivo and patch-clamp electrophysiology, see *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank members of the E.O.G. and S.N.B. laboratories for comments on the study, Sue Ann Mentone for immunohistochemistry, Chao Ma for advice on ex vivo recordings, Kara Marshall and Ellen Lumpkin for advice on whole-mount histology, Uwe Schröer for lending equipment for light-sheet microscopy, Ardem Patapoutian for the gift of plasmid, and Jeffrey Laursen and Charles Pederson for goose tissues. E.R.S. was supported by NIH Training Grant T32HD007094 (to P.G.G.) and a Postdoctoral Fellowship from the Arnold and Mabel Beckman Foundation. E.O.A. is an Edward L. Tatum Fellow and was supported by the Gruber Foundation. This work was funded by NIH National Institute of Diabetes and Digestive and Kidney Diseases Grant R01DK104046 (to P.G.G.), by fellowships from the Arnold and Mabel Beckman Foundation and Rita Allen Foundation, by NIH National Institute of Neurological Disorders and Stroke (NINDS) Grant 1R01NS091300-01A1 (to E.O.G.), and by National Science Foundation Career Award 1453167 and NIH NINDS Grant 1R01NS097547-01A1 (to S.N.B.).

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