Magnetic field changes activate the trigeminal brainstem complex in a migratory bird

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The upper beak of birds, which contains putative magnetosensory ferro-magnetic structures, is innervated by the ophthalmic branch of the trigeminal nerve (V1). However, because of the absence of replicable neurobiological evidence, a general acceptance of the involvement of the trigeminal nerve in magnetoreception is lacking in birds. Using an antibody to ZENK protein to indicate neuronal activation, we here document reliable magnetic activation of neurons in and near the principal (PrV) and spinal tract (SpV) nuclei of the trigeminal brainstem complex, which represent the two brain regions known to receive primary input from the trigeminal nerve. Significantly more neurons were activated in PrV and in medial SpV when European robins (*Erithacus rubecula*) experienced a magnetic field changing every 30 seconds for a period of 3 h (CMF) than when robins experienced a compensated, zero magnetic field condition (ZMF). No such differences in numbers of activated neurons were found in comparison structures. Under CMF conditions, sectioning of V1 significantly reduced the number of activated neurons in and near PrV and medial SpV, but not in lateral SpV or in the optic tectum. Tract tracing of V1 showed spatial proximity and regional overlap of V1 nerve endings and ZENK-positive (activated) neurons in SpV, and partly in PrV, under CMF conditions. Together, these results suggest that magnetic field changes activate neurons in and near the trigeminal brainstem complex and that V1 is necessary for this activation. We therefore suggest that V1 transmits magnetic information to the brain in this migratory passerine bird.

bird migration | magnetic sense | magnetite | magnetoperception | magnetoreception

Birds and other animals move over great distances. These movements require good orientation and navigation abilities. Information from the Earth's magnetic field has been shown to be one of several sources for orientation and/or navigational information (1–6). In principle, the Earth's magnetic field could provide birds and other animals with two fundamentally different kinds of information. The direction of the magnetic field lines forms the basis for a magnetic compass sense (1, 3, 6, 7), and magnetic intensity and/or inclination could provide positional information for a putative magnetic map or signpost sense (2, 5, 8–10); but how do birds and other animals sense information from the Earth's magnetic field?

In recent years, mounting behavioral and anatomical evidence has been accumulating that birds, at least, might have two independent magnetic senses: (*i*) iron-mineral-based sensors located in the upper beak, which are innervated by the ophthalmic branch of the trigeminal nerve (V1) (8, 9, 11–16), and (*ii*) a light-dependent chemical sense which is embedded in parts of the visual system (7, 9, 16–21). However, considerable scientific skepticism remains regarding both of these proposed magnetic senses because, so far, in birds, the studies that have reported changes in neurophysiological activity in response to magnetic field changes differ in their conclusions, could not be independently confirmed, and are likely to have been subject to artifactual difficulties (22–24).

Therefore, the aim of this study was to test whether neurons in brain regions innervated by V1 are activated by magnetic field changes in awake, unrestrained European robins and whether V1 is required for this activation. Consequently, we used a nonelectronic technique: behavioral molecular mapping based on quantification of the neuronal activity-dependent marker ZENK (19, 20, 25–28). The major advantages of a behavioral molecular mapping approach compared with an electrophysiological approach are that we could obtain, in a noninvasive manner, a record of neuronal activation in the brain from awake, unrestrained birds, and that the potential artifacts often associated with the combination of electrophysiology and magnetic field stimuli could be avoided.

We exposed four sham-sectioned birds to a compensated zero magnetic field (ZMF) that did not provide any magnetic information (0 ± 300 nanoTesla [nT]). All sham-sectioned birds underwent the same operational procedure as the sectioned birds: V1 was located and handled with forceps just as in the birds receiving a real section, only in the sham-sectioned birds, the nerve was not cut. Another five sham-sectioned birds and six birds that had ~3 mm of the ophthalmic branch of the trigeminal nerve (V1) surgically removed were exposed to a strongly changing magnetic field (CMF). The CMF condition consisted of two types of magnetic stimulation, which alternated every 5 min. During the first 5 min, the magnetic field turned 90 ° every 30 s around the horizontal axis with approximately the same inclination (67.6 ± 0.8 °) and intensity (48,800 nT ± 400 nT) as the local geomagnetic field in Oldenburg. During the next 5 min, every 30 s, each of the three axes of the magnetic field were varied randomly and independently between −70,000 nT and +70,000 nT resulting in a magnetic field that varied strongly in field intensity (18,500–111,000 nT), horizontal direction (0–359 °) and inclination (−84.9 to +76.6 °). This alternating procedure was repeated continuously for at least 3 h. The randomized aspects of the stimuli were newly generated for each 5-min period.

The CMF stimulation protocol was chosen because the exact nature of the stimuli the putative sensors in the upper beak are tuned to detect is unknown. Consequently, the first 5-min stimulus period was tuned to optimally stimulate any receptor that would sense changes in the horizontal component of the geomagnetic field (i.e., a magnetic compass sensor) and/or any sensor that might detect small changes in magnetic inclination and/or intensity (which is what a biologically relevant magnetic map-sensor should theoretically be tuned to do, if a magnetic map sense is useful over distances of tens of kilometers or less). The second 5-min stimulus period was designed to optimally stimulate any magnetic sensor that would respond best to large changes in any of the three magnetic parameters: inclination, direction, and intensity up to about double the geomagnetic field strength (14). The alternating combination of two types of stimulus periods reduced the risk of

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Table 1. Experimental design

Group name	*N*	Magnetic field	Type of surgery on V1
CMF	5	Changing magnetic field	Sham sections
ZMF	4	Zero magnetic field	Sham sections
CMF sect	6	Changing magnetic field	Real sections
Tracing	3	Changing magnetic field	No sectioning

sensory adaptation and/or long-term potentiating effects, bearing in mind that the birds were stimulated for a full 3 h (the reasoning behind the choice of 3 h is given in *Methods*).

The central processes of V1 neurons in pigeons and ducks (29–31) enter the ipsilateral brainstem in the trigeminal sensory root, which immediately divides into an ascending and a descending tract. The ascending tract turns dorsally to terminate in PrV, whereas the descending tract descends throughout the dorsolateral aspect of the brainstem to terminate in the subnuclei of SpV (Fig. 1*A*). We therefore counted the number of activated neurons in PrV and SpV on both sides of the brain of 18 European robins (*Erithacus rubecula*) subjected to different combinations of experimental treatments (Table 1). The experiments were carried out during the spring or autumn migratory season.

Results

First, we consider the neuronal activation seen in the sham-sectioned birds experiencing different magnetic field conditions. Based on counts of the number of ZENK-positive neurons in the brains of European robins experiencing either a zero magnetic field (ZMF) or a changing magnetic field (CMF), we observed strong magnetic activation of neuronal subpopulations within both PrV and SpV. Within PrV (as defined by acetylcholine esterase staining, Fig. 1*C*), an average of 852 ± 298 (SD) ZENK-expressing neurons were counted when birds experienced a CMF (Fig. 1 *B* and *D*), compared with an average of only 325 ± 92 ZENK-expressing neurons in birds experiencing a ZMF (Fig. 1 *B* and *E*). Because we counted only every second slice, the absolute number of activated neurons is likely to be approximately twice these averages. The number of counted neurons represents a 162% increase in the CMF condition compared with the ZMF condition. The great majority of this labeling occurred in a crescent-shaped region described for present purposes as ventral PrV. However, on the basis of our tracing experiments, it was not clear whether this region, in addition to PrV proper, also received a primary projection from the ophthalmic nerve (V1), and the region may, in fact, lie ventral to PrV proper.

Within the 12 counted sections of SpV (Fig. 1 *G* – *N* and Fig. S1), an average of 804 ± 332 neurons was activated in the CMF condition compared with an average of only 326 ± 92 neurons in the ZMF condition (*t* test, $t = 2.76$, $df = 7$, $P = 0.028$). On closer examination, it was obvious that activation of the medial parts of SpV was responsible for this significant difference in activation, whereas the lateral part of SpV showed no obvious difference between the ZMF and CMF conditions (statistical data given below). Therefore, for further analyses, we divided SpV into lateral and medial parts (Fig. 1 *G–N)*, and ZENK-positive cells were counted separately in the two parts. Within the 12 counted sections of medial SpV, an average of 513 ± 304 neurons were activated in the CMF condition (Fig. 1 *B, H,* and *L*), compared with an average of only 120 ± 44 neurons in the ZMF condition (Fig. 1 *B, I,* and *M*), which represents more than a 300% increase in the CMF condition.

Second, we consider the effects of bilateral lesion of V1. Bilateral section of the ophthalmic branch of the trigeminal nerve (V1) significantly reduced the number of ZENK labeled neurons in PrV (Fig. 1 *B* and *F*) and in medial SpV (Fig. 1 *B, J,* and *N*) when the robins were exposed to the CMF condition. In contrast, sham surgery had no such effect (Fig. 1 *B* and *D* for PrV; Fig. 1 *B* and *L* for medial SpV). Sectioning of V1 may also have slightly reduced the number of activated neurons in the tegmentum ventromedial to SpV (Fig. 1 *N*), but not significantly so.

Significantly more neurons were ZENK positive in the sham-sectioned birds experiencing a changing magnetic field compared with the sham-sectioned birds experiencing a zero magnetic field and compared with the V1-sectioned birds experiencing the CMF condition (PrV: one-way ANOVA, $t = 4.061$ and $t = 5.259$ respectively, $P < 0.01$; medial SpV: one-way ANOVA, $t = 3.299$ and $t = 3.827$ respectively, $P < 0.01$). No significant differences were observed between the sham operated ZMF group and the V1-sectioned CMF group (PrV: one-way ANOVA, $t = 0.714$, $P = 0.49$; medial SpV: one-way ANOVA, $t = 0.162$, $P = 0.87$). No significant differences between treatments occurred in lateral SpV (one-way ANOVA, $F = 1.555$, $P = 0.25$) between the CMF (237 ± 124 activated neurons), the ZMF (188 ± 53 activated neurons) and the V1-sectioned birds (132 ± 97 activated neurons).

Third, we assess whether an effect of a changing magnetic field on general neuronal activation occurred in our experiments. In addition to the lack of a significant difference in activation observed in lateral SpV, we quantified neuronal activation in the optic tectum, as the visual input should have been similar in all birds. We observed no significant differences between the three magnetic field groups in the number of ZENK-positive neurons in control sections taken from a 200-μm-thick slice of the optic tectum (CMF: 464 ± 78 neurons; ZMF: 409 ± 100 neurons; CMF V1-sectioned: 468 ± 67 neurons; one-way ANOVA, $F = 0.766$, $P = 0.49$; Fig. 1*B*). In addition to ZENK-positive neurons being found in PrV, SpV, and the optic tectum, scattered labeled neurons occurred within the radix of the trigeminal nerve (Fig. 1*D*) and in the tegmentum ventromedial to SpV (Rt, Fig. 1 *H–J* and *L–N*).

Fourth, neuronal tract tracing of V1 in European robins (Fig. 2) showed terminations of the trigeminal ophthalmic nerve (V1) within the ipsilateral SpV and PrV. No tracer signal was observed contralateral to the injected side. We found clear spatial proximity and regional overlap of the ophthalmic nerve terminal field and positive ZENK labeling (Fig. 2) in a corresponding section of SpV from a different robin experiencing CMF. (Double labeling in the same individual was considered unacceptable, because we could not rule out the possibility that injection of tracer into the nerve would damage it and so influence its normal function, thereby detrimentally influencing neuronal activation and thus ZENK expression.) ZENK-positive neurons were also found in neighboring parts of SpV that did not include the terminal zone.

Fifth, we considered potential alternative explanations for the observed differences in neuronal activation. Because V1 is known to be activated by mechanical stimulation of the upper beak, we tested whether there was any correlation between mechanical stimulation of the beak and the number of activated neurons within SpV and PrV. Observations using an infrared light–based video camera during the experiments suggested that the magnetic field condition did not systematically influence the birds' motor activity. In the 10 birds for which behavioral videos exist, we quantified how much mechanical contact the beak of each bird experienced during the 3-h magnetic stimulation period. Because we do not know how strong a mechanical contact needs to be to putatively activate PrV and SpV neurons, we made two separate counts: one count including "total beak contacts," i.e., contacts between the beak and the wall or the perch, and any grooming behavior; and another count of "hard beak contacts" only, i.e., contacts between the beak and the cage wall, which occurred only during flights. There was no significant correlation between the number of "total" or "hard" beak contacts" and the number of activated neurons within SpV and PrV (Fig. S2). Among the CMF birds, the bird showing the largest number of activated neurons in PrV (1,319 neurons) and medial SpV (860 neurons) showed by far the lowest number of beak contacts (192 total, 111 hard). In contrast, the CMF bird which showed the lowest number of activated neurons (778 neurons in PrV and 144 neurons in medial SpV) experienced almost eight

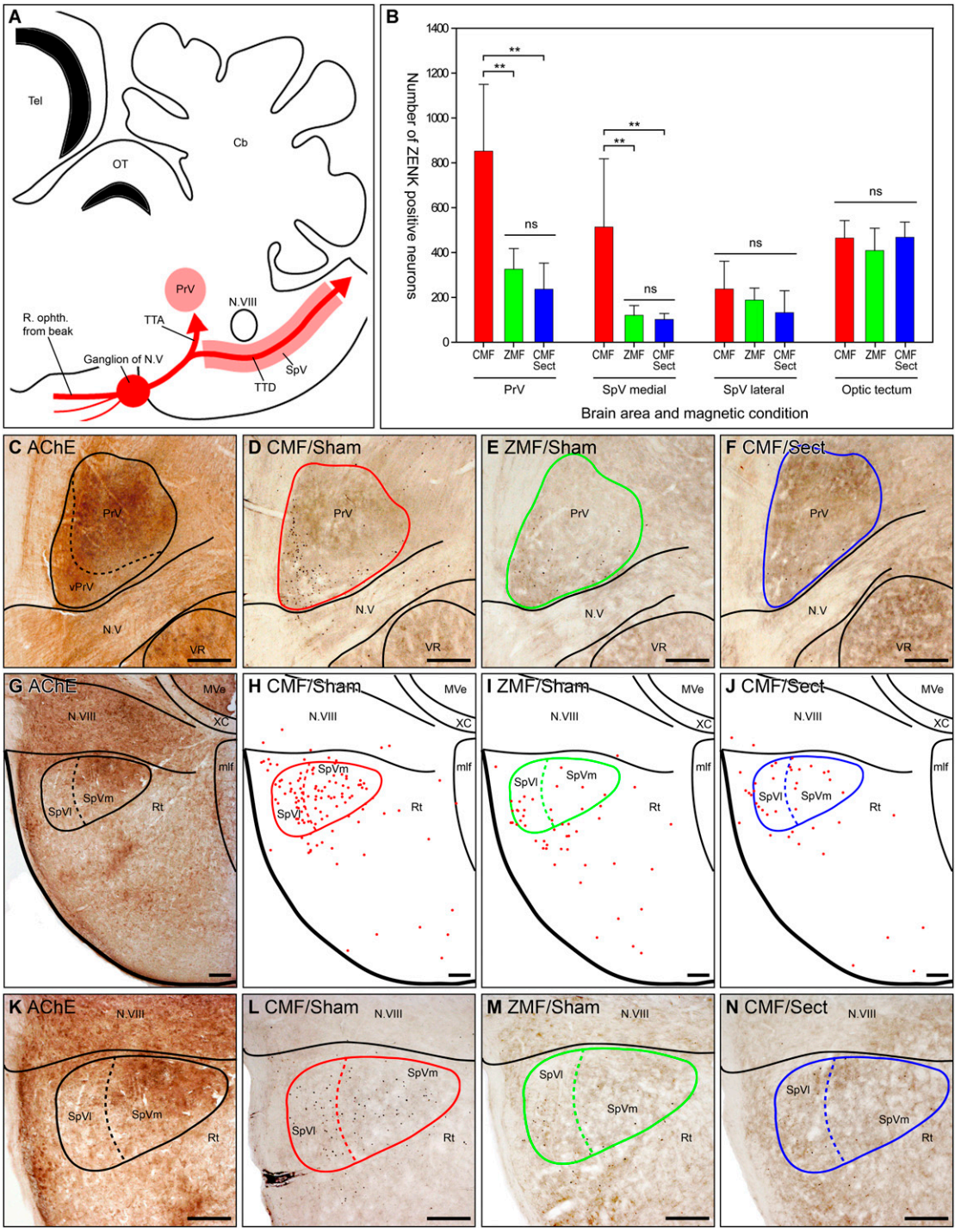

Fig. 1. Magnetic field changes induce ZENK activation in the trigeminal system. (*A*) Schematic illustration of the avian trigeminal sensory system; dorsal is up and anterior is left. Neuronal somata of all three branches of the trigeminal nerve are located in the trigeminal ganglion. Their afferents give rise to an ascending (TTA) and a descending tract (TTD), which terminate in PrV and SpV, respectively. (*B*) Quantification of ZENK activated neurons (black spots in *D–F* and *L–N*) in PrV, the optic tectum, and in medial and lateral SpV. Sham-sectioned birds experiencing changing magnetic field (CMF) conditions are shown in red; sham-sectioned birds experiencing zero magnetic field (ZMF) conditions are shown in green; and birds with sectioned V1 experiencing CMF conditions are shown in blue. $^{**}P < 0.01$, ns, no significant difference. (*C–F*) Frontal brain sections show strongly increased nuclear ZENK expression in PrV, particularly in a crescent-shaped structure ventral (to) PrV, when the birds experienced CMF conditions and were tested with an intact ophthalmic branch of the trigeminal nerve (V1) (*D*). This activation disappeared when the magnetic field stimuli was removed in birds with an intact V1 (*E*) and in birds experiencing the CMF condition when V1 was cut (*F*). (*G–N*) Frontal brain sections through SpV at the level of the vestibulo-cochlear nerve (N. VIII) also show strongly increased ZENK expression in medial (SpVm) but not in lateral (SpVl) parts of SpV in birds with an intact ophthalmic nerve experiencing CMF conditions compared with the other two conditions. (*H–J*) Schematic illustration (original data supplied as Fig. S1) of ZENK expressing neurons (red dots). (*K–N*) Magnified detail of SpV (original data; black spots are ZENK-positive neurons). Quantified areas are encircled in red (*D, H,* and *L*; sham-sectioned, CMF), green (*E, I,* and *M*; sham-sectioned, ZMF), and blue (*F, J,* and *N;* V1-sectioned, CMF). Acetylcholinestarase (AChE) activity (*C, G,* and *K*) helped to define anatomical boundaries. (Scale bars, 200 μm.) Cb, cerebellum; LSO, lateral superior olivary nucleus; mlf, medial longitudinal fascicle; N.V, trigeminal nerve; N.VIII, vestibulo-cochlear nerve; OT, optic tectum; PrV, principal sensory nucleus of the trigeminal nerve; Rt, reticular formation; SpVl/m, spinal trigeminal nucleus, lateral/medial portion; Tel, telencephalon; TTA, ascending branch of the sensory trigeminal tract; TTD, descending branch of the sensory trigeminal tract; vPrV, ventral (to) PrV; XC, cochlear decussation.

times as many beak contacts (1,431 total; 852 hard). Thus, the number of beak contacts did not significantly influence the neuronal activation of PrV and the quantified parts of SpV, whereas the magnetic field condition had a highly significant effect on the number of activated neurons in both SpV and PrV.

Discussion

The tracing results combined with the neuronal activation data suggest that neuronal activation in response to the changing magnetic field is not confined to those parts of the trigeminal brainstem complex that receive the terminations of the ophthalmic branch of the trigeminal nerve (V1). This is unlikely to be explained by magnetic activation mediated by the other two trigeminal branches, because the activation in the ZMF and V1-sectioned CMF condition was very similar. If magnetic information had been transmitted through either of the other two branches of the trigeminal nerve, the V1-sectioned CMF group should have shown higher neuronal activation (as V2 and V3 were left intact) than the ZMF group, which we did not observe. Therefore, our findings suggest that the ophthalmic branch of the trigeminal nerve (V1) is the only trigeminal branch to mediate the effects of the magnetic stimuli that were observed in PrV and SpV and that the "extra" labeling outside the primary V1 terminal zone probably represents magnetic activation via a multisynaptic mechanism. These results are entirely consistent with previous anatomical studies, which have shown that the ophthalmic branch of the trigeminal nerve (V1) is the only branch to innervate candidate ferromagnetic structures in the upper beak (8, 13).

It is almost certain that magnetic information is transmitted to higher brain centers. The majority of projections from PrV terminate in nucleus basorostralis (Bas) (32); but, unfortunately, this region does not express ZENK (26). Primary projections from SpV terminate within other parts of the hindbrain (31), but these projections are not known for European robins. Thus, at the current time, our method could not examine any putative effects of magnetic stimuli in these brain regions.

The absolute increase in the number of activated neurons in the CMF condition compared with the ZMF and sectioned CMF condition should not be considered as an accurate estimate of the total number of neurons in PrV and SpV being activated by magnetic stimuli. The counts likely underestimates the true number of magnetically activated neurons, as the randomness of the magnetic stimuli is likely to have triggered excitatory (increases in ZENK expression) as well as inhibitory (no change or reduction of ZENK expression) responses from cells in the target nuclei. It is therefore likely that stimulation with the specific magnetic stimuli to which the sensors are tuned to respond in nature could have led to a stronger activation than that brought about by the changing magnetic field stimuli used in the present experiment. However, at the present time, these optimal magnetic stimuli are unknown.

We suspect that magnetic information from the upper beak is used in a map or signpost sense (9, 10, 33, 34). Mora et al. (14) showed in a conditioning experiment that pigeons required intact ophthalmic branches of the trigeminal nerve (V1) to detect a strong magnetic anomaly, although Gagliardo et al. (35–37) showed that V1 sectioned pigeons of all ages and levels of homing experience homed as well as control birds. Together, these results suggest that, although pigeons can detect magnetic information, V1 is not generally required for successful homing in this species. In European robins, we showed that the ophthalmic branch of the trigeminal nerve is neither necessary nor sufficient for successful magnetic compass orientation, whereas a visual brain area named Cluster N is necessary for successful magnetic compass orientation (7). Similar results suggesting the absence of a relation between the trigeminal nerve and magnetic compass orientation have been reported in other species, such as Bobolinks, where anesthetic blockade of the trigeminal nerve also failed to affect compass orientation (38). The results of Zapka et al. (7) exclude the possibility that V1 carries primary magnetic compass information in European robins. Thus, the magnetic activation seen in the present experiment is unlikely to have been triggered by magnetic compass information alone. It is more likely that V1 carries information about magnetic intensity and/or magnetic inclination in European robins; but, at the present time, the functional significance of the magnetic activation carried by V1 remains unclear.

We found no magnetic field dependent neuronal activation in the optic tectum. Here, it is important to realize that the optic tectum, which was chosen as a control region to assess whether an effect of a changing magnetic field on general neuronal activation occurred in our experiments, is unlikely to be involved in light-dependent magnetic sensing. The reason is that the optic tectum is part of the tectofugal visual pathway, whereas it has been shown that "Cluster N," which is required for magnetic compass orientation (7), is part of the thalamofugal visual pathway (21). Thus, the light-dependent magnetodetection hypothesis is neither supported nor excluded by the present study.

In conclusion, this study shows that a changing magnetic field condition leads to significant changes in neuronal activation in brain areas receiving primary trigeminal input and that, in European robins, the ophthalmic branch of the trigeminal nerve (V1) is required for this magnetically induced increase in neuronal activation. Strictly speaking, however, these data cannot prove that V1 transmits magnetic information to the brain or that the trigeminal brainstem complex traffics in magnetic information. More direct methods, such as electrophysiological recordings from trigeminal neurons in response to magnetic stimulation, would be required to prove this; however, as pointed out earlier here, such methods are very prone to the production of artifactual results (22–24); hence, the necessity for the present, strongly indicative study using more

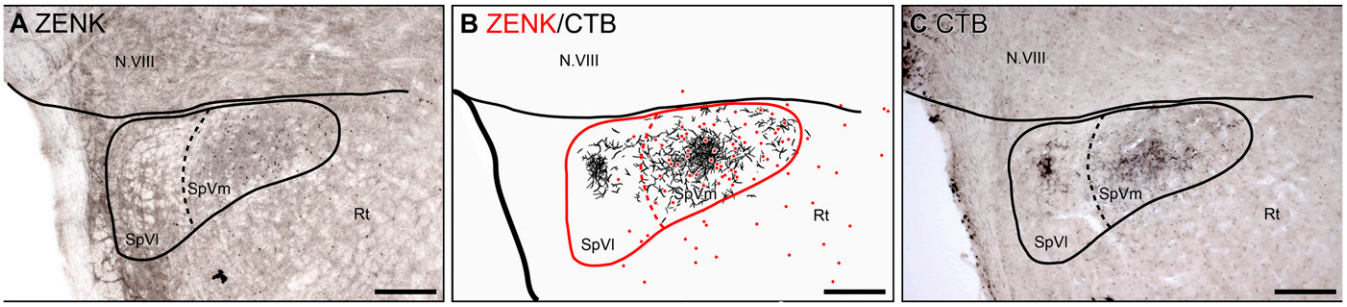

Fig. 2. Tract tracing of V1 shows spatial proximity and regional overlap of V1 nerve endings and magnetically activated neurons in SpV. (*A*) Staining against ZENK protein in a nonsectioned CMF bird (black spots are ZENK-positive neurons). (*C*) Cholera-toxin–labeled V1 nerve terminal fibers in SpV (black label). Heavy metal intensification used also enhances visualization of cell bodies somewhat, as can be seen in Fig. 2*C*; but this is purely a nonspecific effect that can easily be distinguished from the specifically CtB labeled fibers. (*B*) Schematic overlay of *A* and *C*. Black hairlines indicate the CtB-positive terminal nerve fibers shown in *C*; red circles with white borders indicate ZENK-activated neuronal nuclei from *A*. (Scale bars, 200 μm.) Abbreviations as in legend to Fig. 1.

indirect methods. The data also suggest that the ophthalmic branch of the trigeminal nerve (V1) in European robins innervates a primary magnetic sensor in the upper beak and support the idea that iron-mineral–based structures found in the upper beak of birds (8, 12, 13) including European robins (15), can sense information from the ambient geomagnetic field.

Methods

Lesions and Magnetic Field Exposures. We exposed 18 European robins (*Erithacus rubecula*) with or without intact ophthalmic branches of the trigeminal nerve (V1) to specific magnetic fields, as summarized in Table 1. In six birds, ~3 mm of V1 was surgically removed bilaterally under general anesthesia (Fig. 1). Access to the nerve was gained within the orbit by gentle retraction of the eyeball, and refusion of the nerve was prevented by sealing the cut ends with surgical glue. After 5–7 days of recovery, the robins were placed singly in a 40-cm-diameter cylindrical cage, fitted with a 20-cm-diameter round perch (39), in which the bird was free to move. The birds were placed in the cages around sunset and spent most of the time on the circular perch from which they were not able to touch the sides of the cage. The light intensity was set to a value typically used for orientation cage experiments with night-migratory songbirds (2 mW/m^2, equivalent to moonlight). The light was produced by incandescent light bulbs (spectrum given in the online supplementary material accompanying ref. 7).

The magnetic field stimuli were produced by double-wound, 3-axial, Merritt 4-coil systems (40) placed inside wooden huts 4 × 4 × ~2.5 m. The magnetic stimuli were as described earlier here. The power supplies were placed outside the experimental room. The power supplies were always on; any remaining noise from the power supplies was the same, irrespective of magnetic field condition, because their noise does not depend on how much current they send through the coils.

The behavior of the birds was monitored on a video screen to ensure that all birds used in this study were awake during the vast majority of the relevant 3 h. The behavior of 10 of the 14 birds was stored on video tapes (for the remaining four birds, the video recorder malfunctioned). An observer who did not know which magnetic field and operation the bird had been exposed to later used these videos to quantify how many times each bird touched anything within the cage with its beak.

Behavioral Molecular Mapping. Behavioral molecular mapping is based on the detection of immediate early genes such as ZENK (acronym for zif268, Egr-1, Krox 24), which is driven by neuronal activity. ZENK is expressed in most, but not all, neuron types. The exceptions in birds are some thalamic neurons, telencephalic thalamorecipient neurons, and globus pallidus neurons (26–28). Thus, in most other neuron types, which constitute roughly two-thirds of the brain, ZENK expression follows neuronal firing. ZENK protein can be detected in neurons ~15 min after onset of neuronal firing, with peak expression after 60–90 min (26–28). Therefore, accumulation of ZENK protein marks neurons showing increased activity during ~120 min before the animal is killed (26–28). A high level of ZENK protein expression can be kept for several hours, given that the stimulus is not too monotonous. We chose to expose our birds to the given magnetic stimulus for 3 h because we wanted to make sure that any ZENK activation from placing the bird into the setup and any ZENK activation because of the reduction in light intensity from daylight to 2 mW/m^2 (which both happened at time 0) had subsided by the time that we collected the birds.

Immediately following at least 3 h of exposure to either a changing magnetic field or a zero magnetic field, our birds were deeply anesthetized and transcardially perfused with saline followed by 4% paraformaldehyde dissolved in PBS. At this stage, we first confirmed that V1 had not rejoined in the V1-sectioned birds. None of the sectioned nerves had rejoined. Brains were then extracted, cryoprotected, and serially sectioned at 40 μm in the frontal plane using a freezing microtome. Six parallel series of brain slices were produced for each individual. Every second of the six series was stained free-floating with an antibody against the neuronal activity marker ZENK and a commercially available ABC-Kit (Vector ABC Elite Kit, Vector Laboratories). Slices were sequentially incubated with an antibody raised against ZENK (Santa Cruz) and/or CtB (Sigma), a biotinylated secondary antibody, and an avidin-coupled peroxidase complex. Peroxidase activity was detected using a 3′-3-diaminobenzidine reaction. To ensure comparability between experimental groups, each staining procedure, which involved one series of brain slices from each of four individuals, included at least one series of brain slices from each experimental group. This secured that the inevitable variations in the intensity of the background between staining runs were averaged across groups and thus could not affect the overall conclusions. Stained sections were mounted on glass slides and cover-slipped for microscopic analysis. The borders of relevant structures and brain regions in the robins were determined for each bird by staining one series (i.e., every sixth brain section) for acetylcholine esterase activity (41) (Fig. 1 *C*, *G*, and *K*).

The number of ZENK-positive neurons was counted in every second section through PrV and through SpV at levels of the vestibulo-cochlear nerve (N.VIII) (12 SpV slices in total per individual). Labeled neurons on both sides of the brainstem were counted; because there was no difference between the sides, the reported results reflect the total number of neurons counted bilaterally. The number of ZENK-positive neurons in PrV and SpV was determined by a researcher who was uninformed as to the magnetic treatment and operations that each bird had received. The 55 microscope slides with brain slices were blindly assigned numbers from 1 to 55. When the observer started with a new slide, he/she first looked at the activation in the midbrain auditory torus and in the optic tectum, both of which are not supposed to be influenced by manipulations of the trigeminal system, and both of which were always activated in all birds mainly because of self-motion and the same background noise. Based on the intensity of the staining in these putatively irrelevant regions, we estimated the overall ZENK staining intensity of that particular slide; based on this, the ZENK staining intensity that a nucleus was required to show to be counted as "positive" on that slide was determined. This level was then used as a criterion for counting the ZENK-positive nuclei in the target brain regions. Thereby, comparability between slides was optimized. The validity of this method was confirmed by the fact that the counted number of ZENK-positive neurons in a given brain region was highly consistent between brain slices despite the fact that three sets of brain slices from a given individual were placed on three different microscope slides and thus underwent the procedure described above three independent times. Furthermore, the differences in ZENK activation between the CMF group and the ZMF and sectioned CMF groups are so great that even if small counting inconsistencies had occurred, the overall conclusions would not have been affected. We could have chosen slides for illustration (Figs. 1 and 2 and Fig. S1), which would have made the differences in neuronal activation between the groups appear even more pronounced than shown in the figures; however, we chose to show the most representative slices, which means that the chosen pictures best illustrates the average result seen in all of our studied birds. All results were confirmed by independent counts made by two additional observers. As a control, ZENK-positive neurons were counted in a defined part of the optic tectum, which consistently showed ZENK activity in all birds.

Neuronal Tracing. To identify terminal zones of the central projections of V1 in robins, three additional birds received, under general anesthesia, an injection of 1 μL 1% cholera toxin subunit B (CtB) dissolved in PBS into the nerve as it passes medial to the eye within the orbit. From 3 to 5 days later, these birds experienced CMF conditions, followed by immediate perfusion and further tissue processing as described above. CtB immunoreactivity was detected using an appropriate antibody (21, 42). It can be noted that injections of CtB into V1 do not label any cell bodies in the brain that project their axons into V1, because V1 is purely a sensory nerve. Injection of CtB into V1 at the point used in this study retrogradely labels sensory cell bodies in the trigeminal ganglion (the ganglion is shown in Fig. 1*A*), and, by way of transganglionic transport, the central processes of these cells terminate throughout PrV and SpV. To maximize the visualization of the fibers and terminations that make up these central processes, the ratio of signal (CtB staining) to noise (nonspecific background staining) was enhanced by a commonly used technique of intensifying the CtB label with heavy metals during the visualization step of the immunohistochemical procedure. The CtB labeling of V1 fibers and terminations is thereby rendered as black punctuate staining within the trigeminal brainstem complex, as shown in Fig. 2*C*. Further details of the immunohistochemical detection of ZENK and CtB are as previously described (21).

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