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Presence of stable carbon centric free radicals and ferromagnetic elements in the antennae and the wings of nocturnal silk moth: A magnetic nanostructure for magneto sensing

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ABSTRACT

This work addresses an interesting, interdisciplinary problem of nature- "the mechanism by which animal senses the earth's magnetic field and navigate". Currently there are two existing theories trying to explain, "How the animal senses the magnetic field of earth". One theory is based on the presence of biogenic magnetic materials in the body of the animals. Such magnetic materials which are present inside the body, orients itself according to the earth's weak magnetic field and convey the information to the nervous system to develop the navigational map. The second theory is based on a light dependent photochemical reaction. A photochemical reaction leads to the generation of radical pairs, which helps in sensing the weak magnetic field of the earth. In this work, we are proposing a new model of magnetoreception. Unlike the existing radical pair system of magnetoreception, where a light-dependent reaction is essential to generate free radicals, here we show the presence of a large pool of stable carbon-centric free radicals in the nano-domains of the antennae and the wings of silk moth. This stable pool of carbon-centric free radicals is intrinsic in the nano-domains of these anatomical structures and responds to weak magnetic fields similar to that of Earth's (50 μT) even in the absence of light. Hence we are proposing that nocturnal animals in their navigation could utilize such a light independent mechanism. We further observed the presence of ferromagnetic elements (Fe, Ni, Co, Mn) in these structures. In conclusion, we have discussed how carbon centric free radicals along with other ferromagnetic components present in the antennae and the wings of the nocturnal silk moth, might help them to avoid the bats.

Keywords: Antennae, Carbon Radical, Electron Spin Resonance, Ferromagnetism, Magneto-Sensing, Nocturnal Insect, Paramagnetism, Silk Moth, Vibrating Sample Magnetometer, Wing.

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1. INTRODUCTION

A diverse range organisms, like homing pigeon, European robin, loggerhead sea turtle, brown bat, Caribbean spiny lobster, red-spotted newt, salmon, rainbow trout, honey bees have the ability to detect Earth's magnetic field for orientation and navigation. Physicochemical mechanisms that underlie the magnetoreception in different organisms are still poorly understood.(1) Currently there are two principal mechanisms trying to explain animal magnetoreception. First one is based on the presence of iron based magnetic particles (biogenic magnetite) in the cellular structures of organisms.(2–10) Second one is based on the photochemical reactions leading to the generation of radical pairs, which responds to Earth's magnetic field.(11–22) Biogenic magnetite is a permanent magnetic material, which could be operational at any time of the day. In contrast, the photochemical reactions leading to the generation of radical pairs, is a light dependent reaction and could only work during the day or in dim light. It is obvious that birds and insects, which predictably utilize such radical pair for magnetoreception, do navigate during daytime or at dim light. Since radical pair generation is a light dependent reaction, one can wonder, whether radical pair mechanism has any role in the navigation of nocturnal species. In this work, we proposed a novel mechanism of magnetoreception in nocturnal insect, whereby we show it is possibly using a pool of stable free radicals along with biogenic magnetite for navigation. We use electron spin resonance (ESR) spectroscopy to demonstrate the presence of stable carbon-centric free radicals in the antennae and wings of nocturnal silk moth. Magnetic analysis showed that such stable; carbon centric free radicals have soft magnetic memory and responds to very weak magnetic fields similar to that of Earth's (50 μT). These experiments establish the feasibility of a complimentary role of carbon centric free radicals and biogenic magnetite based system in the magnetoreception and navigation of nocturnal silk moth. We have further discussed, how the carbon centric free radicals and the biogenic ferromagnetic components present in the antennae and the wings of the nocturnal silk moth, might help it to sense the sound waves generated by the bats and eventually help it to escape from the bats.

2. MATERIALS AND METHODS

2.1. Cocoon Collection and Rearing of Silk Moth

The semi-domesticated varieties of DABA Tasar silkworm (Antheraea mylitta Drury) cocoons were collected from the state of Chhattisgarh in India. The details of this silkworm rearing were described previously.(23) The moths which are used for the study are born after metamorphosis of the silk worm. The moths developed inside cocoons, which are fresh and can be treated like coming out from a highly dust free and pure natural environment. Initially the cocoons were placed in humidity and temperature controlled chamber in a dust free environment. As soon as the adult moths emerges out of the cocoon, these were transferred to a dust free sterilize jar. Soon after that, using fine non-metallic sterilized instruments the antennae and part of the wings are removed and transferred to a clean sterile glass vial for further analysis. This whole procedure is carried out with utmost care so as to avoid any kind of contamination from the environment. During this study the samples were collected from 25 adult moths which were reared in the clean environment of the incubator. The weight of individual antennae of adult moth is ~0.4 mg. Therefore it is not easy to do experiment using such a small amount of probe for further reaction from individual moth. Hence data were collected by pooling samples from six silk moths of same species.

2.2. Characterizations

Photographs: Regular photographs of the silk moth and other anatomical structures have been taken using Nikon D60 digital SLR camera and then image was optimized using Photoshop software.

Field emission scanning electron microscopy (FESEM) and energy dispersive X-ray spectroscopic (EDX) analysis: Morphology analysis of antennae and wings of silk moth was performed using SUPRA 40VP Field Emission Scanning Electron Microscope (CARL ZEISS NTS GmbH, Oberkochen (Germany) equipped with energy dispersive X-ray (EDX) facility.

X-ray diffraction (XRD) analysis of antennae: The XRD data of antennae and bulk calcium oxalate was measured by ISO Debye Flex-2002 (Rich-Seifert & Co) diffractometer.

Magnetic studies: The ADE, EV7 model of vibrating sample magnetometer (VSM) was used to study the magnetic hysteresis properties of the antennae and the wing samples. These measurements were performed at a room temperature of 27 °Centigrade.

2.3. Electron Spin Resonance (ESR) Studies

ESR spectra of both antennae and wings of silk moths were taken in a Bruker EMX EPR Spectrometer with a typical microwave frequency of 9.8 GHz and a microwave power of 0.200 mW at room temperature (27 °Centigrade). The modulation amplitude was maintained at 5 G and the time constant 10 ms. Spectra processing (numerical integration, digital filtering, baseline correction, parameter calculation, etc.) were performed using Bruker Win-EPR and Simfonia software.

3. RESULTS AND DISCUSSION

3.1. Nocturnal Moth *Antheraea Mylitta* Druary

We studied the magnetic properties of the antennae and wings of the wild silk moth, *Antheraea mylitta* Druary. It is part of a natural fauna of tropical India and commonly called Indian tasar silkworm. It is nocturnal in nature.[24] It belongs to the family Saturniidae (superfamily Bombycoidea). These are comparatively large moths with a wingspan of 3–15 centimeters (Fig. 1(a)). They have a relatively small head and hairy bodies (Fig 1(b)). They have feathery or hair-like antennae (Fig. 1(c)).

FESEM images of the antennae showed the presence of microscopic spiny structures and cuboidal shaped crystals. The spiny structures are 10-20 micron tall and having a cross section of 1–2 micron (Fig. 2(a)). The cuboidal shaped crystals are of varied sizes ranging from 200 nm to 1 micrometer (Fig. 2(b)). We previously observed similar cuboidal shaped crystals of calcium oxalate in silk cocoon (23). XRD analysis of the antennae indicated that indeed these cuboidal shaped crystals are of calcium oxalate (Fig. 2(c)). These calcium oxalate crystals are synthesized in the body of the larvae and secreted during the metamorphosis (23). The exact physiological role is still under investigation. The FESEM images of the wing showed a net-like structure (Fig. 2(d)). On further magnification, well spaced "D" shaped pit-like structures are observed all along the surface of the wings (Fig. 2(e)). The EDX analysis of the antennae and wings showed the presence of metals like Fe, Co, Ni and Mn in appreciable amount (Table I). Histogram of EDX analysis of all the elements present in the anteanne showed that among all the trace elements, calcium is present in a significant amount. Among the major elements, carbon and oxygen is observed in bulk concentrations (Fig. 2(f)). It is difficult to get visible powder-XRD peak for very negligible amount of ferromagnetic materials present in silk moth antennae. Though Fe may contribute in the formation of biogenic magnetite (Fe_3O_4), the role of other ferromagnetic metals (Co, Ni and Mn) is yet to be ascertained.

3.2. ESR Studies

We removed the antennae and the wings from the live adult moth, and independently subjected them to room temperature ESR spectroscopy. ESR spectra of antennae and wings are shown in Figure 3(a), (b) respectively. Both spectra showed the presence of a stable free-radical species. This free-radical species has a characteristic "g" value of 2.0075, which is related to carbon materials.

In biological environment, the radical species can originate from atoms like N, C and O to display such ESR signal close to free electron value. Nitrogen centered free-radical species should recognize its nuclear moment to display triplet splitting in the ESR signal. Oxygen free-radical species would lead to the generation of reactive oxygen species and will be very short lived. A pool of trapped, stable carbon radicals is the best possible alternative. Stable carbon radicals are known to present in carbonaceous matters including graphitic carbon and in irregularly bend carbon nanotubes. These structures retain stable carbon-centered free radicals for a long period of time. The carbon-centric free radicals are trapped in the network structure and exist due to spin frustration.[25–27] Such carbon-centric free radicals are extremely stable and could not be reduced or oxidized by conventional reducing or oxidizing agents.[25–27] Apart from carbon other elements do also show stable free radicals. One such example is mineral Lapise Lazuli, which contains sulfur containing free radical trapped in the silicate matrix. Just like the carbon centric free radical, this S^{3-} anion radical remains fixed under aluminosilicate matrix to retain its stability.[28–29] However, due to spin-orbit coupling its 'g' value deviates from the free electron value. However, due to spin-orbit coupling its 'g' value deviates much more from the free electron value which rules out presence of sulfur centric free radical in silk moth's antennae and wings.

ESR studies showing the presence of biogenic magnetite in antennae and the wings. Along with the strong radical signal, the ESR spectra of the antennae and wings show the presence of a weak broad resonance, centered on a "g" value of ~2.1. This "g" value corresponds to one of the "g" value due to magnetite (Fe_3O_4). The other "g" value of magnetite could not be identified properly because its intensity is weak in the expected range of magnetic field 1000–2000 G (Fig. 3(a, b)). Thus ESR study showed the presence of a carbon-centered free radical along with a

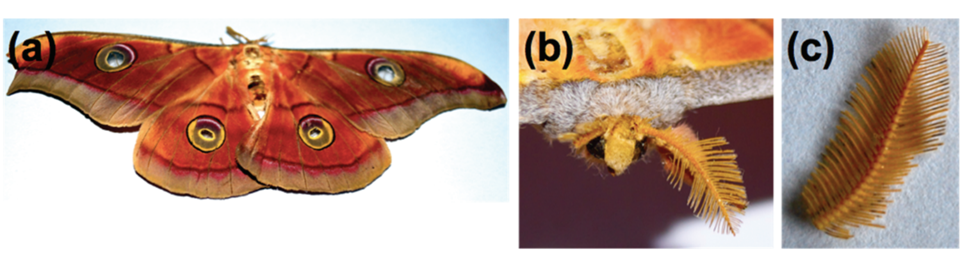

Fig. 1. Anatomy of silk moth (a) Large silk moth with a wingspan of 3–15 centimeters (b) Silk moth has a small head and hairy bodies (c) Feathery or hair-like antennae.

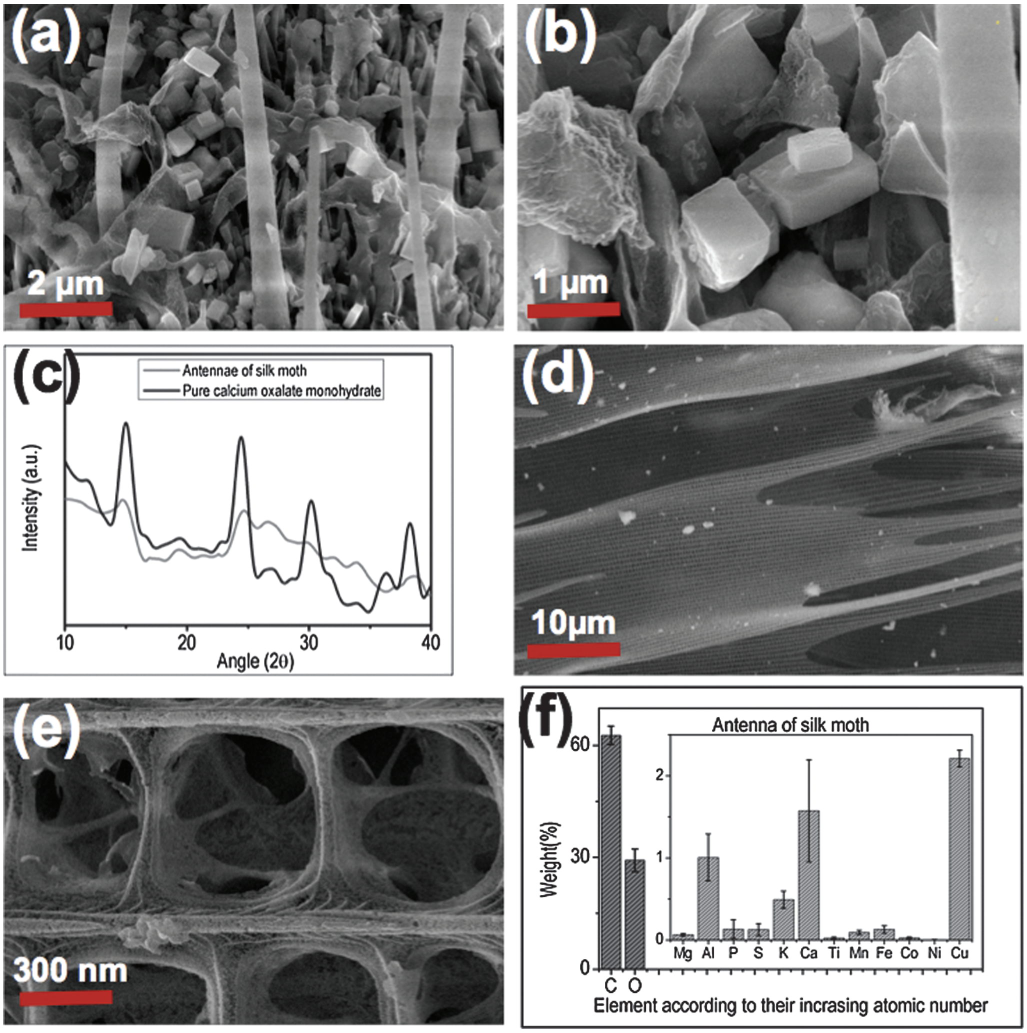

Fig. 2. FESEM, XRD and EDX of antennae and wing of silk moth (a) FESEM image of the antennae showing spiny structures which are 10–20 micron tall and having a cross section of 1–2 micron (b) FESEM image of the antennae showing the presence of cuboidal shaped crystals at the base of the antennae (c) The XRD pattern of antennae of silk moth and pure calcium oxalate monohydrate sample (d) FESEM images of the wing showed a net-like structure (e) High resolution FESEM image showing a well spaced "D" shaped pit-like structures, which are observed all along the surface of the wings (f) EDX analysis of the major and minor elements present in significant amount in the silk antennae. The high concentration of the copper is due to the brass stub used for placing the sample inside the SEM.

ferromagnetic material (Fe_3O_4) in the antennae and wings of silk moth.

3.3. Magnetism Studies

Vibrating sample magnetometer (VSM) was used to characterize the magnetic properties of antennae and wings as a function of magnetic field. The freshly clipped antennae and wings from live adult moth are used for this study. Room temperature measurement of the magnetic hysteresis (M–H) loop, coercivity and the saturation magnetization values for the antennae and the wings are shown in Figure 4(a, b) and the values are presented in Table II. Both antennae and wings showed characteristic narrow M–H loop and low coercivity values indicating the presence of soft magnetic material in their cellular

Table I. Using EDX, the weight percentage of Fe, Co, Ni and Mn transition metals was obtained from the antennae and wings of silk moth. The values are expressed as average weight percentage ± standard error.

Different parts of silk moth	Weight percentage of Iron (Fe)	Weight percentage of Cobalt (Co)	Weight percentage of Nickel (Ni)	Weight percentage of Manganese (Mn)	Average data from total number of samples
Antennae	0.1283 ± 0.043	0.0258 ± 0.0107	0.0028 ± 0.0028	0.0912 ± 0.025	5
Wings	0.1455 ± 0.0389	0.01767 ± 0.01253	0.0363 ± 0.0207	0.0158 ± 0.0075	6

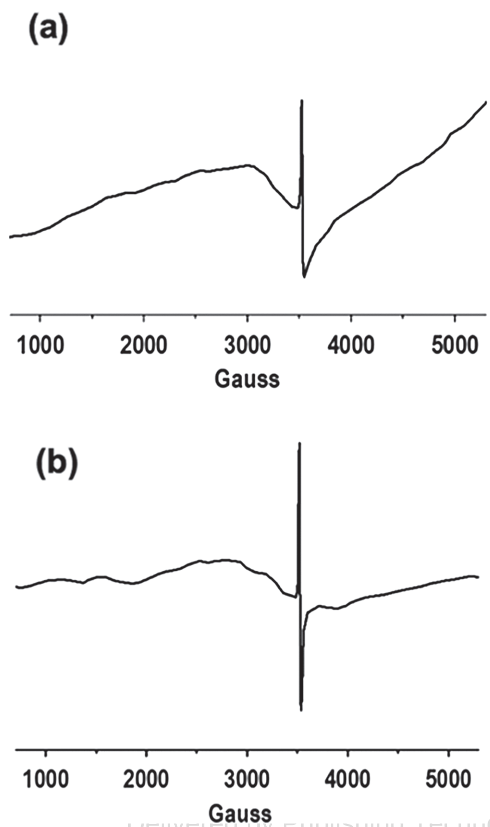

Fig. 3. ESR spectra of the (a) antennae, and (b) wing, of silk moth.

structures. The VSM data indicated that the magnetic materials present in both antennae and the wings respond to a very weak magnetic fields (Fig. 4(a) inset, 4(b) inset) similar to that of Earth's magnetic field (50 μT).

What we can observe is that the antennae shows a lower coercivity value as compared to that of the wings. A low coercivity value suggests how rapidly the material gets magnetized and demagnetized in a varying magnetic field. The antenna is the primary sensory apparatus of the insect. In real time, the antenna is continuously exposed to newer and direction oriented signals. These signals need to be relayed to the nervous system at a rapid pace. In order to achieve a rapid signal turnover rate, antennae needs to equip itself with a soft magnetic memory system. Hence it is not surprising that an antenna has a lower coercivity value signifying a softer magnetic system as compared to the wings. Based on our ESR and VSM data, we presume that magnetorecepetion in the antennae and the wings are carried out by a concerted activity of iron based magnetite and carbon-centered radical species.

Since our EDX and ESR data indicated the presence of magnetite and carbon-centric radical in the antennae as well as in the wings, it was difficult to determine the magnetic contribution of each of these two components from the gross measured M–H loop. In order to address this question, we separately measured the M–H loop of pure magnetite and a multipodal carbon nanotube, which

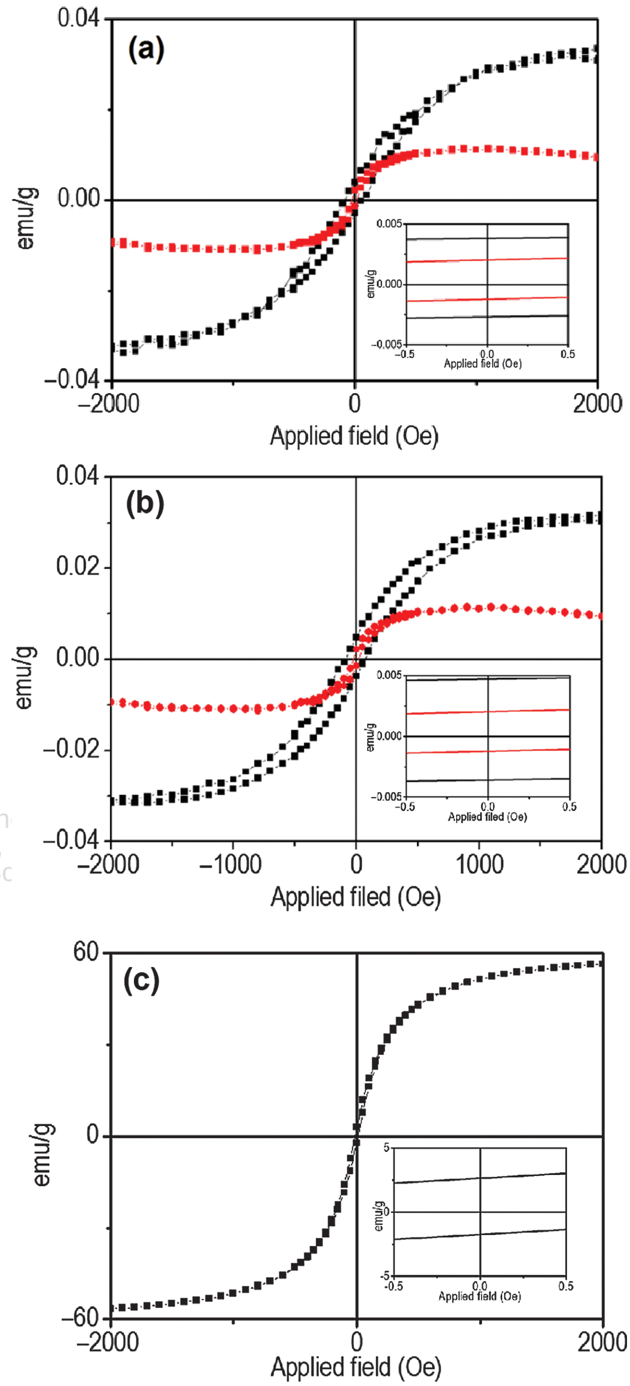

Fig. 4. Magnetic hysteresis loop of (a) antennae of silk moth (black streak) and defective carbon (red streak) measured at room temperature. The inset shows a more detailed view of magnetic property at 0.5 gauss (earth's magnetic field) (b) wings of silk moth (black streak) and defective carbon (red streak) measured at room temperature. The inset shows a more detailed view of magnetic property at 0.5 gauss (earth's magnetic field) (c) Magnetite, and the inset shows a more detailed view of magnetic property at 0.5 gauss.

is known to possess a stable carbon-centric radical under identical condition.[26] The coercivity and the saturation magnetization values of magnetite and carbon-centered free radical are tabulated in Table II. The M–H loop of

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Table II. Saturation magnetization and coercivity values of antennae, wings of silk moth, defective carbon nano-tube and Magnetite.

	Coercivity (H_c) in (Oe)	Saturation magnetization (M_s) in (emu/g) at 2000Oe
Antennae	93.60	0.0328
Wings	111.16	0.031
Defective carbon nano tube	25	0.008
Magnetite	13.5	56

carbon-centered free radical was seen to be accommodated within the volume of the M–H loop of antennae and wing (Fig. 4(a, b)) and they show magnetic field sensitivity at a value as low as 50 μT. Similarly, the M–H loop of pure magnetite (Fig. 4(c) and inset) could also be accommodated within the volume of the M–H loop of antennae and wing. Such preliminary comparison help us to suggest that the observed M–H loop of antennae and wing should accommodate the contribution made by magnetite as well as carbon-centered radical and the individual contribution would be difficult to ascertain. The influence of trace amount of Co, Ni and Mn as observed by EDX analysis of antennae and wings has not been taken into account. The magnetization may be arising out of additional components from these elements. However, do they work together in unison or in tandem is difficult to predict. Nevertheless, the presence of permanent magnetic materials like magnetite and a stable carbon-centered radical is established in the antennae and wings of nocturnal silk moth.

3.4. Possible Role of Carbon Centric Free Radicals and Ferromagnetic Components (Fe, Ni, Co, Mn) in Intercepting the Ultrasonic Sound Waves of the Bats

Bats feed on nocturnal moths. During evolution moths have devised strategies to avoid the bats to increase their chances of survival. During night, bats locate its prey by echolocation. Echolocation is a technique by which bat sends out strong ultrasonic sound waves and which upon intercepting the target reflects and travels back to the bats. The reflected sound waves help the bats to locate the direction and the distance of the target. This highly sophisticated sonar system equipped the bats with the ability to locate and capture nocturnal flying insects. Bats virtually see their target by this method.[30–34] Certain nocturnal moths have ears that can detect the ultrasonic waves by which bats locate their prey. As the bat approaches these moths, they take evasive action. They abandon their flight and take a sharp dive or make erratic loops or

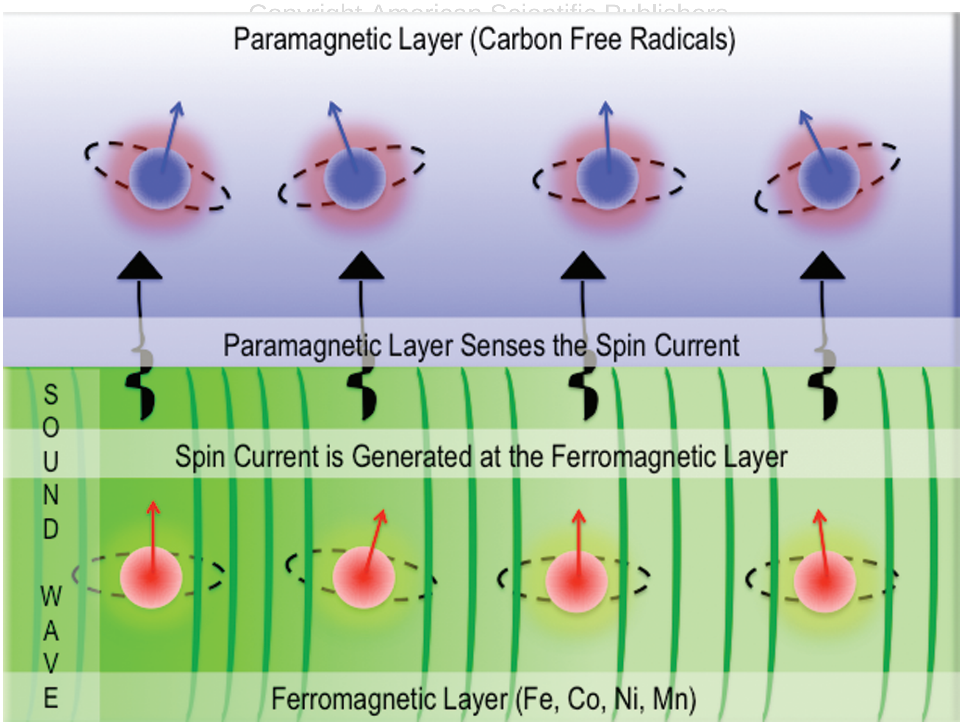

Fig. 5. Proposed model by which ultrasonic sound waves emitted by the bats may be intercepted by the ferromagnetic/paramagnetic bilayer magnetic nanostructure in the antennae and wings of nocturnal silk moth. It is possibly govern by a phenomenon termed as acoustic spin pumping. When the sound waves of ultrasound frequencies, interacts with the ferromagnetic layer, there is an energy transfer event leading to the generation of spin current or sound waves or magnons at this layer. A spin current drifts out from the ferromagnetic layer into the adjacent paramagnetic layer. Spin angular momentum gained by the components of the paramagnetic layer i.e., stable carbon centric free radicals, helps the moth to recalibrate its position in space in order to avoid the attacking bat.

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flies at top speed away from the ultrasound source.[30–34] This significantly increases their chances of survival. There are some moths, which have the ability to jam the sonar of the bats. These moths generate sounds, which could interfere with, or "jam," bat sonar.[35] In the light of our findings, we asked the following question: Whether the ferromagnetic (Fe, Co, Ni, Mn) and the paramagnetic (Carbon centered free radical) materials form some kind of bilayer nanostructure to intercept the ultrasonic sound waves? Here, we are proposing a novel strategy by which a moth may be able to intercept the ultrasonic sound waves generated by the bats. We are treating the antennae and the wings as simple ferromagnetic/paramagnetic bilayer structure (Fig. 5).

When a sound wave is injected into this ferromagnetic/paramagnetic bilayer of the antennae and the wings, the energy is transferred from the sound waves to spin waves, or magnons, in the ferromagnetic material, which leads to the generation of spin current. This phenomenon of generation of spin current is termed as acoustic spin pumping. Spin current is essentially the flow of spin, in different magnetic nanostructures.[36–43] Further these spin currents could be detected by the adjacent paramagnetic materials (Carbon centered free radicals) by inverse spin-Hall effect. This change in the spin currents of paramagnetic material may help the moth to evade the attacking bat. Devices, which convert sound energy to electrical energy using acoustic spin pumping, have already been demonstrated.[43] Nocturnal silk moth may be an example from nature, which utilizes this phenomenon of acoustic spin pumping to avoid the bats.

4. CONCLUSIONS

Current research reviews on nocturnal navigation and orientation have mostly focused on the presence of highly evolved visual system in these species.[44–45] Very little has been addressed to understand, how during the night, these species might use a light independent, free radical based magnetoreception system. Here we are proposing a new model of free radical based magnetoreception in the nocturnal insects. Unlike the existing radical pair system of magnetoreception, where a light-dependent reaction is essential to generate free radicals, here we show the presence of a pool of stable carbon-centric free radical in the antennae and the wings of silk moth. These stable carbon-centric free radicals are intrinsic to these anatomical structures and responds to weak magnetic fields similar to that of Earth's (50 μT) even in the absence of light. In summary, our results demonstrate the presence of a carbon-centered radical and along with magnetite-based system in the antennae and wings of silk moth. Based on these observations, we have propose a complimentary role of carbon-centered radical and magnetite based system in the magnetoreception of nocturnal insects which might further help them in developing smarter survival strategies.

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