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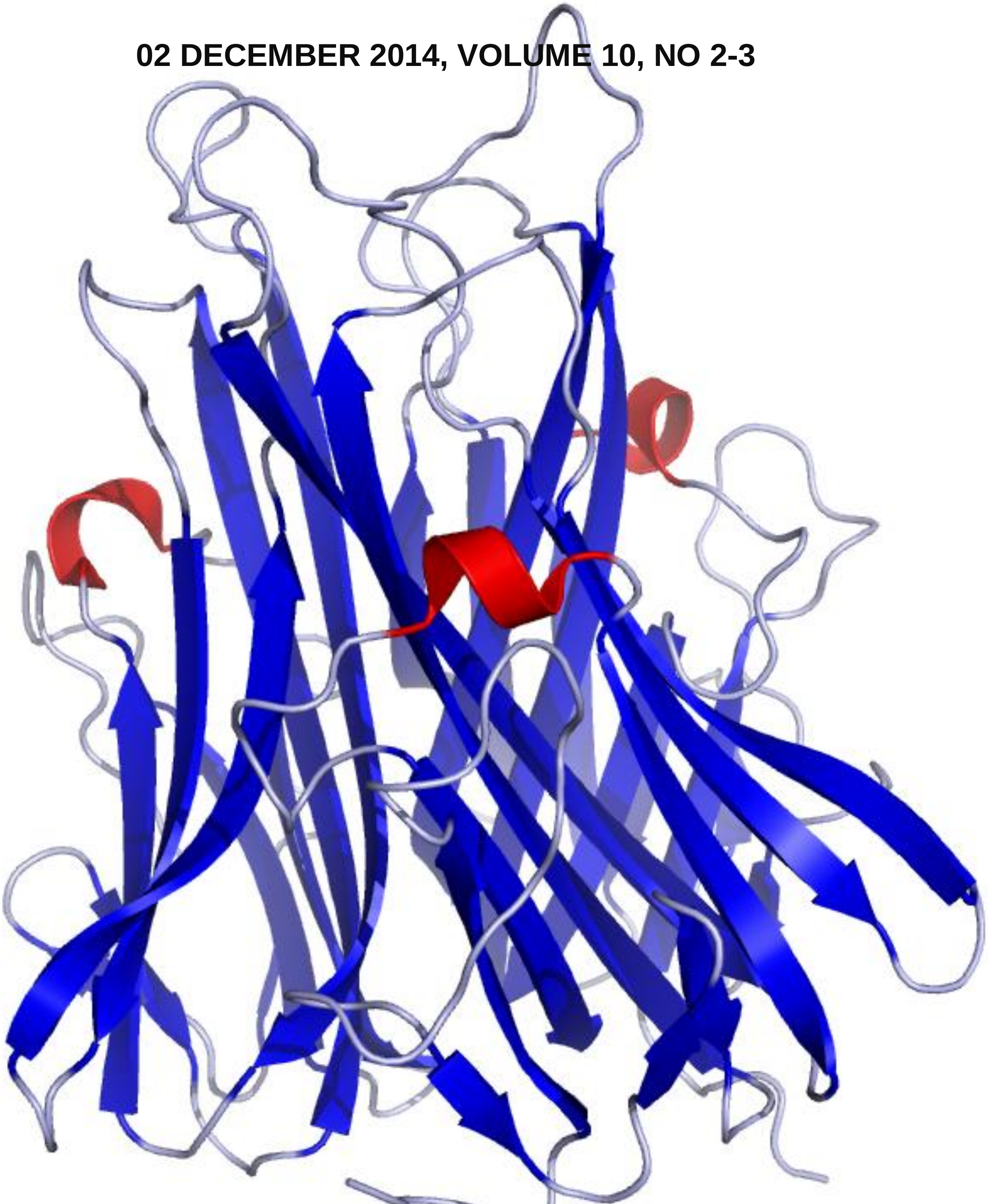

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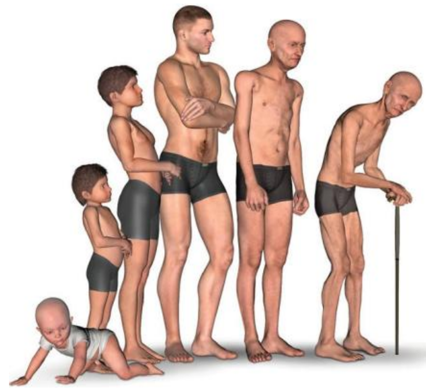

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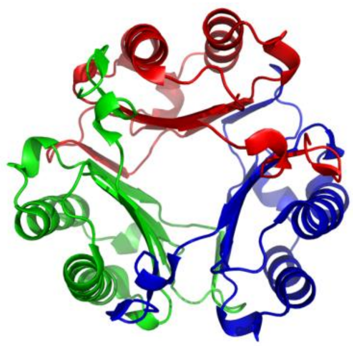

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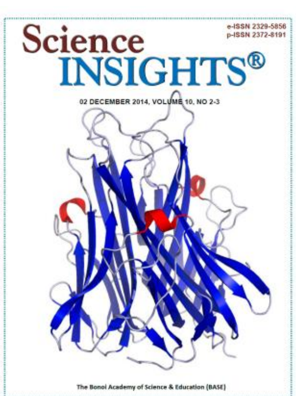

COVER

MIF blockade alleviates SNI-induced thermal hypersensitivity and allodynia in accompanying with decrease in spinal TNF-α level. Neutralization of TNF-α produces identical effect on nociception as MIF inhibition did. However, MIF alone plays no role in nociceptive responses in normal animals. These data suggest that proinflammatory cytokine MIF involves in peripheral nerve injury-induced hypersensitivity by potentiating TNF-α signaling. See page 283.

Image: BASE illustrating group

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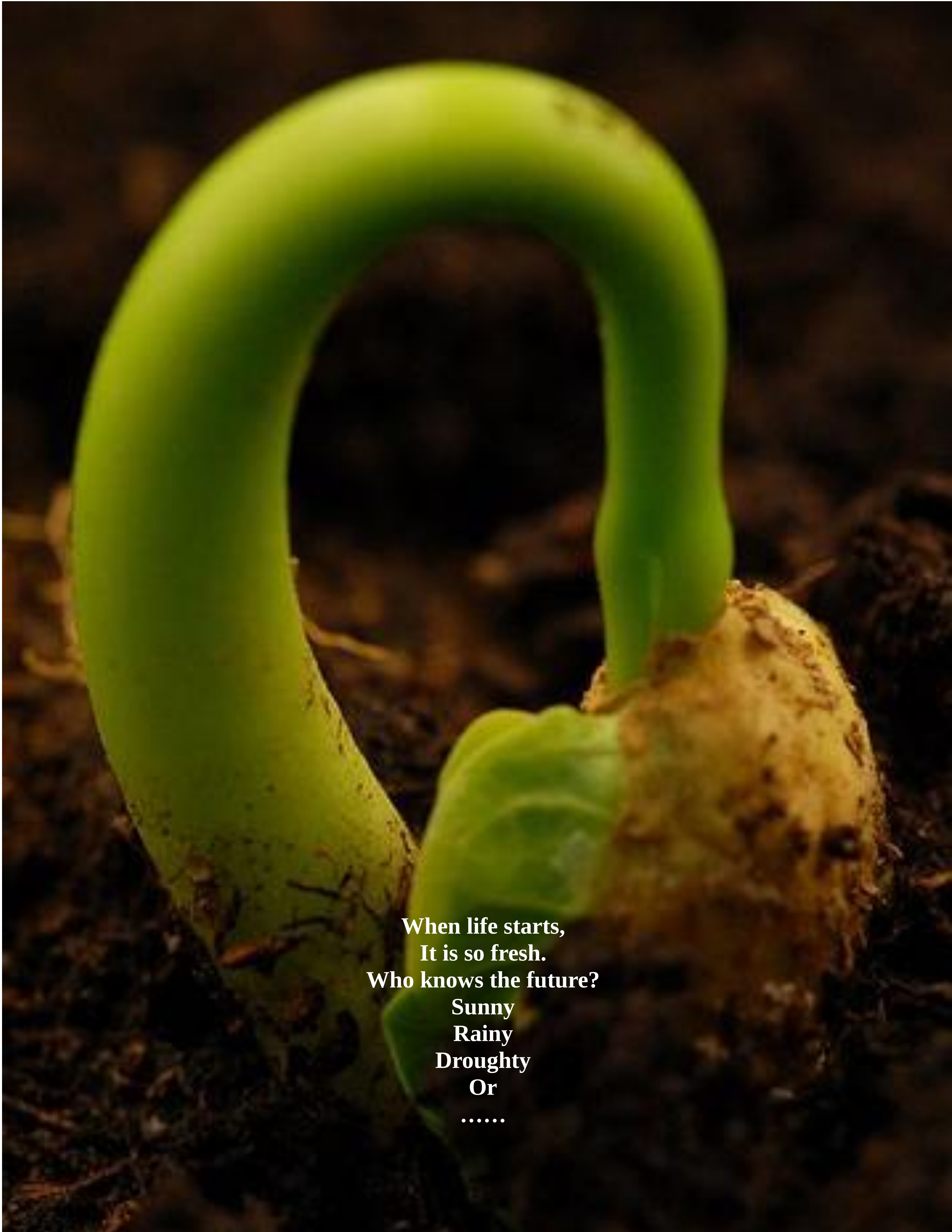
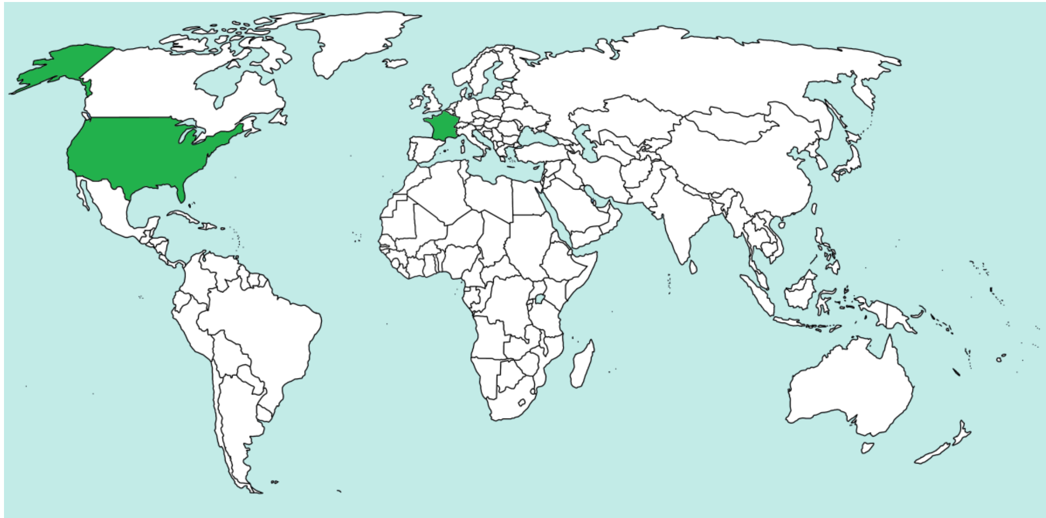
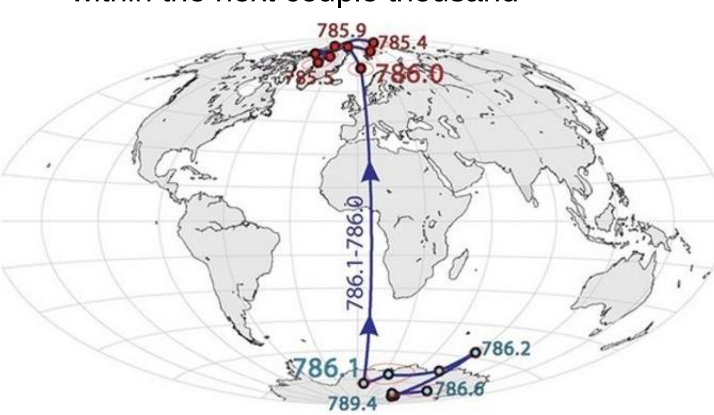

When life starts,
It is so fresh.
Who knows the future?
Sunny
Rainy
Droughty
Or
……



Paris, FRANCE

Earth's Magnetic Field Could Flip

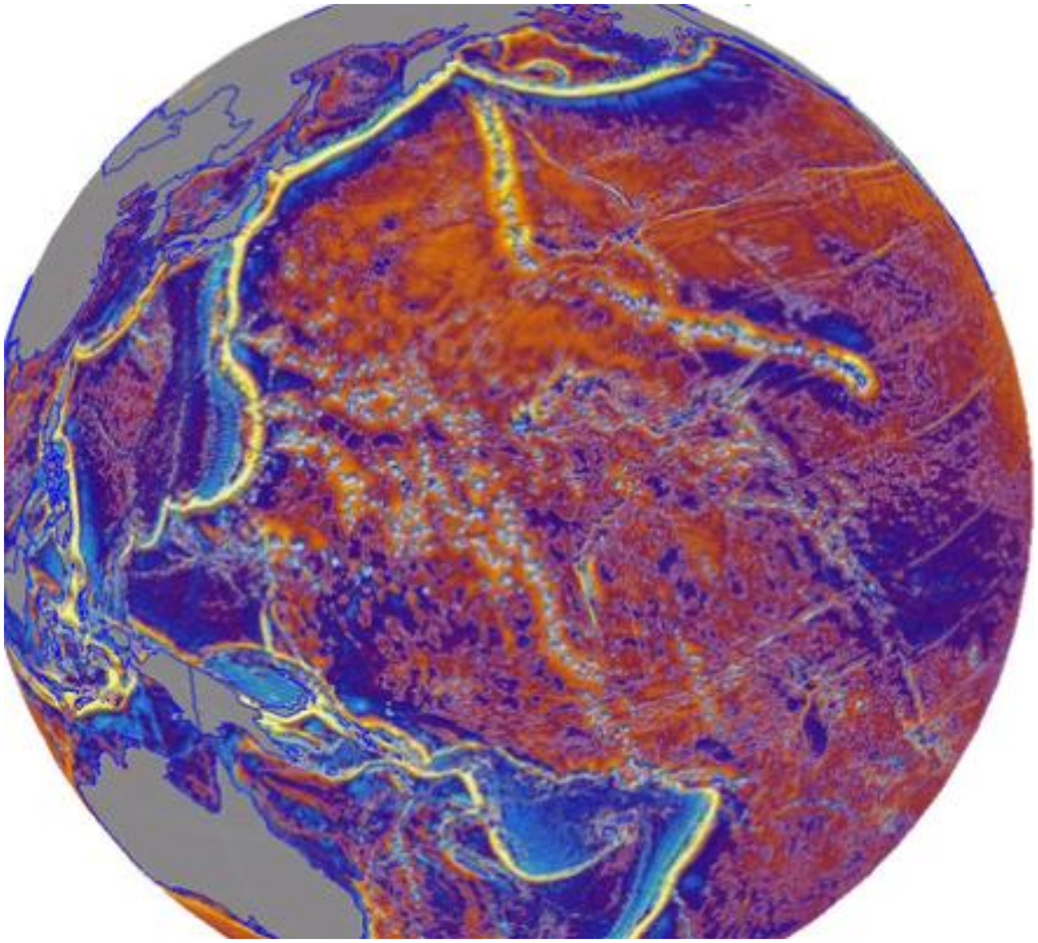
A pilot looking down at her plane controls and realizing magnetic north is hovering somewhere over Antarctica may sound like a scene from a science-fiction movie, but new research suggests the idea isn't so far-fetched in the relatively near future. A magnetic field shift is old news. Around 800 000 years ago, magnetic north hovered over Antarctica and reindeer lived in magnetic south. The poles have flipped several times throughout Earth's history. Scientists have estimated that a flip cycle starts with the magnetic field weakening over the span of a few thousand years, then the poles flip and the field springs back up to full strength again. However, a new study shows that the last time the Earth's poles flipped, it only took 100 years for the reversal to happen. The Earth's magnetic field is in a weakening stage right now. Data collected this summer by a European Space Agency (ESA) satellite suggests the field is weakening 10 times faster than scientists originally thought. They predicted a flip could come within the next couple thousand years. It turns out that might be a very liberal estimate, scientists now say. "We don't know whether the next reversal will occur as suddenly as this [previous] one did, but we also don't know that it won't," Paul Renne, director of the Geochronology Center at the University of California, Berkeley, said in a statement. Geologists still are not sure what causes the planet's magnetic field to flip direction. Earth's iron core acts like a giant magnet and generates the magnetic field that envelops the planet. This helps protect against blasts of radiation that erupt from the sun and sometimes hurtle toward Earth. A weakening magnetic field could interrupt power grids and radio communication, and douse the planet in unusually high levels of radiation. While the ESA satellite studied the magnetic field from above, Renne and a team of researchers studied it from below. The researchers dug through ancient lake sediments exposed at the base of the Apennine Mountains in Italy. Ash layers from long-ago volcanic eruptions are mixed into the sediment. The ash is made of magnetically sensitive minerals that hold traces of Earth's magnetic field lines, and the researchers were able to measure the direction the field was pointing. Renne and colleagues then used a technique called argon-argon dating — which works because radioactive potassium-40 decays into argon-40 at a known rate — to determine the age of the rock sediment. The layers built up over a 10,000-year period, and the researchers could pinpoint where the poles flipped in the rock layers. The last flip happened around 786 000 years ago.■ By Kelly Dickerson. Original article on*Live Science*.



La Jolla, USA

New Uncharted Mountains Hidden Beneath the Sea

A new topographic map of Earth's mysterious ocean floor reveals thousands of towering volcanoes, hidden gashes where supercontinents ripped apart and other never-before-



seen features once veiled by miles of water and thick sediment. The topography of Earth's seafloor is as corrugated and bumpy as a book set in Braille. By reading these peaks and ridges, scientists can chronicle the birth of new ocean crust and the past wanderings of Earth's continents. However, even though the seafloor carries the pivotal clues to plate tectonics, the dry surface of Mars has been detailed more clearly than the ocean's watery depths. The new map, released in the journal Science, promises to fill in some of the blanks. Compared with the previous map, from 1997, the resolution is twice as accurate overall and four times as better in coastal areas and the Arctic, said lead study author David Sandwell, a marine geophysicist at the Scripps Institution of Oceanography in La Jolla, California. As with the earlier 1997 map, scientists expect to improve their knowledge of Earth's geologic history based on new features revealed by the map's finer scale. Altimetry measures sea surface height from space by timing how long it takes a radar signal to reflect off the ocean and return. The ocean surface has subtle highs and lows that mimic both seafloor topography and Earth's gravity field. The world's volcano count jumped tremendously thanks to the new map. The number of seamounts soared from around 5,000 to about 20,000, Sandwell said. Seamounts are small, conical volcanoes that are usually inactive or extinct. In the deep ocean, tall seamounts attract a riot of marine life. The map captures all seamounts more than 0.9 miles (1.5 kilometers) tall. Some of the new seamounts appear in linear chains, but many do not. That suggests the volcanoes did not erupt above a mantle plume, a blob of hot rock that rises from the deep mantle (the layer under the crust). Some scientists think mantle plumes don't exist, but, as with climate change, the majority of researchers agree on the concept but argue about the details. The motion of Earth's tectonic plates created some of the most distinct features visible on the seafloor. These include spreading ridges and fracture zones, where the massive plates pull apart and lava oozes to the surface. Plates are created at spreading ridges and recycled at deep ocean trenches. The ocean trenches appear as deep blue troughs in the gravity map. For the first time, the global seafloor topography captures the abyssal hills, the most common surface feature on Earth, the study reports. While the secrets of the origin are still debated, scientists think a combination of faulting and volcanism at spreading ridges creates the hills. The corrugated ridges and valleys cover up to 30 percent of Earth's surface, by some estimates. "They're the most common landform on the planet, and I'm always amazed that people have never heard of them," Sandwell said. Along coastlines, the data uncovered faults and fractures buried under thick piles of mud and sand pouring off the continents. Sandwell and his colleagues highlighted the new details seen in fracture zones that extend from South America to Africa. "We can see these transform faults or fracture zones all the way up to the continental margins that are currently buried by sediments, and you couldn't before," Sandwell said. Though the two continents seem to fit together like puzzle pieces, "squiggles" in the fractures tell of tectonic complexity during their breakup, he added. "These are a new thing," Sandwell said. Analyzing such details around each continent will improve reconstructions of past plate motions. In the Gulf of Mexico, the researchers identified a defunct spreading ridge now entombed under miles of sediment. The spreading ridge opened the gulf about 150 million years, when the Yucatan Peninsula pivoted counterclockwise from North America. "Because of a major improvement in accuracy, this new gravity field will lead to more discoveries of tectonic features, especially in regions with thick sediments," Hwang wrote. Most of the new ridges, faults and volcanoes were undiscovered because 80 percent of the ocean floor has never been charted by ships. The new topography will improve depth estimates in much of the ocean, the researchers said.■ By Becky Oskin, Original article on *Live Science*.

Philadelphia, USA

The "Astoundingly Huge" Dinosaur Discovered in Argentina

Say hello to *Dreadnoughtus schrani*, a newly discovered dinosaur that was so formidable, it was named after a battleship that prowled the seas during the early 20th century. "It is by far the best example we have of any of the most giant creatures to ever walk the planet," Dr.

Jennifer Hall, Carnegie Museum of Natural History

Kenneth Lacovara, a paleontologist at Drexel University's College of Arts and Sciences and the scientist who discovered the skeleton, said in a written statement. "With a body the size of a house, the weight of a herd of elephants, and a weaponized tail, *Dreadnoughtus* would have feared nothing." The titanosaur's skeleton, which dates back 77 million years, was discovered in Southern Patagonia in Argentina, and unearthed over the course of four digs between 2005 and 2009. *Dreadnoughtus schrani* was substantially more massive than any other supermassive dinosaur for which mass can be accurately calculated. The skeleton is also the most complete of its kind, comprising 43.5 percent of the dinosaur's bones. It contains nearly all bones from the forelimbs and hind limbs, most of the tail vertebrae and numerous ribs. Lacovara and his colleagues have digitally scanned the dinosaur's bones and constructed a "virtual mount" of the skeleton. The scientists hope their research on Dreadnoughtus will shed new light on the anatomy of supermassive dinosaurs, and how they walked and grew.■ By Macrina Cooper-White from the journal *Scientific Reports*.

Boston, USA

Puppy-Sized Spider in Rainforest

The South American Goliath birdeater (*Theraphosa blondi*) is the world's largest spider, according to Guinness World Records. Its legs can reach up to one foot (30 centimeters) and it can weigh up to 6 oz. (170 grams). Piotr Naskrecki was taking a nighttime walk in a rainforest in Guyana, when he heard rustling as if something were creeping underfoot. When he turned on his flashlight, he expected to see a small mammal, such as a possum or a rat. "When I turned on the light, I couldn't quite understand what I was seeing," said Naskrecki, an entomologist and photographer at Harvard University's Museum of Comparative Zoology. A moment later, he realized he was looking not at a brown, furry mammal, but an enormous,

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puppy-size spider. Some sources say the giant huntsman spider, which has a larger leg span, is bigger than the birdeater. But the huntsman is much more delicate than the hefty birdeater — comparing the two would be "like comparing a giraffe to an elephant," Naskrecki said. The birdeater's enormity is evident from the sounds it makes. "Its feet have hardened tips and claws that produce a very distinct, clicking sound, not unlike that of a horse's hooves hitting the ground," he wrote, but "not as loud." When Naskrecki approached the imposing creature in the rainforest, it would rub its hind legs against its abdomen. At first, the scientist thought the behavior was "cute," he said, but then he realized the spider was sending out a cloud of hairs with microscopic barbs on them. When these hairs get in the eyes or other mucous membranes, they are "extremely painful and itchy," and can stay there for days, he said. But its prickly hairs aren't the birdeater's only line of defense; it also sports a pair of 2-inch-long (5 centimeters) fangs. Although the spider's bite is venomous, it's not deadly to humans. But it would still be extremely painful, "like driving a nail through your hand," Naskrecki said. And the eight-legged beast has a third defense mechanism up its hairy sleeve. The hairs on the front of the spider's body have tiny hooks and barbs that make a hissing sound when they rub against each other, "sort of like pulling Velcro apart," Naskrecki said. Yet despite all that, the spider doesn't pose a threat to humans. Even if it bites you, "a chicken can probably do more damage," Naskrecki said. Despite its name, the birdeater doesn't usually eat birds, although it is certainly capable of killing small mammals. "They will essentially attack anything that they encounter," Naskrecki said. The spider hunts in leaf litter on the ground at night, so the chances of it encountering a bird are very small, he said. However, if it found a nest, it could easily kill the parents and the chicks, he said, adding that the spider species has also been known to puncture and drink bird eggs. The spider will eat frogs and insects, but its main prey is actually earthworms, which come out at night when it's humid. "Earthworms are very nutritious," Naskrecki said. Birdeaters are not very common spiders. "I've been working in the tropics in South America for many, many years, and in the last 10 to 15 years, I only ran across the spider three times," Naskrecki. After catching the specimen he found in Guyana, which was female, Naskrecki took her back to his lab to study. She's now deposited in a museum.■ By Tanya Lewis. Original article on *Live Science*.

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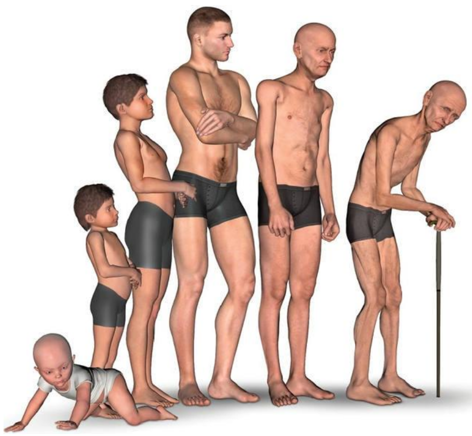
NEUROSCIENCE

The On/Off Switch for Aging Cells

Scientists at the Salk Institute in the USA have discovered an on-and-off "switch" in cells that may hold the key to healthy aging. This switch points to a way to encourage healthy cells to keep dividing and generating, for example, new lung or liver tissue, even in old age. In our bodies, newly divided cells constantly replenish lungs, skin, liver and other organs. However, most human cells cannot divide indefinitely–with each division, a cellular timekeeper at the ends of chromosomes shortens. When this timekeeper, called a telomere, becomes too short, cells can no longer divide, causing organs and tissues to degenerate, as often happens in old age. But there is a way around this countdown: some cells produce an enzyme called telomerase, which rebuilds telomeres and allows cells to divide indefinitely. "Previous studies had suggested that once assembled, telomerase is available whenever it is needed," says senior author Vicki Lundblad, professor and holder of Salk's Ralph S. and Becky O'Connor Chair. "We were surprised to discover instead that telomerase has what is in essence an 'off' switch, whereby it disassembles." Understanding how this "off" switch can be manipulated–thereby slowing down the telomere shortening process–could lead to treatments for diseases of aging (for example, regenerating vital organs later in life). Lundblad and first author and graduate student



Timothy Tucey conducted their studies in the yeast Saccharomyces cerevisiae, the same yeast used to make wine and bread. Previously, Lundblad's group used this simple single-celled organism to reveal numerous insights about telomerase and lay the groundwork for guiding similar findings in human cells. "We wanted to be able to study each component of the telomerase complex but that turned out to not be a simple task," Tucey said. Tucey developed a strategy that allowed him to observe each component during cell growth and division at very high resolution, leading to an unanticipated set of discoveries into how–and when–this telomere-dedicated machine puts itself together. Every time a cell divides, its entire genome must be duplicated. While this duplication is going on, Tucey discovered that telomerase sits poised as a "preassembly" complex, missing a critical molecular subunit. But when the genome has been fully duplicated, the missing subunit joins its companions to form a complete, fully active telomerase complex, at which point telomerase can replenish the ends of eroding chromosomes and ensure robust cell division. Surprisingly, however, Tucey and Lundblad showed that immediately after the full telomerase complex has been assembled, it rapidly disassembles to form an inactive "disassembly" complex—essentially flipping the switch into the "off" position. They speculate that this disassembly pathway may provide a means of keeping telomerase at exceptionally low levels inside the cell. Although eroding telomeres in normal cells can contribute to the aging process, cancer cells, in contrast, rely on elevated telomerase levels to ensure unregulated cell growth. The "off" switch discovered by Tucey and Lundblad may help keep telomerase activity below this threshold.■

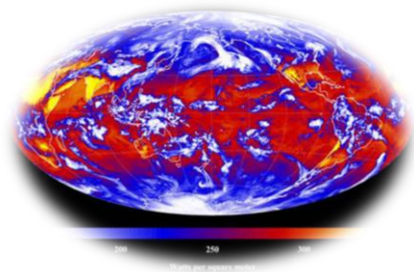
Genes & Development 2014; doi:10.1101/gad.246256.114

EARTH

The Missing Piece of the Climate Puzzle

In classrooms and everyday conversation, explanations of global warming hinge on the greenhouse gas effect. In short, climate depends on the balance between two different kinds of radiation: The Earth absorbs incoming visible light from the sun, called "shortwave radiation," and emits infrared light, or "longwave radiation," into space. Upsetting that energy balance are rising levels of greenhouse gases, such as carbon dioxide (CO_2), that increasingly absorb some of the outgoing longwave radiation and trap it in the atmosphere. Energy accumulates in the climate system, and warming occurs. But in a paper out this week in the Proceedings of the National Academy of Sciences

MIT researchers show that this canonical view of global warming is only half the story. In computer modeling of Earth's climate under elevating CO_2 concentrations, the greenhouse gas effect does indeed lead to global warming. Yet something puzzling happens: While one would expect the longwave radiation that escapes into space to decline with increasing CO_2, the amount actually begins to rise. At the same time, the atmosphere absorbs more and more incoming solar radiation; it's this enhanced shortwave absorption that ultimately sustains global warming. "The finding was a curiosity, conflicting with the basic understanding of global warming," says lead author Aaron Donohoe, a former MIT postdoc who is now a research associate at the University of Washington's Applied Physics Laboratory. "It made us think that there must be something really weird going in the models in the years after CO_2 was added. We wanted to resolve the paradox that climate models show warming via enhanced shortwave radiation, not decreased longwave radiation." Donohoe, along with MIT postdoc Kyle Armour and others at Washington, spent many a late night throwing out guesses as to why climate models generate this illogical finding before realizing that it makes perfect sense — but for reasons no one had clarified and laid down in the literature. They found the answer by drawing on both computer simulations and a simple energy-balance model. As longwave radiation gets trapped by CO_2, the Earth starts to warm, impacting various parts



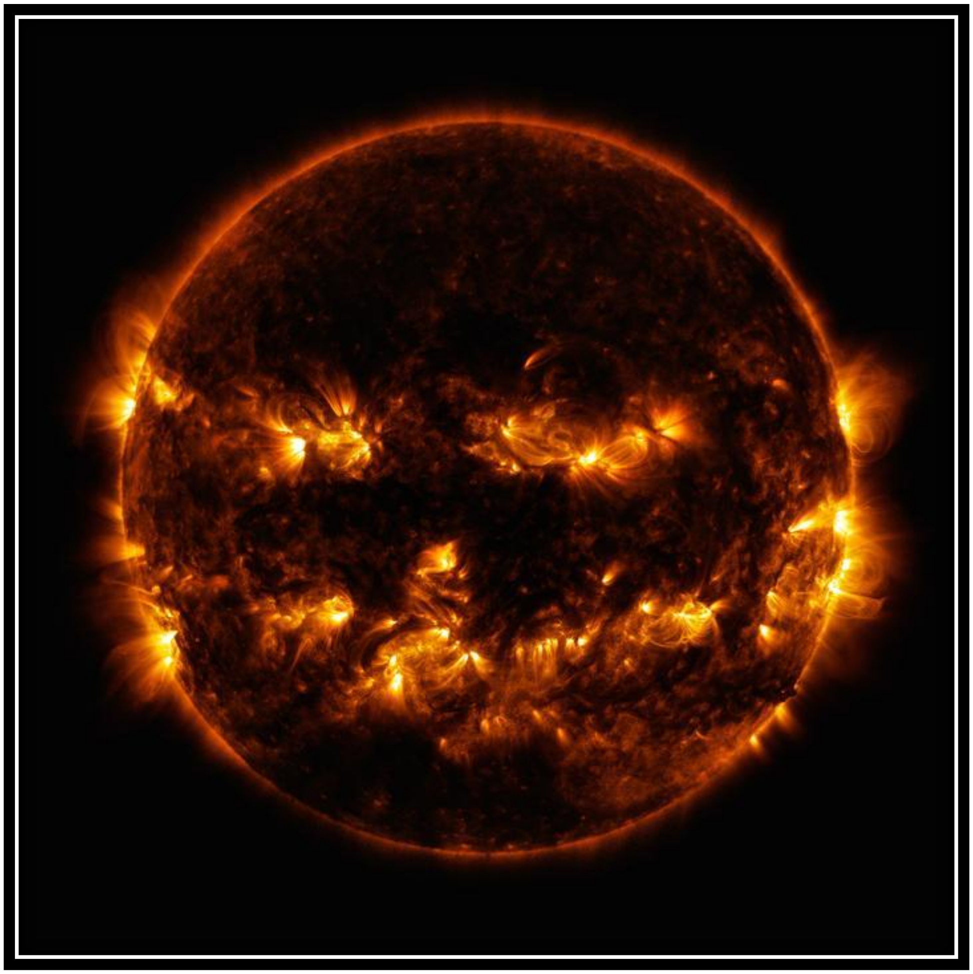
of the climate system. Sea ice and snow cover melt, turning brilliant white reflectors of sunlight into darker spots. The atmosphere grows moister because warmer air can hold more water vapor, which absorbs more shortwave radiation. Both of these feedbacks lessen the amount of shortwave radiation that bounces back into space, and the planet warms rapidly at the surface. Meanwhile, like any physical body experiencing warming, Earth sheds longwave radiation more effectively, canceling out the longwave-trapping effects of CO_2. However, a darker Earth now absorbs more sunlight, tipping the scales to net warming from shortwave radiation. "So there are two types of radiation important to climate, and one of them gets affected by CO_2, but it's the other one that's directly driving global warming — that's the surprising thing," says Armour, who is a postdoc in MIT's Department of Earth, Atmospheric and Planetary Sciences. Out in the real world, aerosols in air pollution act to reflect a lot of sunlight, and so Earth has not experienced as much warming from shortwave solar radiation as it otherwise might have. But the authors calculate that enough warming will have occurred by midcentury to switch the main driver of global warming to increased solar radiation absorption. The paper is not challenging the physics of climate models; its value lies in helping the community interpret their output. "While this study does not change our understanding of the fundamentals of global warming, it is always useful to have simpler models that help us understand why our more comprehensive climate models sometimes behave in superficially counterintuitive ways," says Isaac Held, a senior scientist at NOAA's Geophysical Fluid Dynamics Laboratory who was not involved in this research. One way the study can be useful is in guiding what researchers look for in satellite observations of Earth's radiation budget, as they track anthropogenic climate change in the decades to come. "I think the default assumption would be to see the outgoing longwave radiation decrease as greenhouse gases rise, but that's probably not going to happen," Donohoe says. "We would actually see the absorption of shortwave radiation increase. Will we actually ever see the longwave trapping effects of CO_2 in future observations? I think the answer is probably no." The study sorts out another tricky climate-modeling issue — namely, the substantial disagreement between different models in when shortwave radiation takes over the heavy lifting in global warming. The authors demonstrate that the source of the differences lies in the way in which a model represents changes in cloud cover with global warming, another big factor in how well Earth can reflect shortwave solar energy. Originally reported from *SciTech*.■

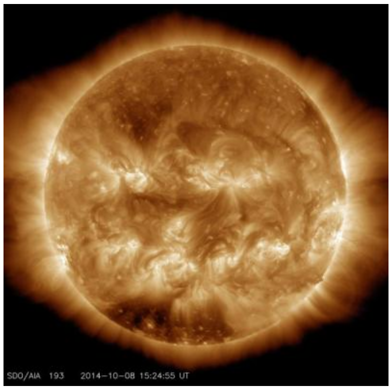
PNAS 2014; doi: 10.1073/pnas.1412190111

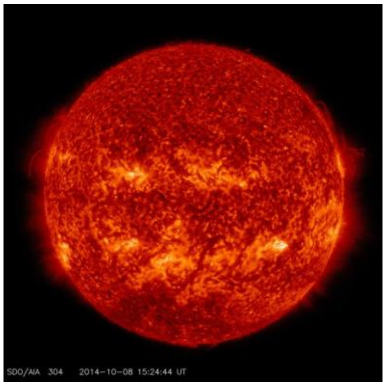

Who feeds us?

The Sun

By NASA (2014, USA)







Active regions on the sun combined to look something like a jack-o-lantern's face on Oct. 8, 2014. The active regions appear brighter because those are areas that emit more light and energy — markers of an intense and complex set of magnetic fields hovering in the sun's atmosphere, the corona. This image blends together two sets of wavelengths at 171 and 193 Angstroms, typically colorized in gold and yellow, to create a particularly Halloween-like appearance. (From NASA's Goddard Space Flight Center)


When you face the eruption
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Pathological Contribution of Spinal Macrophage Migration Inhibitory Factor to Neuropathic Hypersensitivity through Interacting with TNF-α in the Rat

Maria L. Bolick, Qingsong Zhao, Shiqin Xu, Mary K. Pathak, Aili Sunny, Fuzhou Wang

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Pathological Contribution of Spinal Macrophage Migration Inhibitory Factor to Neuropathic Hypersensitivity through Interacting with TNF-α in the Rat

Maria L. Bolick [*,1], Qingsong Zhao [†,1], Shiqin Xu [‡,1], Mary K. Pathak [*], Aili Sunny [*], Fuzhou Wang [*, ‡, Δ]

BACKGROUND Our previous data demonstrated that the proinflammatory cytokine macrophage migration inhibitory factor (MIF), a pleiotropic cytokine produced mainly by nonneuronal tissue, has been implicated in the pathogenesis of inflammatory and neuropathic hypersensitivity, whereas the precise underlying mechanisms are not totally elucidated. The aim of this study was to examine the interacting role for spinal MIF with TNF-α in neuropathic pain.
METHODS After approval by the institutional Animal Care and Use Committee, the randomized Sprague-Dawley rats underwent prophylactic intrathecal administration of recombinant MIF (rMIF), TNF-α, MIF mAb, TNF-α mAb, or in combination prior to the spared nerve injury (SNI). Thermal hyperalgesia with hot plate and tactile allodynia using von Frey filaments were assessed after different interventions. Spinal cord levels of MIF and TNF-α were measured using Western Blotting and immunocytochemistry.
RESULTS Exogenous rMIF potentiated SNI-induced nociceptive behavior that were not evoked by single use of rMIF without SNI, and this potential effect could be blocked by MIF antibody in part. After giving rMIF combined with TNF-α to SNI animals, the perception of thermal and tactile stimuli was maximized. Spinal MIF mAb inhibited TNF-α expression, and vise versa for TNF-α Ab on MIF expression after SNI. rMIF or TNF-α combined to SNI produced more significant effect on the levels of MIF and TNF-α than SNI alone, and this effect could be furthered by administering rMIF and TNF-α together.
CONCLUSION These data demonstrate that proinflammatory cytokine MIF is involved in the peripheral nerve injury-induced hypersensitivity through potentiating spinal TNF-α signaling.■

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Keywords: Macrophage migration inhibitory factor – Tumor necrosis factor – Spinal cord – Inflammation – Neuropathic pain

NOXIOUS challenges can evoke a large number of cytokine productions to prevent and eliminate the original causes. However, a series of debilitating pathological and behavioral events like hyperalgesia is often linked to the immune activation (1). Tumor necrosis factor-α (TNF-α), one of the early proinflammatory cytokines, is released in response to pain evoking conditions (2). TNF-α signaling involves in the sensitization of both primary afferent and spinal cord neurons, and plays essential role primarily in formalin-induced pain condition by inducing peripheral edema and sensitization (3, 4). Meanwhile, peripheral injection of TNF-α can mimic and evoke neural excitability and hyperalgesia (5), and can result in ectopic discharges in primary afferent fibers and rat dorsal horn neurons (6).

Macrophage migration inhibitory factor (MIF), a multi-functional molecule, has been found to be involved in the regulation of the inflammatory responses and other enzymatic activities (7), and strongly associated with some inflammation-based pathology (8-11). Our previous data disclosed the fundamental role of MIF in contributing to the pathogenesis of inflammatory (12) and neuropathic hypersensitivity (13). Interestingly, TNF-α alone can induce local and systemic up-regulation of MIF (14, 15), and high levels of MIF can also induce macrophage TNF-α secretion (15). Accordingly, we surmised that MIF may contribute to the sensitization of afferent fibers and further discharges on dorsal horn neurons by potentiating the TNF-α signaling in peripheral nerve injury-associated hypersensitivity.

MATERIALS AND METHODS

Animals

The animals used in this study and the animal care were presented in our previous studies in detail (12). In brief, after approval by the Institutional Committee of Animal Care and Use, adult male Sprague-Dawley rats weighting 250-350 g were housed in pairs to a plastic cage with soft bedding on a reverse 12:12 h dark/light cycle with lights on at 8:00 AM with free access to food and water throughout the experiment for at least one week before the experiments. Test sessions took place during the light phase between 10:00 AM and 6:00 PM in a quiet room maintained at 22-24 ℃. No food or water was available to the rats during the experiment. Each animal was used only once and was euthanized at the end of the experiment by administrating a lethal dose of pentobarbital for tissue collection.

Procedures

Rats were randomly assigned to one of ten groups with16 each, of which four groups got Sham, spared nerve injury (SNI) (see detailed description below), sole intrathecal (i.t.) injection of recombinant MIF (rMIF, 150 μg/kg) or TNF-α (50 μg/kg), respectively; and another three groups received respective prophylactic i.t. injection of MIF mAb (100 μg/kg), TNF-α mAb (100 μg/kg) and MIF mAb+TNF-α mAb and another three groups got i.t. injection of rMIF, TNF-α, or rMIF+TNF-α prior SNI surgery, respectively. All i.t. drugs were administered 4 days consecutively with once each day before nerve injury.

After the first group allocation, rats in each group were redivided into two subgroups according to a second-step stratified sequence number produced by computer for receiving thermal and tactile nociceptive test separately. Possible effects of repeated testing were minimized via measuring the threshold to respond to noxious stimuli on all tests.

Intrathecal catheterization

Rats were implanted with intrathecal catheter (ALZET Osmotic Pumps, Cupertino, CA, USA) for drug delivery as described previously (16). In brief, after shaving and sterilizing the cephalic-cervical area, a midline incision was made followed by dissection of the paravertebral muscles from the spinous processes. The dura was slit and the catheter was inserted for 7.0 cm caudal from the dural slit and then it was fixed with a drop of tissue glue (B. Braun, Tuttingen, Germany) and was further secured on the fascia of paravertebral muscle. Finally, sodium penicillin 10,000 IU (Shanghai AoBopharmtech, Shanghai, China) was given intramuscularly against infection. The rats would be excluded (~10%) if neurological deficits were exhibited after catheterization. The intrathecal catheter was not removed until the day of sacrifice to prevent evoking unexpected behavioral stress and spinal cord injury.

Spared nerve injury model and behavior test

The spared nerve injury (SNI) model was used as described in our previous work (16). In brief, animals were anesthetized with isoflurane, and the tibial and common peroneal branches of the sciatic nerve were ligated and sectioned distally, but the sural nerve was left intact. For sham surgeries, the sciatic nerve was merely exposed but not ligated or dissected.

Allodynia measurement

Pain thresholds were assessed using the von Frey filaments (Stoelting Co., Wood Dale, IL) prior to surgical procedures and again at different time points thereafter. The testing protocol has been described in our previous study (13, 16). In brief, the filaments were applied to the central surface of the hind paw plantar for a maximal of 10 s to determine the threshold of the stimulus through evoking a withdrawal response. The increment of stimulus was based on the response of the rat to the current filament, if the paw withdrew, the same hair was again used 60 s later; but if not, the next stronger hair was presented. If the rat withdrew its paw in two consecutive trials with the same filament, no further filaments were tested. Withdrawal responses were used to determine the absolute threshold, i.e. the 50% withdrawal threshold, by fitting to a Gaussian Integral Psychometric Function via a Maximum Likelihood method.

Thermal pain behavior

Thermal hyperalgesia was assessed using an Analgesiometer (Eddy's Hot Plate; Naugra Export, Ambala Cantt, Haryana, India) as described in our early studies (13, 16). Briefly, the temperature on the hot plate was set at 55±0.1 ℃, and the paw withdraw

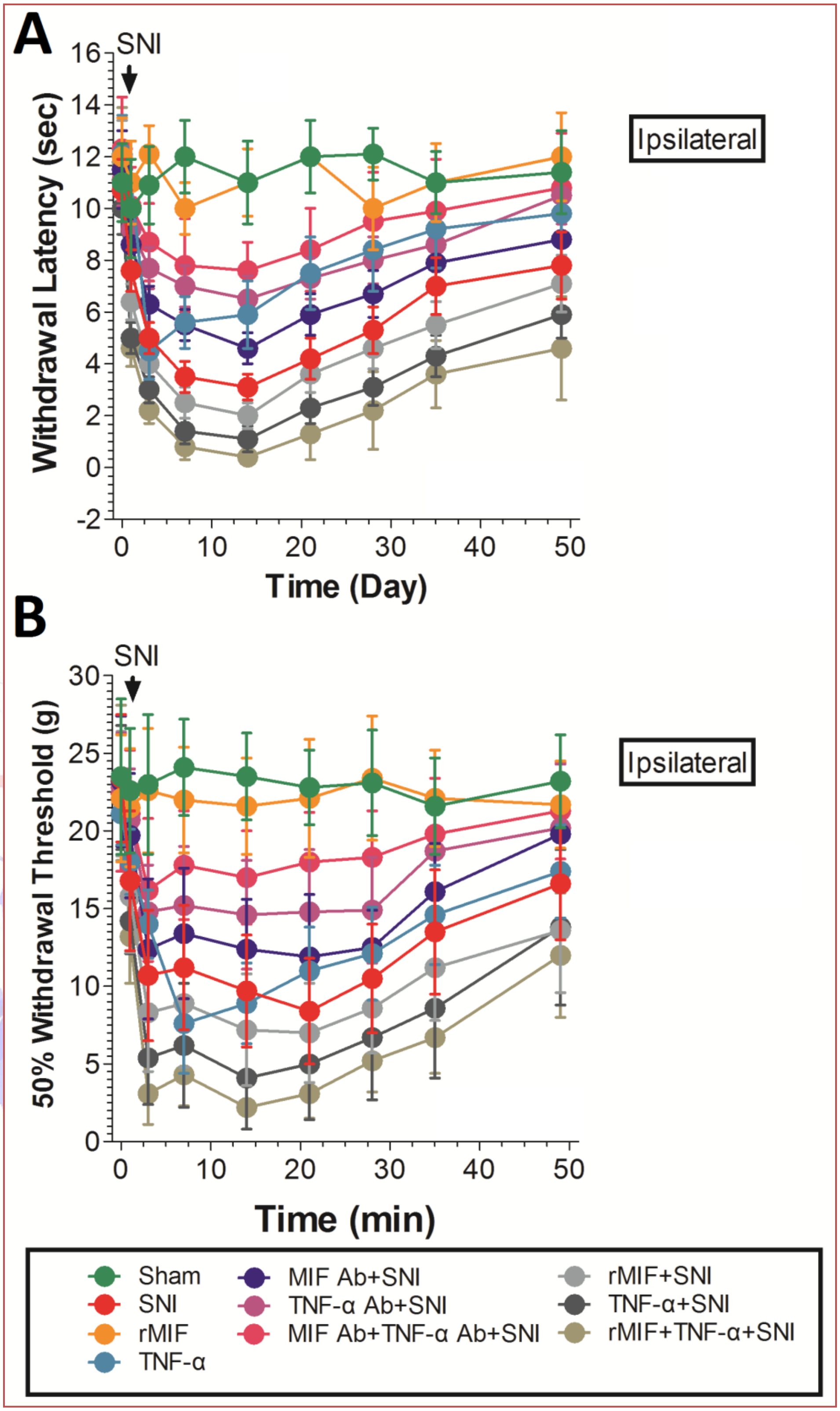

Figure 1. Nociceptive behavior responses after treatments. Experiments of thermal hyperalgesia (A) and tactile allodynia (B) were performed after SNI injury. MIF Ab plus TNF-α Ab produced the most significant alleviation of these two nociceptive behaviors than MIF Ab or TNF-α Ab alone ($P < 0.05$). The effect of TNF-α Ab on these behavior responses was more significant than MIF Ab ($P < 0.01$). Prophylactic rMIF or TNF-α amplified the pain behavior compared with sole SNI ($P < 0.05$), and an additive effect was produced after using rMIF and TNF-α together to the nerve injury ($P < 0.01$). Single use of rMIF without SNI did not produce any nociceptive effect, but TNF-α did.

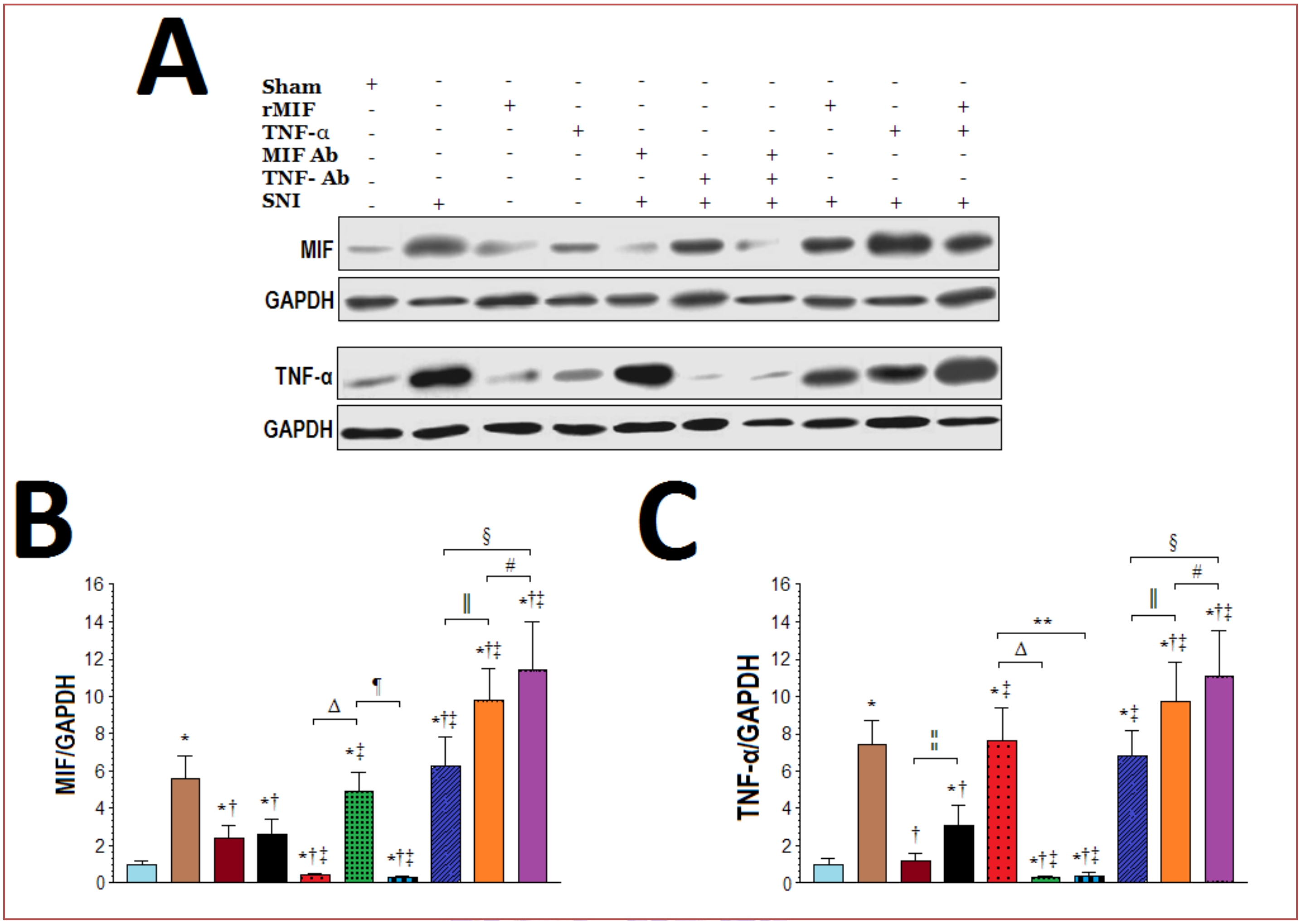

Figure 2. Immunobloting of MIF and TNF-α in the spinal cord. Spinal expression of MIF and TNF-α was detected by Western Blotting. MIF Ab could not inhibit TNF-α expression, and vise versa for TNF-α Ab on MIF expression after SNI. Nevertheless, rMIF or TNF-α combined to SNI produced more significant effect on the levels of MIF and TNF-α than SNI alone ($P < 0.001$), and this effect could be furthered by administering rMIF and TNF-α together ($P < 0.0001$). rMIF itself did not induce further expression of TNF-α, but TNF-α could increase spinal MIF. *, versus saline; †, versus SNI; ‡, versus single rMIF or TNF-α.

al latency of withdrawal were observed by recording the number of seconds required for reaction to the thermal stimuli. The maximal duration of stimulation was set at 22 s as a cut-off time to avoid tissue damage. Each animal was tested three times repeatedly at an interval of 15 min, and each time the test was carried out by a different investigator.

Immunoblotting for MIF and TNF-α expression

Lumbar spinal tissues were collected and the dorsal part was homogenized in buffer (10 mM Tris, 5 mM EDTA [ethylenediaminetetraacetic acid], 2% Triton X-100, 0.2 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml leupeptin and aprotinin) and mechanically disrupted. Samples were analyzed by SDS-PAGE by means of a transfer buffer (25 mM/l Tris, 192 mM/l glycine, and 20% methanol) in a wet-transfer apparatus. Blots were blocked with 5% non-fat dry milk in PBS with 0.1% Tweens-20 and then incubated with mouse anti-rat MIF or TNF-α Ab (all two were 1:1,000, Sigma-Aldrich, USA). After repeated washing, rabbit anti-mouse secondary antibody (1:4,000, horseradish peroxidase-conjugated) incubation was performed, developed with a chemiluminescence system, and followed with film exposure and relative intensity analysis with the Typhoon Imaging System (GE Healthcare, Piscataway, NJ, USA). The immuneblots were washed briefly and then incubated with a monoclonal mouse anti-rat GAPDH (glyceraldehyde 3-phosphate dehydrogenase) antibody (1:10,000, Abcam) for 40 min at room temperature followed by a horseradish peroxidase-conjugated rabbit anti-mouse antibody. GAPDH protein was then visualized and detected as the internal biomarker.

Immunohistochemistry

The L5 segment of the spinal cord was removed by laminectomy 20 min after the drug injection into the plantar surface of the hind paw and fixed with fresh 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at

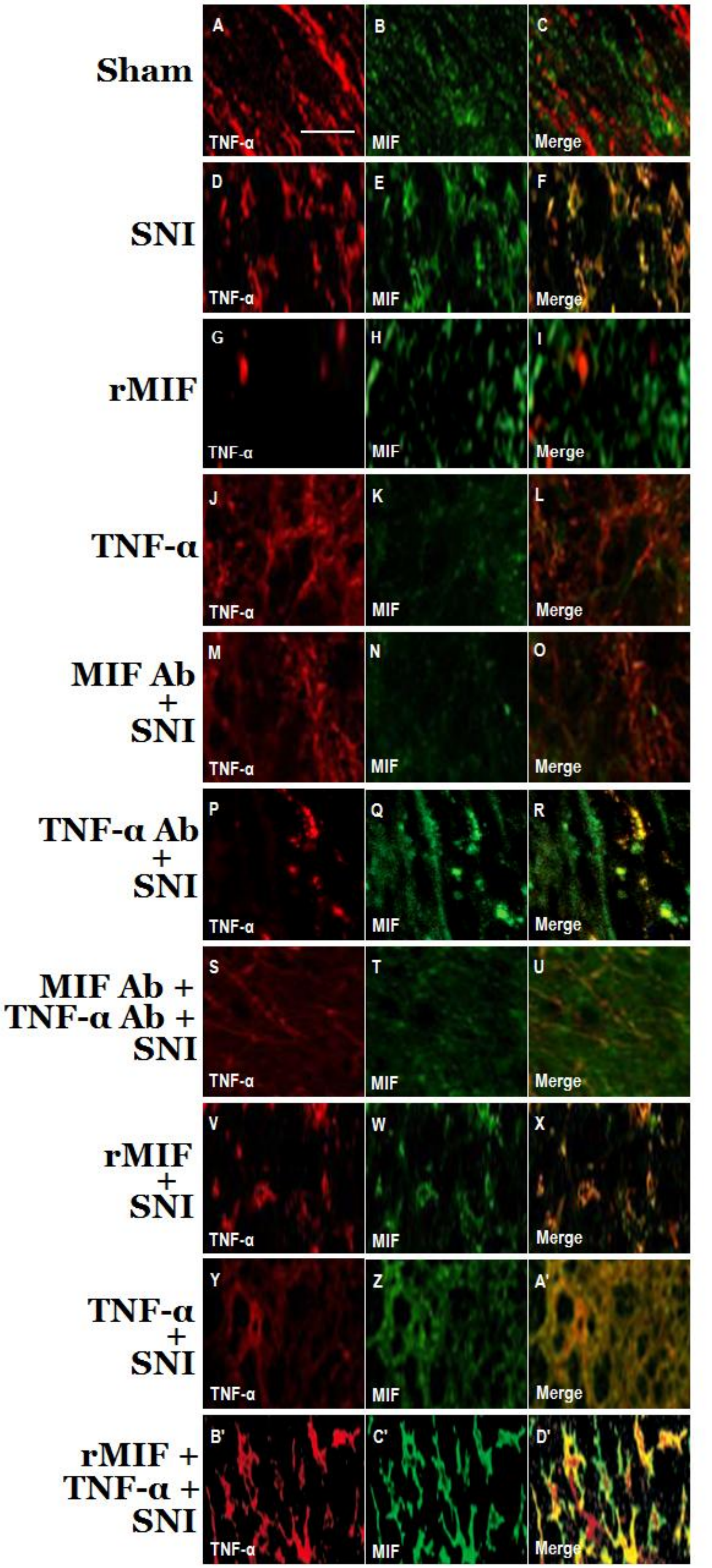

Figure 3. Immunolabeling of TNF-α and MIF expression in the ipsilateral spinal cord. In the Sham animals (A-C), MIF and TNF-α did not co-exist in the ipsilateral spinal dorsal horn. MIF was in glial cells, but TNF-α was in surrounding tissue of glial cells. In the SNI rats (D-F), TNF-α transferred into glial cells after peripheral nerve injury and co-located with MIF. In the rMIF injected animals (G-I), rMIF itself did not induce further expression of TNF-α in the dorsal horn. In the TNF-α-treated rats (J-L), TNF-α increased spinal level of MIF in part. In the MIF Ab + SNI animals (M-O), MIF Ab did not inhibit TNF-α expression in the ipsilateral spinal cord. In the TNF-α Ab + SNI animals (P-R), TNF-α Ab did not block MIF expression in the ipsilateral dorsal horn. In the MIF Ab + TNF-α Ab + SNI animals (S-U), MIF and TNF-α were blocked by both antibodies. In the rMIF + SNI (V-X), and the TNF-α + SNI (Y-A′) rats, prophylactic administration of rMIF or TNF-α to SNI produced additive effect on the spinal levels of TNF-α and MIF. In the rMIF + TNF-α + SNI animals (B′-D′), combined use of rMIF and TNF-α maximized the effect of SNI's role in the spinal levels of TNF-α and MIF.

4 ℃ overnight and then transferred to 30% sucrose in phosphate buffered saline for 48 h. Transverse sections (20-μm thick) of L5 spinal cord were prepared using a cryocut microtome (Leica CM3050S). The floating sections were incubated with 3% H_2O_2 for 30 min and blocked in solution containing 5% normal goat/horse serum, 5% fetal bovine serum, 2% bovine serum albumin, and 0.1% Triton X-100 for 2 h at room temperature. For double immunofluorescent staining, floating sections were incubated overnight at 4 ℃ with a mixture of mouse anti-rat TNF-α Ab (1:500, Santa Cruz Biotechnology, Santa Cruz, USA) and rabbit anti-rat MIF Ab (1:500, Sigma-Aldrich, USA), The sections were then incubated for 1 h at room temperature with a mixture of fluorescein isothiocyanate-conjugated anti-mouse IgG and cyanine 3 (Cy3)-conjugated anti-rabbit IgG antibodies (1:200, Jackson ImmunoResearch). The sections were mounted with Vectashield (Vector Laboratories). Fluorescent images

were viewed with an Olympus fluorescence microscope (Olympus America Inc., Center Valley, PA), and the images were shot with a digital camera.

Statistical analysis

Analyses were performed using GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA). Data are presented as the mean ± standard error of the mean (SEM). Nociceptive data were analyzed with two-way ANOVA. The ANOVA tests were always followed by the Bonferroni *post hoc* tests for multiple comparisons. One-way ANOVA was used to analyze the intergroup difference in the tissue levels of MIF and TNF-α. All reported P values are two-sided and a P value of less than 0.05 was considered to be statistically significant.

RESULTS

Effect of prophylactic MIF and TNF-α blockades on the nociceptive behavior responses

The time course of the nociceptive responses to different treatments is presented in the Figure 1. Prophylactic administration of MIF Ab plus TNF-α Ab produced the most significant alleviation of tactile and thermal nociception than MIF Ab or TNF-α Ab alone ($P < 0.05$). The effect of TNF-α Ab on these behavior responses was more significant than MIF Ab ($P < 0.01$). If used rMIF or TNF-α simultaneously, the behavioral responses were amplified compared with those rats only with SNI injury ($P < 0.05$), and an additive effect was produced after using rMIF and TNF-α together ($P < 0.01$). Single use of rMIF without SNI did not produce any nociceptive effect. However, sole TNF-α injection evoked changes in rat behavior.

Spinal expression of MIF and TNF-α

Lumbar spinal cord was analyzed for protein expression of MIF and TNF-α depicted in the Figure 2. MIF Ab could not inhibit TNF-α expression in the experimental tissue, and TNF-α Ab also did not block MIF expression in the experimental spinal cord. Nevertheless, combined administration of rMIF or TNF-α produced additive effect on increasing tissue levels of MIF and TNF-α, and this effect could be furthered by adding rMIF and TNF-α together. Moreover, rMIF itself did not induce further expression of TNF-α, but TNF-α could increase spinal level of MIF.

Immunolabeling of TNF-α and MIF in the ipsilateral dorsal horn

Double staining of MIF and TNF-α in the ipsilateral dorsal horn demonstrated a strong fluorescence for the two proteins, but interestingly, MIF and TNF-α did not co-exist at the same site. MIF was in glial cells, but TNF-α was in surrounding tissue of glial cells. A more interesting thing was that TNF-α transferred into glial cells after SNI stimulation and co-located with MIF. MIF Ab could not inhibit TNF-α expression in ipsilateral spinal cord, and TNF-α Ab also did not block MIF expression in the ipsilateral dorsal horn. However, adding rMIF or TNF-α to SNI produced additive effect on increasing spinal levels of TNF-α and MIF, and this effect could be furthered by giving rMIF and TNF-α together. Finally, rMIF itself did not induce further expression of TNF-α in dorsal horn, but TNF-α could increase spinal level of MIF in part. (Figure 3)

DISCUSSION

We herein reported a pathologic role for MIF in facilitating TNF-α in sensitizing SNI-induced hypersensitivity. Rat recombinant MIF potentiates SNI-induced nociceptive behavior that were not evoked by single use of rMIF without SNI, and this potential effect could be blocked by MIF antibody in part. After giving rMIF simultaneously with TNF-α to SNI animals, the perception of exogenous stimuli was maximized. We report that underlying these behavior changes is the elevation of the levels of MIF and TNF-α in corresponding spinal cord tissues.

SNI-associated hypersensitivity was assessed through thermal and allodynia pain behaviors. These two types of behaviors were enhanced by sole intrathecal use of rMIF or TNF-α, and an additive effect on these behavioral responses would be evoked by giving rMIF and TNF-α together. On the contrary, these pain behaviors could be alleviated by MIF Ab or TNF-α Ab alone, and the blockade effect would reach maximal extent when combining MIF and TNF-α antibodies. Given rMIF itself did not evoke any changes in the nociceptive responses without SNI, but TNF-α was. Therefore, we conclude that spinal MIF functions as a promoting molecule to TNF-α in SNI-induced neuropathic hypersensitivity. Meanwhile, the SNI-induced nociceptive state is the prerequisite of MIF as a contributing factor to the pronociceptive role of TNF-α.

Although the effect of other proinflammatory cytokines including interleukin-1β (IL-1β) and interleukin-6 (IL-6) on central sensitization and hyperalgesia was evaluated and found IL-6 regulates inhibitory neurotransmission, and IL-1β regulates both excitatory and inhibitory neurotransmission, these effects are separate with TNF-α signaling (17, 18). In our study, we found a dependency link between MIF and TNF-α in sensitizing central perception of noxious stimuli. In the spinal cord, TNF-α evokes MIF release and MIF in turn promotes TNF-α production, TNF-α Ab cannot block MIF and MIF Ab cannot inhibit TNF-α.

Cytokine mechanisms of central sensitization give additional explanation for the longstanding uncertainty surrounding the mechanism of this widely used pain model. Accumulating evidence suggests that TNF-α signaling plays an important role in central sensitization development. Overexpression or exogenous administration of TNF-α promotes excitatory neurotransmission (18, 19), inducing acute peripheral mechanical sensitization by acting on TNF receptor 1 (TNFR1) in primary afferent neurons which results in p38-dependent modulation of tetrodotoxin-resistant sodium channels (20), and inducing the functional expression of cyclooxygenase-II (COX-2) in dorsal root ganglion cells (21). TNF-α also enhanced AMPA-induced current and NMDA current in dorsal horn neurons (18) that was in agreement with a previous report from hippocampal neurons (22). These studies investigated the role of TNF-α in pain and potential mechanisms. In our study, we provide evidence that MIF facilitates TNF-α signaling by a feed-back modulation in SNI-induced hyperalgesia. Whether MIF also exerts effects on neurotransmission or on ion channels in the primary afferent nerve fibers and spinal dorsal horn neurons remain to be determined.

MIF and TNF-α inhibitors are effective for the treatment of inflammatory pain conditions, including rheumatoid arthritis (RA) and inflammatory bowel diseases (23-27). In addition, TNF-α blocking therapy reduced systemic MIF levels in RA (28). Our findings together with previous reports showed amelioration of pain in a variety of models suggested that agents that specifically target on MIF or TNF-α signaling may be effective in specifically targeting on pain hypersensitivity.

Some limitations should be acknowledged before concluding our study. First, previous data demonstrated that MIF is a subsequent product of the peripheral immune cells and the endocrine cells of the anterior pituitary gland (29). In our study, we did not seek the original source of MIF after nerve injury. Second, a change of gradient of the dosage of MIF and TNF-α or their antibodies delivered separately or in combination may be useful to analyze their precise relationship. Third, we only tested ipsilateral changes in MIF and TNF-α levels in spinal dorsal horn, how about the contralateral changes is meaningful for future studies. Therefore, further studies are needed to clarify these points.

In sum, MIF blockade alleviates SNI-induced thermal hypersensitivity and allodynia in accompanying with decrease in spinal TNF-α level. Neutralization of TNF-α produces identical effect on nociception as MIF inhibition did. However, MIF alone plays no role in nociceptive responses in normal animals. These data suggest that proinflammatory cytokine MIF involves in peripheral nerve injury-induced hypersensitivity by potentiating TNF-α signaling.■

Conflict of Interests

None

Acknowledgements

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Author Contributions

Maria L. Bolick, Qiangsong Zhao, and Shiqin Xu (Study Design, Data Collection, Data Interpretation, Manuscript Preparation, Literature Search); Mary K. Pathak and Aili Sunny (Data Collection, Data Interpretation, Manuscript Preparation, Literature Search); Fuzhou Wang (Study Design, Data Collection, Data Interpretation, Manuscript Preparation, Literature Search, Funds Collection)

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Common Animals That Medieval People Got Completely Wrong

MEDIEVAL people lived in a time when superstition was fact, and unbelievable things were easily accepted. Nowadays, it's hard to get such animals as the adorable beaver and the ever-loyal dog wrong, but back then fabulous tales were considered nonfiction. To be fair, they couldn't exactly hop on a computer and verify what they'd heard. In fact, many of them never even learned to read. The few that were lucky enough to be taught how to read often stayed in much the same place for most of their lives. After all, travel wasn't quite as advanced as it is today.

Compounding this problem was the fact that very few new works of literature were written during those days. However, stories were well beloved, since they helped relieve the monotony and boredom. People loved stories about animals, and the information often came from "bestiaries"—books that detailed accounts and descriptions of animals. However, they weren't exactly written but rather copied. People had to rely on the texts of such noted and intelligent people as Pliny the Elder and Augustine of Hippo, who died quite a while before the Middle Ages.

BEES

Remember the "birds and the bees" talk? Well, they must have used something besides bees in medieval times. Why? Because, apparently, they didn't think bees were "born" like normal animals. They were born from the decaying bodies of oxen or calves. Sometimes, it was said they came from worms that spontaneously formed in the bodies of cattle. They also believed the little buggers chose the noblest bee among them to be their king (as opposed to a queen) and that they had wars.

The bees were also thought to have a code of laws based on customs. There was no need

for things like punishing the wrongdoers or a death code; the lawbreakers efficiently handled everything themselves. They were thought to punish themselves by committing suicide by stinger, because bee criminals seemingly couldn't bear to live with their guilty conscience. Also, bees were classified as birds. The smallest of birds, actually. Birds that started out as worms; maybe the ancients appreciated irony as much as we do.

MOUSE

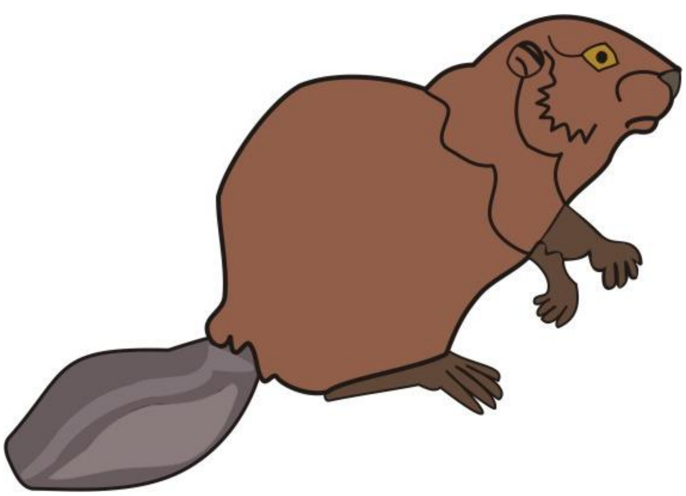
The bee wasn't the only animal that medieval people thought "just happened." Everyone knows what a mouse is. It's hard not to, considering they are just about everywhere. As a matter of fact, there are so many of them that people decided they must come from a source that was just as common as they were. According to the experts of the day, mice came fromthe ever-present dirt. That's actually where their name came from—they got*mus* (mouse) from *humus* (dirt).

However, not all experts thought that mice were just little dirtballs that decided to grow legs and a tail. Pliny the Elder wrote that mice

conceived either by licking each other or by tasting salt. Licking seems a bizarre way to reproduce, but he's the expert. Pliny the Elder also taught that Egyptian and Alpine mice had the rather unique quality of walking on two legs. If you're scared of mice, imagine a bipedal, mangy little mouse coming at you.

BEAVER

Ah, the adorable little bucktoothed beaver, the bane of trees everywhere, hated by river- and stream-owners alike. If you've ever done any beaver research at all, you know they were hunted for their skins. But, according to our group of medieval experts, hunters couldn't care less for the skin of a beaver. They were after a much stranger, much more disgusting prize: the testicles. The poor animal's testicles were apparently used in making medicine.



See, the beaver must have been quite a life-loving creature. When it was being hunted and knew it would be unable to get away, it made a terrible choice. It would sacrifice its testicles to live. How? It was quite simple. Those teeth that can slice through tree trunks with ease? It seems they were the perfect self-castration tool. The beaver would bite off its own testicles and throw them at the hunter. It would give up its manhood to continue living. But what if the beaver had to go through the stress of being hunted for its "goods" again? It was an easy matter of lifting its leg to let the hunters know that they were wasting their time chasing a eunuch beaver.

CUCKOO

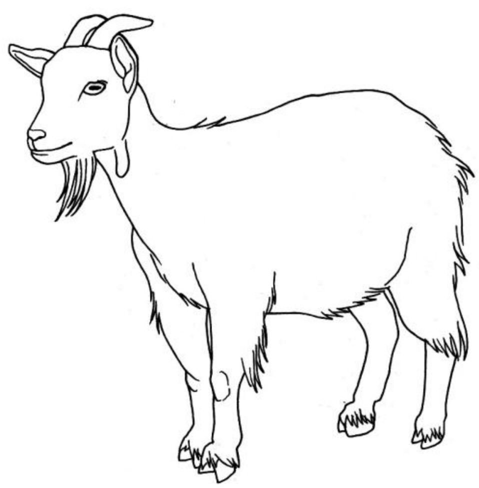
The cuckoo bird. That little bird that comes out of old clocks at the top of every hour? According to the bestiaries, it had some weird habits. For example, the cuckoos were weak and short-winged. This made it hard for them to fly long distances. So the lazy little birds had a solution to this problem: They would piggyback on a kite (the bird, not the toy). We have no idea how they could have done that.

That wasn't the only example of the cuckoo's laziness. It appears these birds didn't like work that much; as a matter of fact, they didn't even take care of their own young. They'd simply leave their eggs in the nest of another bird and then go on their happy little way. They were not only lazy, but also terrible parents. However, it seems they did do one productive thing: Their saliva supposedly produced cicadas, those annoying little insects that love to keep you up all summer.

It should be noted that some birds actually do abandon their young in others' nests, and so do several species of the cuckoo (including the common cuckoo). So the medieval writers actually did get something almost right.

GOAT

Goats are fairly common animals. We've all known about goats since we were little kids, largely thanks to those wonderful stories about trolls under bridges. The medieval drawings and descriptions of goats were actually quite accurate. Well, except for one thing. Apparently,



the he-goats were very lusty creatures, and this caused their blood to be extremely hot. So hot, in fact, that it would dissolve diamonds—the hardest natural substance in the world. In the authors' own words: a stone "so hard that it can be wrought neither by iron nor fire." We wonder how they went about testing this theory. Also, how'd they go about killing goats if they thought their blood was hot enough to melt diamonds?

IBEX

Then there's the goat's relative: the ibex. Also called "mountain goats," the ibex are best known today for hopping around on mountains, oblivious to the fact that a single slip would send them to their deaths. They also possess spiral horns, used for protection against predators and fighting for dominance.

However, the ancients believed there was another use for the horns. They couldn't believe that an animal could be amazing enough to hop around on mountains without a built-in defense against falling. They believed that if the ibex were ever to slip, it would simply land on its horns (which they assumed were hard enough to not break). The ibex would just shake the fall off, unharmed, and go back to its simple life of being a ninja goat.

PELICAN

The pelican seems like an innocent enough creature. A pelican was the gentle, if slightly crazy, friend of Ariel in *The Little Mermaid*. You see them near the sea all the time. They usually seem completely harmless (though they *can* be rather aggressive). But to people living in medieval times, they must have been terrifying.

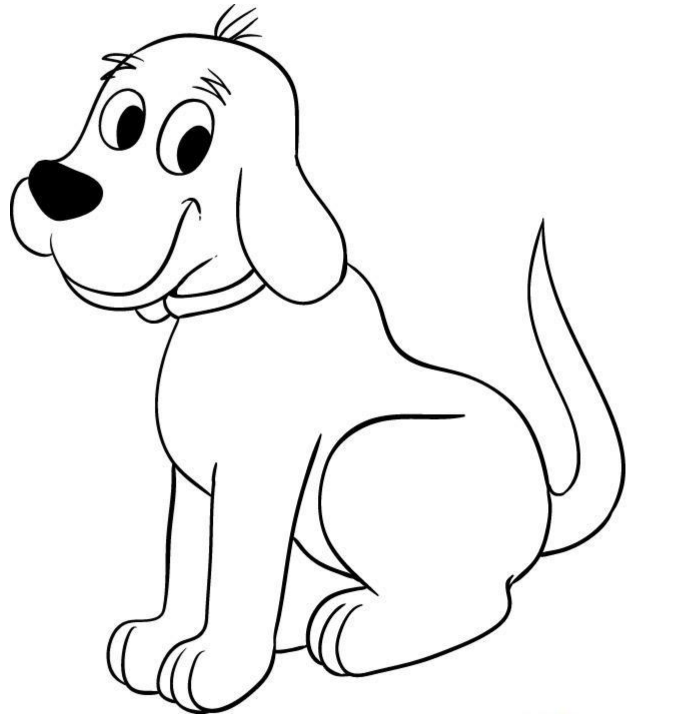
Pelicans have baby pelicans. That's normal. But, according to medieval bestiaries, when

their babies pecked at them, the pelicans would lose their anger and strike the babies. Not just a little swat, either; they'd strike hard enough to kill. Yes, they killed their own babies. But it all ended well; after three days, the mom would peck herself until she bled. When the blood of their mother fell on the dead babies, they'd come back to life. But that's not the only thing the "experts" got wrong. They also thought that one kind of pelican ate crocodiles. Crocodiles! That'd have to be one horrifying bird, a far cry from the adorably clunky one we know today.

DOG

The loyal dog, man's best friend. All of us know what a dog is. We've hunted with them, shared our food with them, taken care of them, and loved the furry goofballs since someone first threw a piece of meat to a wolf. But, if you believe the medieval outlook on them, they're even more awesome. First, it was believed that dogs were unable to live without men. That said, they were extremely useful creatures. They could cure their own wounds by licking them. But, more amazingly, binding a young dog to a patient would heal any internal wounds.

There are stories told of the loyalty and bravery of dogs. Ever heard of King Garamantes's dogs? The story goes that this king was captured by his enemies. He was rescued but not by his army or his knights: He was rescued by his 200 dogs. On top of that, the dogs then escorted the king back to his lands, fighting off any who tried to take him again.



And then there's the story of a man who was murdered. His dog loyally kept vigil over his corpse. The crowd that had gathered around the dead man couldn't get the dog to leave. The murderer, thinking he could throw suspicion off himself, joined the crowd and went up to the body, feigning emotion. The dog saw him and, recognizing the man as the murdering cutthroat, attacked him. Almost immediately, the killer confessed to the murder so the crowd would get the enraged dog off of him.

WEASEL

The weasel is a small, ferret-like creature. Most of us probably have at least a vague notion of what it looks like. Medieval folk had far less knowledge of this creature. Or, rather, they had a strange, strange notion of the weasel. First off, it was a dirty creature that wasn't to be eaten. Not many people today crave weasel sandwiches, so that part isn't too far off. But then things start to get rather . . . disturbing.

See, the weasel apparently conceived through the mouth and gave birth through its ear. (Some experts claimed the opposite: Weasels conceived through the ear and gave birth through the mouth. It's hard to say which is

worse.) If the babe came out the right ear, it would be a male; if it was birthed out the left ear, it would be a female. Oh, and if their baby was injured while exiting the ear/birth canal? Nothing to it: Since the weasel was skilled with medicine, it could revive its young.

PANTHER

Ever heard of a panther? A picture of a real one can be found here. Looks different than the one above, doesn't it? Believe it or not, that picture's closer to the truth than any of the other stuff medieval experts believed about the panther. They described it as a "gentle, multicolored beast whose only enemy is the dragon."

Here's how the typical panther's life went, according to our medieval sources: The panther would feast—on dragons, presumably. After it was done with its scaly meal, it would find itself a cozy cave and take a long catnap; a three-day-long catnap, actually. Then, when it woke, the panther would give a loud roar. While it was roaring, a sweet smell would come out of its mouth. All the animals of the panther's habitat would follow this odor to the roaring panther, apparently bewitched.

Well, almost all. There was an exception: the mighty dragons. The huge, terrifying dragons would cower in their little hidey-hole, because, for some unknown reason, the panther scared the life out of them. Oh, and the animals that went to the panther after its nap? No one really talked about what came of the bewitched forest creatures... but there were only so many dragons for the "gentle" panther to eat. ■ *Read more on the original article: Listverse*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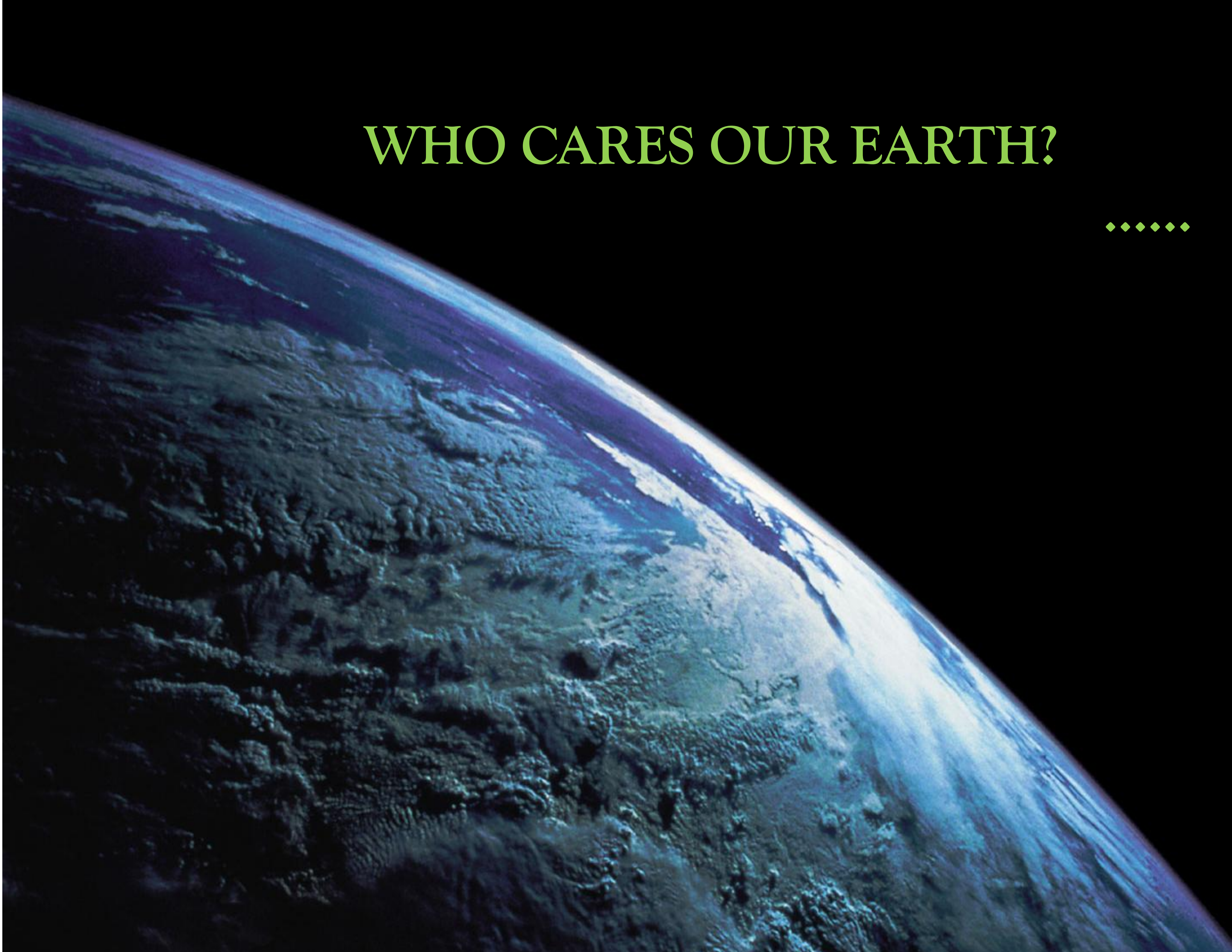
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