**Report**

**A Putative Mechanism for Magnetoreception by Electromagnetic Induction in the Pigeon Inner Ear**

**Highlights**
- Magnetic stimuli activate neurons in the caudal vestibular nuclei
- Magnetic stimuli induce a voltage in a model of a semicircular canal
- Electoreceptive molecules are expressed in vestibular hair cells
- We postulate that pigeons detect magnetic fields by electromagnetic induction

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**In Brief**
Nimpf, Nordmann et al. confirm that magnetic stimuli result in neuronal activity in the vestibular nuclei of pigeons. Hypothesizing that this is attributable to electromagnetic induction within semicircular canals of the inner ear, they demonstrate the presence of known electrosensory molecules in vestibular hair cells.
A Putative Mechanism for Magnetoreception by Electromagnetic Induction in the Pigeon Inner Ear

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SUMMARY

A diverse array of vertebrate species employs the Earth’s magnetic field to assist navigation. Despite compelling behavioral evidence that a magnetic sense exists, the location of the primary sensory cells and the underlying molecular mechanisms remain unknown [1]. To date, most research has focused on a light-dependent radical-pair-based concept and a system that is proposed to rely on biogenic magnetite (Fe₃O₄) [2, 3]. Here, we explore an overlooked hypothesis that animals detect magnetic fields by electromagnetic induction within the semicircular canals of the inner ear [4]. Employing an assay that relies on the neuronal activity marker C-FOS, we confirm that magnetic exposure results in activation of the caudal vestibular nuclei in pigeons that is independent of light [5]. We show experimentally and by physical calculations that magnetic stimulation can induce electric fields in the pigeon semicircular canals that are within the physiological range of known electrosensory systems. Drawing on this finding, we report the presence of a splice isoform of a voltage-gated calcium channel (CaV1.3) in the pigeon inner ear that has been shown to mediate electrosensation in skates and sharks [6]. We propose that pigeons detect magnetic fields by electromagnetic induction within the semicircular canals that is dependent on the presence of apically located voltage-gated cation channels in a population of electrosensory hair cells.

RESULTS

Magnetically Induced Activation in the Pigeon Vestibular Brainstem

We set out to replicate a previous study conducted by Wu and Dickman, who reported that magnetic stimuli induce neuronal activation in the vestibular nuclei of pigeons [5]. To perform this experiment within the laboratory environment, we built a room constructed of mu metal surrounded by an aluminum Faraday cage to shield against static and oscillating magnetic fields (Figure 1A). This setup allowed us to perform experiments in a controlled, magnetically clean environment [7]. Magnetic fields were generated using a double-wrapped, custom-built 3D Helmholtz coil system situated in the center of the shielded room (Figure 1B). To reduce movement during the experiments, birds were head fixed using a surgically implanted plastic head post, and the body was immobilized using a 3D printed harness (Figure 1C). We applied the same stimulus as Wu and Dickman, exposing adult pigeons to a 150–25 T, rotating magnetic field (n = 22) or to a zero magnetic field (n = 23) for 72 min (Figures 1D–1F). We performed this experiment both in darkness (n = 30) and under broad-spectrum white light (n = 15). Birds were then perfused, the brains were sliced, and matched sections containing the vestibular nuclei (3 sections per bird) were stained with sera against the neuronal activity marker C-FOS. To minimize variation, all staining was performed simultaneously, all slides were scanned with the same exposure settings, and C-FOS-positive neurons were counted using a machine-learning-based algorithm. Using established anatomical coordinates, we segmented the medial vestibular nuclei (VeM) and compared the density of C-FOS-positive cells of the experimental groups [8]. We observed an increase in the density of C-FOS-positive cells in both the light and dark when exposing birds to magnetic fields. In the light, the average density in controls was 35.32 ± 10.20 cells/mm² (n = 8; mean ± SD) whereas with the magnetic treatment it was 44.38 ± 5.72 cells/mm² (n = 7; mean ± SD). In the dark, the average density for control birds was 34.70 ± 10.82 cells/mm² (n = 15; mean ± SD) whereas with the magnetic treatment it was 50.52 ± 27.34 cells/mm² (n = 15; mean ± SD). An application of a two-way ANOVA revealed a significant effect of the magnetic treatment but no interaction between magnetic treatment and lighting conditions (two-way ANOVA; magnetic: p = 0.0176, F = 6.125; magnetic by light: p = 0.5675, F = 0.332) (Figures 2A and 2B; Table S1). To explore this in more detail, we employed our spot-detection algorithm coupled to an elastic registration to generate heatmaps showing regional...
differences in the density of C-FOS-positive cells. This revealed an enrichment of activated neurons in the dorsomedial part of the VeM in animals exposed to magnetic stimuli (Figure 2C). Previous tracing experiments have shown that the dorsomedial VeM is innervated by projections from both the semicircular canals and the otolith organs located in the inner ear [9].

A Model for Electromagnetic Induction in the Pigeon Inner Ear

As we did not observe an interaction between the presence of light and magnetically induced neuronal activation, our results are consistent with a magnetic sensory system based on either magnetite or electromagnetic induction [10]. We have previously reported the discovery of an iron-rich organelle in both vestibular and cochlear hair cells that is associated with vesicular structures, but because it is primarily composed of ferrihydrite it lacks the magnetic properties to function as the hypothesized torque-based magnetoreceptor [11–13]. Moreover, a systematic screen for magnetite in the pigeon lagena using synchrotron-based X-ray fluorescence microscopy and electron microscopy has failed to identify extra- or intracellular magnetite crystals [14]. In light of these findings, we focused on electromagnetic induction [15]. First proposed by Camille Viguier in 1882, this hypothesis predicts that as a terrestrial animal moves through the Earth’s static magnetic field a voltage is induced within the conductive endolymph of the semicircular canals [4, 16].

To test the viability of this hypothesis, we built a simple model of a pigeon semicircular canal by filling a plastic tube with artificial pigeon endolymph (see STAR Methods; Figure 3A) [17]. The tubing was closed on both sides with electrodes connected to a nanovoltmeter (forming a loop with a diameter of 21 cm), placed in the center of our magnetic coil system, and exposed to the same rotating magnetic stimulus applied to our birds (i.e., 150 μT rotating 360° with 6°-step changes every 2 s). After each rotation the plane was shifted by 15° (E) and the procedure was repeated until a full rotation around each axis was achieved (12 planes per x, y, and z axis, respectively) (F).

Figure 1. Experimental Setup for the Magnetic Activation Study

(A) The magnetic activation experiments were performed inside a magnetically shielded room consisting of a Faraday cage to shield from broadband anthropogenic magnetic noise and a mu-metal cage to shield from the geomagnetic field.

(B and C) Pigeons were placed in the center of a double-wrapped 3D Helmholtz coil system (B) located in the magnetically shielded room and immobilized by head fixation and a molded body harness (C).

(D–F) Schematic illustration of the magnetic stimulus used in this study. The magnetic field vector was rotated 360° within a plane by 6° steps every 2 s (D). After each rotation the plane was shifted by 15° (E) and the procedure was repeated until a full rotation around each axis was achieved (12 planes per x, y, and z axis, respectively) (F).
Electroreceptive Molecules Are Expressed in the Pigeon Inner Ear

If magnetoreception in pigeons relies on the conversion of magnetic fields into an electric signal, the magnetosensory apparatus might resemble known electroreceptive epithelia on a cellular and molecular level. Ampullae of Lorenzini are electroreceptive organs found in cartilaginous fish that consist of specialized sensory cells located at the base of gel-filled canals [22]. Recent work in little skate (Leucoraja erinacea) has shown that the voltage-sensitive calcium channel Ca\textsubscript{V1.3}/CAC-N\textsubscript{1D} and the large-conductance calcium-activated potassium channel BK/KCNMA1 are enriched in these cells and facilitate electrosensation [23]. Given the ontogenetic proximity of electroreceptive, auditory, and vestibular hair cells, we asked whether these electroreceptive molecules are present in the pigeon semicircular canals [24]. To function as electroreceptors, we would expect these channels to be apically located in hair cells where they would be exposed to the endolymph (Figures 4A–4C). To test this prediction, we performed fluorescence immunohistochemistry on pigeon ampullary hair cells (n = 3 birds). Staining with sera against the BK channel revealed an enrichment of apical staining in hair cells that are positive for the marker otoferlin (Figures 4A, 4D, 4F, 4G, and S2D–S2I) [25, 26]. Consistent with previous studies, we observed goblet-shaped Ca\textsubscript{V1.3} staining at hair cell ribbon synapses [27] and a punctate enrichment at the apical membrane that has not previously been reported (Figures 4E and 4G). High-resolution confocal imaging further revealed that these Ca\textsubscript{V1.3}-rich plaques are most pronounced at the base of the kinocilium, and that Ca\textsubscript{V1.3} is localized in the kinocilium itself (Figures S2A–S2C).

In sharks and skates, Ca\textsubscript{V1.3} is characterized by a low threshold of activation attributable to a 10-amino acid lysine-rich insertion located in the intracellular loop between IVS2 and IVS3 of the alpha subunit (Figure 4H). It has been shown that mutating the charged lysine residues in this insertion to neutral glutamine residues results in a channel with a higher threshold for activation [6, 23]. Interestingly, Hudspeth and colleagues have reported a similar insertion in Ca\textsubscript{V1.3} that is expressed in the hair cells of chickens [28]. To ascertain whether this charged insertion is expressed in pigeons, we drew on the available...
gel electrophoresis showed that CaV1.3 is absent in the respiratory concha, muscle, and liver but is otherwise broadly expressed. Strikingly, we only observed PCR products consistent with a larger splice isoform in the cochlea and vestibular system (Figure 4I). Cloning of these PCR products revealed an insertion of 10 amino acids rich in lysine residues with notable homology to that reported in sharks and skates (Figure 4J). We refer to this variant as the RA splice isoform. In skates it has been shown that CaV1.3 works in conjunction with the BK potassium channel, which has a unique conductance profile due to the expression of an alternatively spliced exon [23]. This variant is distinguished by the presence of an arginine residue at position 340 (R340) and an alanine residue at 347 (A347), which are located intracellularly near the pore of the channel (Figure 4K). We refer to this variant as the KKER splice isoform.

In skates it is present in numerous animals including skates, sharks, bats, turtles, rainbow trout, and birds (Figures 4I and S4A). In the case of the KCNMA1 RA isoform, a BLASTp search revealed that it is present in numerous animals including skates, electric eels, numerous fish and many bird species, rodents (including Mus musculus), primates (including Homo sapiens), bats, dolphins, and whales (Figures 4L and S4B). We conclude that the RA splice isoform of KCNMA1 is widely distributed in vertebrates whereas the KKER isoform of CACNA1D is often found in phyla that are known to possess an electric or magnetic sense.

**DISCUSSION**

In 1882, Viguier speculated that “the geomagnetic field determines, within the endolymph of the canals, induced currents, whose intensities vary dependently of both the canals’ positions in relation to inclination and declination, and the intensity of the magnetic field” [4]. In this manuscript, we have explored this hypothesis, one that has largely been ignored by the scientific community since its proposition. We present data that replicates the work of Wu and Dickman, demonstrating magnetically induced neuronal activation in the vestibular nuclei that is not dependent on light. We show that changing low-intensity magnetic stimuli (150 μT) can induce electric fields that lie within the window of physiological detection and that the molecular machinery necessary to detect such fields is present in the pigeon inner ear. Our data are consistent with a model whereby pigeons detect
Figure 4. Electroreceptive Molecules Are Expressed in the Pigeon Inner Ear

(A–C) Schematic illustrating induction-based magnetoreception in the pigeon inner ear.

(A) A semicircular canal with sensory hair cells located at the base of the gelatinous cupula at the crista ampullaris (ca).

(D–G) Immunofluorescence images showing the expression of electroreceptive molecules in the pigeon inner ear.

(H) Schematic of the electroreceptive molecule Ca,1,3.

(I) Gel electrophoresis showing the expression of Ca,1,3 in different species.

(J) List of species and the sequences of the Ca,1,3 molecule:

- Chain catshark (Scyliorhinus retifer)
- Little skate (Leucoraja erinacea)
- Rock pigeon (Columba livia) - exon 28b
- Zebrafish (Danio rerio)
- African clawed frog (Xenopus laevis)
- Mouse (Mus musculus)
- Human (Homo sapiens)

(K) Schematic of the electroreceptive molecule BK.

(L) Gel electrophoresis showing the expression of BK in different species.

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magnetic fields by electromagnetic induction within the semicircular canals relying on the presence of apically located voltage-gated calcium channels in a population of electroreceptive hair cells.

We have replicated the study of Wu and Dickman with only minor changes to their experimental protocol [5]. Specifically, we employed double-wrapped coils to control for heat and vibration when delivering the magnetic stimuli and a plastic head post and glue in preference to metal screws to head fix the bird and conducted the experiment in both darkness and light. We have further been able to refine the region activated by magnetic stimuli by elastic registration and mapping of C-FOS-positive cells to the dorsomedial part of the VeM. Our results, coupled with the initial study, support the contention that the vestibular system is involved in processing magnetic information in the absence of light. Wu and Dickman had argued that the primary sensors likely reside in the lagena, because extirpation of the cochlear duct abolishes magnetically induced activity in the vestibular nuclei. These results, however, are also consistent with magnetosensation by electromagnetic induction, because removal of the cochlear duct would compromise the integrity of the entire endolymphatic system and its ionic constituents.

Magnetosensation by induction has only been considered viable in elasmobranch fish, because surface-electrosensitive epithelia could detect voltages induced as the animal moves through the conductive seawater within the Earth’s static magnetic field [21, 22]. In birds, induction has largely been dismissed due to the high electrical resistivity of the air and the lack of a conductive circuit [1]. Our physical modeling and anatomical measurements suggest that the semicircular canals possess the requisite dimensions and properties to function as a physiological dynamo. Moreover, the apical location and the expression of splice isoforms of Ca_{1.3}/CACNA1D and BK/KCNMA1 indicate that hair cells in pigeons may also function as electroreceptors. It is known that hair cells are closely related to electroreceptive cells on a developmental, cellular, and molecular level [24]. The ancestral lateral line system of vertebrates consists of both mechanosensory hair cells and electroreceptive ampullary organs, which develop from lateral line embryonic placodes [30]. Both cell types have apical ciliary protrusions and similar gene expression profiles, and share characteristic ribbon synapses [31, 32]. It is therefore conceivable that hair cells in Aves have maintained or acquired an electroreceptive capacity that is exploited for magnetic detection.

A critical issue in considering the validity of the inductive hypothesis is how magnetic information would be distinguished from vestibular input assuming that amphilary hair cells are both mechanically and electrically sensitive [4, 16]. The anatomical framework of the semicircular canals provides an elegant solution to this problem. Imagine a magnetic vector with an orientation that is perpendicular to the plane of a semicircular canal. Rotation in the plane of the canal leads to inertia-based fluid displacement and mechanic stimulation of hair cells but minimal electromagnetic induction. In contrast, rotation perpendicular to the plane does not result in fluid movement but generates electric field changes [16]. In this way, magnetic and vestibular information originating from the same sensory epithelia could be distinguished from each other, so long as the animal has a third reference frame (e.g., visual input). A second important element to this model is the presence of the cupula within the crista ampullaris. Acting as a physical barrier it enables the separation of positively charged cations (Na⁺, K⁺) as the animal moves its head through the magnetic field [16]. The permeability of this structure to cations has yet to be determined, but we assume that its gelatinous composition and its positive charge impede cation flow [33, 34].

An experimental paradigm that permits investigators to distinguish between magnetoreception based on induction and magnetite is to fix a strong magnet (e.g., 10 mT) to the bird. Such a field would immobilize any magnetite chains, rendering them unresponsive to the application of an Earth-strength field. In contrast, even a small magnetic vector superimposed onto a larger fixed field would result in a changing magnetic stimulus and permit magnetoreception by electromagnetic induction. Although this experiment has yet to be performed in a controlled laboratory-based assay in pigeons, a number of groups have attached magnets to birds to assess their effect on homing and navigation. Keeton glued bar magnets onto the backs of pigeons, initially reporting that this interfered with their homeward orientation on overcast but not sunny days [35], which was...
replicated by loaë [36]. However, it should be noted that Keeton himself generated contradictory results when performing a larger study between 1971 and 1979 [37]. Several groups have attached magnets to the heads of albatrosses and observed no effect on navigation, leading the authors to speculate that the birds do not have a magnetite-based magnetosensor, or that they do not rely on magnetic cues for orientation [38, 39]. Although these studies have largely ignored the possibility that birds might rely on electromagnetic induction as a mechanism, we would urge caution when interpreting their results. There are numerous uncontrolled environmental variables, and it is unclear whether the magnets were truly fixed in position given they were glued to the skin of the birds.

Finally, we wish to acknowledge that there are alternative explanations for our results. First, it is conceivable that the magnetically induced activation that we observe in the VeM is a consequence of multimodal sensory integration and not due to primary sensors located in the inner ear. Second, we have assumed that the light-independent magnetic activation we report excludes a radical-pair-based mechanism; however, it is possible that a chemical-based compass may exist and does not depend on light. Moreover, our results do not preclude the existence of a light-based magnetoreceptor in pigeons, because they may possess more than one magnetosensory system. Third, the long isoform of CaV1.3 (although critical for electroreception in sharks and skates) may merely tune hair cells to auditory and vestibular stimuli in birds. Despite these caveats, the putative mechanism that we present in this paper enables us to make several predictions. Should pigeons rely on CaV1.3 to detect magnetic fields by electromagnetic induction, we expect that magnetically induced neuronal activation will be compromised by (1) pharmacological intervention with CaV1.3 antagonists such as nifedipine; (2) hair cell ablation with antibiotics; and (3) genetic deletion of the long isoform of CaV1.3. In contrast, neuronal activation will be preserved if a strong dipole magnet is fixed on the head of the animal, immobilizing any magnetite particles but still permitting induction from an applied changing stimulus. These predictions will serve as a basis for future experiments and the interrogation of a hypothesis that has been forgotten but not yet falsified.

STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2019.09.048.

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AUTHOR CONTRIBUTIONS

Conceptualization, S.N., G.C.N., and D.A.K.; Investigation, S.N., G.C.N., D.K., E.P.M., L.L., A.P.-A., L.U., A.W.-W., and P.V.; Formal Analysis, S.N., G.C.N., D.K., E.P.M., M.N., and L.L.; Software, T.L.; Resources, M.J.M. and M.C.; Writing – Original Draft, S.N., G.C.N., D.K., E.P.M., M.J.M., P.V., D.K., and L.L.; Funding Acquisition, D.A.K. and S.N.; Supervision, D.A.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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# STAR METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies** | | |
| Rabbit anti C-FOS | Santa Cruz | Cat#sc-25; RRID: AB_2231996 |
| Rabbit anti CACNA1D | Alomone labs | Cat#ACC-005 |
| Mouse anti MaxiKalpa | Santa Cruz | Cat#sc-374142; RRID: AB_10919141 |
| Mouse anti acetylated Tubulin | Sigma Aldrich | Cat#T6793; RRID: AB_477585 |
| Goat anti Otoferlin | Santa Cruz | Cat#sc-50159; RRID: AB_785002 |
| Mouse anti Syntaxin-1 (SP6) | Santa Cruz | Cat#sc-20036; RRID: AB_628316 |
| Donkey anti rabbit Alexa Fluor-488 | Thermo Fischer | Cat#A-21206; RRID: AB_2535792 |
| Donkey anti goat Alexa Fluor-568 | Thermo Fischer | Cat#A-11057; RRID: AB_2534104 |
| Donkey anti mouse Alexa Fluor-647 | Thermo Fischer | Cat#A-31571; RRID: AB_162542 |
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| Paraformaldehyde | Sigma Aldrich | S9378 |
| Diaminobenzidine | Sigma Aldrich | D5905 |
| Hydrogen peroxide | Merck | 822287 |
| D-mannitol | Sigma Aldrich | M4125 |
| Neg-50 frozen section medium | Thermo Fischer | 6502 |
| Triton X-100 | Sigma Aldrich | X100 |
| Donkey serum | Abcam | ab7475 |
| Fluorescent Mounting Medium | Dako | S302380 |
| Alul | NEB | R0137S |
| **Critical Commercial Assays** | | |
| Antenn Unmasking Solution | Vector Laboratories | H-3301 |
| VECTASTAIN Elite ABC HRP Kit | Vector Laboratories | PK-6100 |
| RNasey mini kit | Quiagen | 74104 |
| Quantitect reverse transcription kit | Quiagen | 205313 |
| Phusion Hot Start Flex NA polymerase | NEB | M0535S |
| TOPO-TA Cloning Kit | Thermo Fischer | 45-0030 |
| **Oligonucleotides** | | |
| CACNA1D_27F: CAGGAGTGTTTCAGTTTTGTA | This paper | N/A |
| CACNA1D_29R: TATGGCCGACGATAGACGTC | This paper | N/A |
| KCNMA1_8F: CAGCCACTAAGTATTTG | This paper | N/A |
| KCNMA1_10R: TGGCTACGTGCGCAG | This paper | N/A |
| **Software and Algorithms** | | |
| Panoramtic Viewer, Version 1.15.4 | 3D Histech | N/A |
| Definiens Architect XD | Definiens Software | N/A |
| R | [40] | N/A |
| ITOL v4 | https://itol.embl.de/ | N/A |
| Prism software for graphs and statistical analysis, version 7 | GraphPad Software | N/A |
| FIJI | [5] | N/A |
| **Other** | | |
| Magnetically shielded room | Magnetic shields | N/A |
| 3D Helmholtz coil system | Serviciencia | BH1300-3-A |
| DC power source | Aim Tti | CPX400DP |
| Slide scanner | 3D Histech | Pannoramic 250 FlashIII |
| Nanovoltmeter | Keithley Nanovoltmeter | 2182A |

(Continued on next page)
LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for reagents may be directed to and will be fulfilled by the lead contact, David A. Keays (keays@imp.ac.at). This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Male and female adult rock pigeons (Columba livia) from our Austrian cohort were maintained on a 12:12 light-dark cycle at 25°C in a custom-built aviary. For the magnetic stimulation assay, 45 experimental birds were used. Six animals were used for tissue cDNA preparations and 6 used for immunohistochemical experiments. Animals were housed and experimental procedures were performed in accordance with an existing ethical framework (GZ: 214635/2015/20) granted by the City of Vienna (Magistratsabteilung 58).

METHOD DETAILS

Magnetically shielded room and magnetic coils
The experiments were performed inside a 4.4 m long, 2.9 m wide and 2.3 m high room which was shielded against oscillating electromagnetic fields by a 5 mm thick aluminum layer and against static magnetic fields by a 1 mm thick layer of mu-metal (Figure 1A) (Magnetic Shielding, UK). The ambient magnetic field intensity inside the room was attenuated to 0.3 μT and maximum radio frequency intensities between 0.5 to 5 MHz were below 0.01 nT. Magnetic fields were generated using a double-wrapped, custom-built 3D-Helmholtz coil system (Serviciencia, S. L) situated in the center of the shielded room (Figure 1B). Double wrapped coils permit the generation of magnetic stimuli when current flows in parallel through the coils. In contrast in the control condition current flows antiparallel, generating the same heat and vibration to the experimental situation but no magnetic stimulus [40]. The diameters of the coils were: 1,310 mm (x axis), 1,254 mm (y axis), and 1,200 mm (z axis). The coils were driven by DC power sources (TTI CPX400DP) and a computer situated outside the magnetically shielded room. All cables leading into the room were filtered for radio frequencies.

Subjects and Stimulations
Adult pigeons (Columba livia) underwent a surgical procedure to glue (UHU, 37420) a 3D printed polylaurinlactam non-magnetic head-stud to the pigeon skull to restrict head movement during the experiment (Figures 1C and 1D). The animals were habituated to our shielded room, the body harness, and the head fixation apparatus for 30 minutes on 3 consecutive days prior to the experiment. Pigeons were then exposed to a rotating magnetic field of 150 μT (n = 22) or to a zero magnetic field (n = 23). The magnetic field applied replicated that employed by Wu and Dickman. Briefly, it consisted of 360° rotations with 6° steps every 2 s (120 s in total). After each rotation the plane was shifted by 15° and the procedure repeated. This occurred 12 times (a full 360°) around the x axis and then for the y- and z-axes. For the zero-magnetic field control the same protocol was used but currents ran antiparallel through the double-wrapped coils, producing a null magnetic field. Exposure to magnetic stimuli lasted for 72 minutes and was performed either in darkness (n = 30) or under white light (400-700 nm, intensity of ~760 Lux, n = 15). The experimenters were blind to the stimulation conditions at all times. At the completion of the experiment birds were immediately sacrificed and perfused intracardially with 200 ml of 4% PFA in PBS (Sigma Aldrich, 158127). The brains were dissected, postfixed in 4% PFA for 18 hours at 4°C, dehydrated in 30% sucrose (Sigma Aldrich, S9378) for 3 days at 4°C and sectioned in the coronal plane on a sledge microtome (40 μm).

Immunohistochemistry on brain sections
The vestibular nuclei were identified using a pigeon brain atlas [8]. Three sections between stereotaxic coordinates P 2.25 and P 2.50 were selected for analysis. Sections were mounted on glass slides, dried for 2 days at room temperature followed by 3 washes in PBS (5 minutes each). Antigen retrieval was then performed in a water bath using antigen unmasking solution heated up to 90°C over 1 hour (Vector Laboratories, H-3301). After another washing step (3x5 minutes in PBS), slides were incubated with the C-FOS antibody (1:1500, Santa Cruz, sc-253) in 4% milk/0.3% Triton X-100/PBS for 6 hours at room temperature. Slides were washed 3x5 minutes in PBS, incubated with the secondary antibody (1:1000, anti-rabbit, Vectastain Elite ABC HRP Kit, PK-6100, Vector Laboratories) for 2 hours at room temperature, followed by another washing step and incubation with the AB reagent (Vectastain Elite ABC HRP Kit, PK-6100, Vector Laboratories) for 1 hour. After another round of washing, slides were incubated in 0.06% Diaminobenzidine (Sigma Aldrich, D5905) in PBS supplemented with 0.08% H2O2 (Merck, 822287) for 1 minute, followed by 3x5 minute washes in PBS, dehydration in serial dilutions of ethanol and coverslipping. All sections of all birds underwent the staining procedure at the same time to minimize variation in background staining.

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Cryostat             | Thermo Fischer | MICROM HM 560 |
| Laser scanning confocal microscope | Zeiss | LSM780 |
| Tissue lyser II      | Quiagen | 128091236 |

Continued
Counting and statistical analysis
Slides were scanned on a slide scanner with a 20x objective (Pannoramic 250 Flash III, 3DHistech) and the vestibular nuclei manually segmented (6 bilateral segments from 3 sections) using Pannoramic Viewer (Pannoramic Viewer 1.15.4, 3DHistech). Segments were exported as TIFF files for further analysis. Automated identification and counting of C-FOS positive nuclei was performed by custom made rule-sets using a machine-learning algorithm embedded in the Definiens Architect software (Definiens Architect XD, Definiens Software). The number of C-FOS positive cells per mm² was calculated. To analyze the effects of the magnetic stimulation on neuronal activation we performed two-way ANOVAs, using the factors: magnetic treatment, light condition and their interaction. Prior to execution of this statistical test we checked all four groups using the Shapiro-Wilk normality test and found that all groups did not differ from normality. We used the software R [41] for all statistical analyses. Figures were generated using Graphpad Prism (Prism 7 for Mac OS X).

Generation of cell density maps
The mean distance between C-FOS positive nuclei was used to generate heatmaps. Specifically, we employed the following algorithm: 
\[ I = \frac{1}{2} \left( \frac{1}{2} M D o + \sum M D \right) / (2 + N) \]
where \( I \) in the intensity, \( M D o \) is the mean distance to border of a neighboring positive cell, \( M D \) is the sum of the mean distances, and \( N \) is the number of neighboring cells. Heatmaps were averaged for each treatment group by registration onto a reference template. Section borders were corrected for mapping errors. From averaged heatmaps, a differential algorithm: 
\[ \Delta I = \frac{I_{n+1} - I_n}{I_n} \]
was used to estimate the electric field, \( \Delta I \).

Modeling of electromagnetic induction in an artificial pigeon semicircular canal
Polystyrene tubing (0.8 cm inner diameter, 1.2 cm outer diameter) was used to build a replica of a pigeon semicircular canal with a diameter of 21 cm and a circumference of 69 cm (Figure 3A). The tubing was filled with artificial pigeon endolymph consisting of 141.35 mM potassium, 0.23 mM calcium and 141.81 mM chloride in monoQ H₂O [17]. The osmolarity was adjusted to 293 mOsm/L with D-mannitol (Sigma Aldrich, M4125) and pH was adjusted to 7.4. Both ends of the loop were closed with gold plated electrodes and connected to a nanovoltmeter (Keithley Nanovoltmeter Model 2182A). The tubing was positioned in the center of our Helmholtz coil system and exposed to a rotating magnetic field of 150 μT around the vertical axis (6° step changes every 2 s, 120 s in total). The induced voltage in the artificial semicircular canal was measured using the nanovoltmeter. The voltage measurement was performed every 10 ms with signal integration between two measurement points to enhance the signal-to-noise ratio. Afterward, the recorded signal was filtered with a digital Butterworth filter (library functions butter and filtfilt part of the python package signal in scipy; the used parameters were as follow: order of the filter = 5, critical frequency = 0.15) to reduce the low frequency background from the measurement system.

Physical calculations
To estimate the diameter of each semicircular canal we measured from the center of the bony canal to the vestibule (n = 3 birds), drawing on previously published CT reconstructions of the pigeon inner ear [18]. The induced voltage, \( u \), is defined as 
\[ u = \frac{1}{2} \left( \frac{1}{2} B_0 + \sum B \right) / (2 + N) \]
where \( B \) is the magnetic flux. Using the geometry of the set-up this expression can be written as 
\[ u = \frac{1}{2} \left( \frac{1}{2} B_0 + \sum B \right) / A \]
with \( A \) the area enclosed by a semicircular canal. The electric field, \( E \), in the semicircular canal is calculated with 
\[ E = \frac{u}{2r} \]
where \( r \) is the radius of the semicircular canal and \( u \) the measured induced voltage. We then applied a scaling factor which by dividing the radius of one semicircular canal by the radius of the artificial inner ear. To estimate the electric field, \( \Delta E_n \), generated by natural head movement we employed the following equation: 
\[ \Delta E_n = B_0 \cdot \pi r \]
where \( r \) is the radius of the semicircular canal, \( B_0 \) is the Earth’s field, and \( f \) is the frequency of head scanning.

Immunohistochemistry on semicircular canal sections
Pigeon temporal bones containing intact inner ears were fixed overnight in 4% PFA. Following a wash in PBS, ampullae in the membranous labyrinth were dissected under a stereomicroscope. Tissues were dehydrated in 30% sucrose/PBS overnight, embedded in Neg-50 Frozen Section Medium (Thermo Fisher, 6502) and sectioned (10 μm) on a cryostat (Thermo Fisher, MICROM HM 560). Slides were dried at RT for at least 1 h prior to staining, washed in 3 × 5 min in PBS, and exposed to heat-based antigen retrieval (Vector Laboratories, H-3301). After a 30 min cooling period and 3 × 5 min washes in PBS, slides were incubated overnight with antibodies diluted in 0.3% Triton X-100/PBS (Sigma Aldrich, X100) with 2% donkey serum (Abcam, ab7475). The following concentrations were used: 1:300 Ca,1.3 (Alomone labs, ACC-005), 1:300 BK (Santa Cruz, sc-374142), 1:500 acetylated Tubulin (Sigma-Aldrich, T6793), 1:300 Otoferlin (Santa Cruz, sc-50159), 1:500 Syntaxin 1 (Santa Cruz, sc-20036). The next day, slides were washed 3 x in PBS for 5 min and incubated with donkey anti-rabbit Alexa Fluor-488 (Thermo Fisher, A-21206), donkey anti-goat Alexa Fluor-568 (Thermo Fisher, A-21206), and donkey anti-mouse Alexa Fluor-647 (Thermo Fisher, A-31571), each diluted 1:500, for 1 h at 4°C. After counterstaining with DAPI and 3 × 5 min washes in PBS, slides were mounted with Fluorescent Mounting Medium (Dako, S302380). Images were acquired using a laser scanning confocal microscope (Zeiss, LSM780), and processed in ImageJ [42].

PCR analysis of Ca,1.3 (CACNA1D) splice isoforms
Tissue samples (brain, pineal gland, retina, cochlea, vestibular epithelia, respiratory coochae, skin, heart, muscle, liver, and spleen) were collected from adult pigeons (Columba livia), snap-frozen in liquid nitrogen, and mechanically homogenized with a tissue lyser.
(QIAGEN Tissue Lyser II, 128091236). For the vestibular epithelia the ampullae, lagena, utricle and saccule were pooled. Total RNA was extracted from tissue lysates using the RNeasy mini kit (QIAGEN, 74104) and reverse transcribed with a Quantitect Reverse Transcription Kit in accordance with the manufacturers’ instructions (QIAGEN, 205313). The cDNA libraries were diluted to a working concentration of 1:100 and stored at –20°C. Polymerase chain reaction (PCR) primers flanking exon 28a were designed based on available genomic resources. The primer sequences were F: CAGGAGTGTTCACTGTTGA; and R: TATTGGCAGCATAGTAGTGACGT. PCR amplification of the tissue cDNA libraries was performed with the Phusion Hot Start Flex DNA polymerase (NEB, M0535S). PCR products were analyzed by agarose gel electrophoresis (4%).

**PCR analysis of BK (KCNMA1) splice isoforms**  
To determine the tissue specific expression of different BK (KCNMA1) splice isoforms we designed an assay that relies on PCR amplification of a 239 bp fragment that spans exons 8 to 10 followed by a restriction digest. The primer sequences were F: CAGCCAC TAACGTATGGG; and R: TCGCTACGTGCCAG. We amplified the aforementioned cDNA libraries using Phusion Hot Start Flex DNA polymerase (NEB, M0535S). A restriction digest was then performed using AluI (NEB, R0137S) which discriminates between exon 9a (SI isoform; TTT-GCC-AG^C-TAC) and exon 9b (RA isoform; TTT-GCT-CGC-TAC; no target). PCR products were digested following gel purification for one hour at 37°C and analyzed by agarose gel electrophoresis (2%). Exon 9a and 9b isoforms amplicons from vestibular tissue were TA-cloned into pCR4-TOPO (Thermo Fischer, 45-0030) in accordance with the manufacturer’s protocol and their sequence confirmed.

**Phylogenetic analysis of Ca,1.3 (CACNA1D) and BK (KCNMA1) splice isoforms**  
We explored the taxonomic distribution of the 10 amino acid insertion in the pore-forming alpha1D subunit of Ca,1.3 (CACNA1D) and RA isoform in the BK (KCNMA1) channel using a sequence-similarity based strategy. To identify CACNA1D homologs containing the KKER insertion we performed a BLASTp search with a 50 amino acid long segment of pigeon CaV1.3 (centered around the insertion) against the NCBI non-redundant protein database (NP_990365.1:1264-1313; BLASTP v2.8.1+; limited to the top 10000 target sequences). Only sequences with similarity in 5 out of the 10 amino acid positions were kept. In the case of the BK channel a BLASTp search was performed with the RA isoform ± 20 flanking amino acids against the NCBI non-redundant protein database. We derived species phylogenetic trees using NCBI’s Common Tree and iTOL v4.

**QUANTIFICATION AND STATISTICAL ANALYSIS**  
To analyze the effects of the magnetic stimulation on neuronal activation we performed two-way ANOVAs, using the factors: magnetic treatment, light condition and their interaction. Prior to execution of this statistical test we checked all four groups using the Shapiro-Wilk normality test and found that all groups did not differ from normality. We used the software R [41] for all statistical analyses. One animal was excluded from analysis because of methodological problems (sections detached from the slide and could not be further analyzed). The exact sample sizes, means, and standard deviations can be found in the results section of the paper (one “n” is defined as one animal). We defined a significant result with p < 0.05 throughout the paper. The treatment conditions were randomized on each test day and the experimenters where blind to the applied treatments.

**DATA AND CODE AVAILABILITY**  
The rule-sets used to quantify C-FOS positive cells in the current study have not been deposited in a public repository as they are embedded in the Definiens Architect software but the used parameters are available from the corresponding author on request.