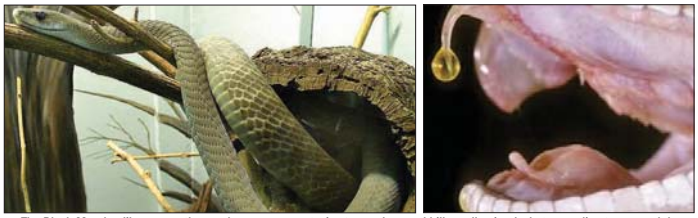
Analgesic in the snake pit

The venom of the deadly Black Mamba has been found to contain a valuable painkiller, says
a ananthanarayanan



The Black Mamba, like many other snakes, uses neurotoxins to paralyse and kill small animals, but according to research by Sylvie Diochot (below) and colleagues, working in France, the venom also contains a very potent painkiller, most likely working to calm down unlucky prey. They analysed 50 different Mamba species before ultimately finding the Black Mamba's pain-killing proteins — called mambalgins.

Sylvie Diochot.

WHILE anaesthesia is an important field in medicine and an integral part of surgery, the control of intense pain is also an area as important as control or cure of disease. The "Gold Standard" in pain control is the opium derivative, morphine, which is unequalled in relief from the agony of many cancers, in heart attacks, water in the lungs and the testing time of childbirth. But morphine has serious side effects and its use is highly addictive, apart from larger doses being needed with continued use.

In this context, the isolation by Sylvie Diochot and colleagues, working in France, of a component of snake venom that is as effective as morphine, yet free of side effects, will influence more fields than only pain control. Morphine is the main extract from opium, which is the sap that oozes out of the poppy seedpod. Its use as a painkiller and as an exhilarant has been known since ancient times. Morphine was first separated from opium in 1804, for use as an analgesic and to help cure opium or alcohol addiction. But it is also widely misused, leading to dependence and addiction and is, again, easily converted into heroin, a more potent and more addictive psychotropic substance.

Morphine appears to act by mimicking the effect of endorphins, or morphine-like substances produced endogenously, ie, within the body itself. Endorphins are produced for pain relief, and to induce relaxation, sleepiness or feelings of pleasure, often as a means of reinforcing learning or behaviour. Morphine acts by connecting to specific parts of the cells in the central nervous system, to bring about its main effects – intense pain relief and deep sedation. But it also brings about euphoria and "disinhibition" – its name itself is in honour of Morpheus, the Greek God of Dreams — which has made it a drug of entertainment as much as one with medical use.

When used causally, there is intense psychological addiction, which goes on to physical addiction and, what is worse, the need for larger and large doses to maintain its mood-altering effects. Dependence is also a major disadvantage in legitimate use as a painkiller. So are other side effects, like the effect on respiration and the suppression of intestinal movements, which leads to constipation. But the efficacy of pain control is unparalleled and the management of several serious conditions is unthinkable without morphine.

Venom

Snake venom, secreted by glands behind the fangs, consists almost entirely of proteins whose function is mainly to immobilise prey or predators. Snakes have evolved highly efficient delivery systems, largely a hypodermic syringe-type set of injecting fangs. The proteins in the venom act both on the cardiac and vascular system as well as the nervous system of the victim. The effects are of inducing blood coagulation, a fall in blood pressure and an interference with the transmission of nerve impulses, which leads to paralysis, including that of the lungs and the heart. These specific actions of venoms of snakes or even of spiders and scorpions on biological processes have led to their use in medicine, for diagnosis or cure.

The way these protein-like toxins act on nerves is that they block or open pathways in the surface of nerve cells. The action of nerves is by transmitting a signal received from one cell to the next nerve cell or a muscle cell. The receipt and dispatch of signals is regulated by changes in the electrical tension between the inside and the exterior of the nerve cell.

The electrical tension is caused by inequalities of concentration of electrically charged atoms, called "ions", of sodium and potassium inside and outside the cells. Under normal conditions, the tension cannot be released as the ions cannot pass through the cell wall or through the groups of special proteins, called "ion channels", in the cell wall. But when primed by an external protein, which has a shape that exactly fits some part, called the "receptor", of the ion channel, the "gate" can be opened to allow a rush of charge in and out of the cell. This flow may then open the gate of a neighbouring cell to start a cascade of transmission, or it may excite a muscle cell, to cause contraction.

The action of protein toxins is by interfering with ion channels. The presence of a toxic protein, for instance, can modify the shape of the receptor so that it no longer reacts to the normal signals and is thus rendered ineffective.

Mambalgin

The venom of the Black Mamba is one of the fastest acting snake venoms in the world. It consists mainly of neurotoxins, or proteins that affect nerve cells, and the Median Lethal Dose (LD50), or the quantity of venom needed to kill 50 per cent of a tested population, is less than 0.2 mg per kilogram. This translates into 10 mg, or a hundredth of a gram, being good enough for a 50-kg person. The main proteins in the toxin consist of smaller protein molecules that spread quickly through the circulatory system of the victim, which is why the venom of the Black Mamba is the fastest acting. And for good measure, the average quantity of venom delivered in a bite is 110 mg.

Till recent times, when anti-venom has been made easily available, the bite of the Black Mamba was 100 per cent fatal in sub-Saharan Africa, where the snake is found.

As toxins that consist of proteins affect nerve cells, there has been interest in their action on ion channels that are involved in the transmission of pain. Recent studies have shown that snake venom is able to activate "Acid-Sensing Ion Channels" that are involved in sensing pain.

Sylvie Diochot, at France's Institute de Pharmocologie Moleculaire and Cellulaire, and her colleagues in different institutes in France surveyed the effect of the components of neuro-toxins drawn from different sources, different species of poisonous snakes, spiders, scorpions, sea anemones, looking for the reverse effect, of blocking the Asic. The different components of the toxins were separated and each was tested for its function of blocking Asic in living cells. The screening of a large number of toxin proteins revealed that two of the proteins in the venom of the Black Mamba did have the effect they were looking for.

These two proteins, which have been named Mambalgin1 and Mambalgin2 (after Mamba Analgin), were found to be nearly identical proteins built from 57 amino acids. Proteins are assembled by living cells with the help of templates that are stored in the cell DNA. The components of segments of DNA spell out the order in which specific amino acids are to be strung to create the protein. This specific formula, which may specify hundreds of amino acids, builds the protein that will fold in a highly specific way — and hence present peculiar and individual molecular features that can induce cells to behave in specific ways.

The DNA of poisonous snakes has, thus, over the course of evolution devised the proteins that are able to break down the neural communication in animals they attack and immobilise and kill them. And in the case of the Black Mamba, there are these two proteins that are able to affect communication of the channels that convey the sensation of pain!

Having found these two proteins that block Asic in cells in an experimental setting, it was found that they had the same effect on Asic in human cells, again, in laboratory tests. The next test was to see if Mambalgins worked on a living animal. seven to 11 weeks old so experimental mice were tested for reaction to pain, both in the normal state and after they had been administered doses of Mambalgins or of morphine. Pain was created with the help of chemical substances injected in the paws of the mice and the analgesics were administered either by spinal injection or injection in the paws. The effectiveness was assessed by observing behaviour, like kicking of the paws, which indicates discomfort.

The results of the trials were that the Mambalgins were quite as effective in blocking pain as morphine. What is more, it was found that while the same dose of morphine, when repeatedly administered, stopped being as effective as it was at the start, there was no such "tolerance" that developed with Mambalgins. Morphine also creates respiratory depression, which was found to not happen with Mambalgins. While any addicting propensity of the Mambalgins has not been mentioned in the paper by the group, published in *Nature*, any alternative to morphine as a reliever of acute pain, and free from major side effects that complicate recovery of patients, would make a great difference to pain management in most cases.

The study shows that Mambalgins work by acting both on the nerve cells that first sense the pain and also on the central nervous system. The evolutionary path through which the Black Mamba has included a powerful analgesic in the combination of lethal proteins in its venom is still not understood. But the discovery of Mambalgins, apart from being pain-relieving agents themselves, will advance the understanding of new routes to the management of pain.

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Strict observance is essential

tapan kumar maitra explains the importance of safety measures when using pesticides

WHEN using pesticides, safety measures are called for to prevent the poisoning of workers by toxicants, contamination of the environment, and the contact of outsiders and animals with pesticides. In all cases when pesticides are to be used, the supervisor must notify the management of the farm in which the work is to be done, the heads of neighbouring farms and the local population at least two days in advance on the time and nature of the work to be done and the safety measures.

Standard warning signs are set up at a distance of at least 300 metres from the boundaries of the section to be treated. Workers may enter sections treated with pesticides to perform field operations in four to six days after the use of polychloropinene and toxaphene; in three weeks after the use of hexachlorobutadiene; and in three to five days after the use of other pesticides.

Special attention must be given to a strict observance of the safety rules when working with virulent, highly toxic and volatile pesticides. When controlling rodents, poisons and highly toxic substances are used to prepare bait. This is prepared either in a specially assigned room provided with an exhaust hood or in special areas at a distance of at least 200 metres from dwellings and livestock shelters. The poisonous substances involved in the preparation of bait are guarded. Their residues are returned to the store or kept in a specially assigned room. The preparation of bait is mechanised. This not only speeds up the work, but also ensures safety. Workers must use means to protect their skin, eyes and respiratory organs.

Bait must be used on the day of preparation. Unused bait is handed in to a toxicant store for storage or are handed over to another farm where rodents are being controlled against a receipt. Small residues of bait unsuitable for use are placed in a pit, fuel is poured over and it is burned. The implements, containers and auxiliary materials used for preparing the bait are washed with soapy, hot water after work. Implements not lending themselves to cleaning are destroyed or neutralised if they are to be used again. Wooden objects are burned, metal ones are crushed and buried.

The area where the bait was prepared is ploughed to a depth of at least 25 cm, the soil layer turned over and covered with slaked lime. Only after this is done can the guard be lifted. To avoid poisoning, open placing or scattering of poisoned bait is prohibited in populated localities and within the boundaries of grazing land for cattle and poultry, around farms within a radius of 500 metres, at places of concentration of beneficial wild animals and birds, and also on the adjoining land within a radius of 200 metres. In these cases, the bait is placed in burrows or other shelters. Aerial scattering of poisoned bait containing zinc phosphide can be done only with special permission from the Union ministry of agriculture, as an exception.

All fumigation jobs, owing to the hazard involved, are performed by skilled specialists and only with permission of a sanitary inspector. All objects being gassed are need to be guarded round the clock up to completion of the work. Gassing may be performed only in premises that meet the requirements of hermetic sealing and arranged not closer than 200 metres from dwellings and 100 metres from farm buildings.

Fumigation is performed at a temperature of the air outside and inside the premises not under 10° Celsius and not over 35° Celsius. To ensure safety, the work is done by a brigade divided into teams of three. They work in overalls, gloves, special shoes and gas masks fitted with filters of a corresponding grade. A restricted zone is marked out around the object and warning signs are put up.

All work involving the admission of a gas into premises is performed with account taken of the properties of the gas and its container. When working with flammable fumigants, the objects being treated are disconnected from the electric mains and special fire safety measures are taken. After gassing, the objects are degassed. The time needed for this is determined by the object, the properties of the fumigant and the method of degassing. Large objects should be degassed gradually to prevent large amounts of gas escaping into the atmosphere. The supervisor issues written permission for use of the object.

Highly toxic substances are often used to treat seeds. Consequently, this work may be done only when using apparatus in good order and serially manufactured machines (PSSh-3, PS-10, etc) outfitted with a cleaning device. Reliable means for protecting the body, eyes and especially respiratory organs are obligatory. The treatment of seeds with the use of adhesives reduces the concentration of pesticide vapour in the working zone to one-ninth of its value without them. It is prohibited to treat seeds in barrels and by scooping. Treatment is performed on an open site or under a canopy at a distance of at least 200 metres from dwellings. Indoors, treatment may be carried out only with good ventilation.

The chemicals and treated grain are strictly accounted for and stored in special premises. It is prohibited to use treated grain for food purposes or as feed for animals or poultry, or to mix it with untreated grain and sell it in any way. Ventilation, washing, cleaning and baking do not free the products of toxicant residues.

Treated grain is stored separately from food products and fodder and needs to be reliably guarded. Such grain is issued for sowing only when ordered by the head of the farm or his/her deputy. Treated seeds are to be transported to the place of sowing in sacks of a dense fabric and only in exceptional cases, upon special permission of a sanitary inspector, is transportation in canvas-covered motor vehicles permitted. It is strictly prohibited to convey people on vehicles laden with treated seeds.

During sowing, workers are provided with individual means of protection. When sowing, the lid of the seedbox must be tightly closed and the seeds in the machine must be levelled out with a shovel (by no means with the hands). Residues of treated grain are returned to the store while small amounts of spilled grain are burned and the ashes buried. The place where the treated grain was kept and its containers are neutralised. Vehicles involved in the transportation of treated grain and sowing machines are thoroughly cleaned and neutralised.

Spraying and dusting should be performed in the morning and evening, and in dull weather during the daytime. Treatment must never be performed before or during a rainfall.

The rates of use of pesticides and treatment periods must be strictly controlled. Specially equipped filling areas must be provided for preparing working formulations of the toxicants. Preparation of the working liquids and filling of reservoirs of spraying machines with virulent and highly toxic pesticides must be completely mechanised. Special machines and aviation equipment are used to prepare aerosols or insecticide smoke pots. Closed premises treated with aerosols must be ventilated for 24 hours.

Grazing of cattle on treated areas and on areas within a radius of 300 metres is permitted not earlier than in 25 days after treatment (for especially toxic and persistent pesticides, this period is longer and is indicated in special instructions). It is prohibited to feed livestock weeds from fields treated with pesticides.

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'A prehistoric library'

A detailed laser-scan survey of Stonehenge has discovered 72 previously unknown Early Bronze Age carvings chipped into five of the giant stones

FOR part of its existence as an ancient temple, Stonehenge doubled as a substantial prehistoric art gallery, according to new evidence. A detailed laser-scan survey of the entire monument has discovered 72 previously unknown Early Bronze Age carvings chipped into five of the giant stones. All of the newly discovered prehistoric art works are invisible to the naked eye — and have only come to light following this survey which recorded literally billions of points micro-topographically on the surfaces of the monument's 83 surviving stones. In total, some 850 gigabytes of information was collected.

Detailed analysis of that data — carried out on behalf of English Heritage — found that images had been engraved on the stones, normally by removing the top one to three millimetres of weathered (darker coloured) rock to produce different sized shapes. Of the 72 newly discovered images revealed through the data analysis, 71 portray Bronze Age axe-heads and one portrays a Bronze Age dagger.

Prior to the laser survey, 46 other carvings (also of axe-heads and daggers) were known or suspected at Stonehenge — mostly identified visually back in the 1950s. The laser-scan survey has now confirmed the existence of those other images and provided more details about them.

The 72 new "rock art" discoveries almost treble the number of carvings known at Stonehenge — and the monument's largely invisible art gallery now constitutes the largest single collection of prehistoric rock carvings in southern Britain. Although now largely invisible to the naked eye, back in the Early Bronze Age the images, composed of then-unweathered (and therefore lighter coloured) stone would have been clearly visible. The revelations are likely to be of huge importance to archaeologists' understanding of a key part of Stonehenge's life as a prehistoric temple. It's known that, when the main phase of the monument was initially built in the middle of the third millennium BC, it was designed primarily as a solar temple, aligned on the mid-winter and mid-summer solstices. But, as Stonehenge evolved over subsequent centuries, the extent to which other religious functions were added is not yet known. Certainly, in the period 1800-1500 BC, vast numbers of individual monumental tombs were constructed in the landscape around Stonehenge and additional features (various circles of ritual pits) were laid out around the monument. The carved axe-heads and daggers also belong to this enigmatic period — and may signify some sort of expansion or change in the great stone circle's religious function.

Digital photogrammetry being used to record 3D images of the micro-topography on the tops of the lintels at Stonehenge.

In Indo-European tradition, axe-heads were often associated with storm deities — and some surviving European folklore beliefs suggest that upwards-facing axe blades were used as magical talismans to protect crops, people and property against lightning and storm damage. It's potentially significant that every single one of the Stonehenge axe-head images have their blades pointing skywards, while the daggers point downwards. The axe-heads — the vast majority of the images — may therefore have been engraved as votive offerings to placate a storm deity and thus protect crops.

It may also be significant that the vast majority of the carvings either face a nearby set of tombs (from roughly the same period) — or the centre of Stonehenge itself. Rare evidence from elsewhere in Britain suggests that axe-head and dagger carvings could have funerary associations.

The laser-scan data shows that many of the axe-head images have exactly the same dimensions as up to half a dozen other images in the prehistoric Stonehenge "art gallery". This in turn suggests that real axe-heads were being used as "stencils" to help produce the images. If that's the case, the largest axe-heads portrayed — up to 46 cm long — depict objects that were far bigger than archaeologists have ever found and which must have been for purely ceremonial or ritual use.

The laser-scan survey was carried out for English Heritage by a Derby-based survey company — the Greenhatch Group — last year. A subsidiary of York Archaeological Trust — ArcHeritage, also operating on behalf of English Heritage — then spent many months analysing and cataloguing the vast quantities of data. "The new discoveries are of huge importance. They also demonstrate how emerging technologies can extract previously unsuspected and crucial information from a monument like Stonehenge," said Marcus Abbott, head of Geomatics and Visualisation at ArcHeritage.

"As the previously invisible images started appearing on our computer screens, we stared in disbelief at the sheer quantity of carvings being revealed – and treble-checked all our data," he added.

The survey and analysis has also yielded other new insights into Stonehenge. It's revealed, through an examination of how finely the stone surfaces were worked, that the entire prehistoric temple was constructed to be viewed primarily from the north-east. That's the side of the monument that is approached by what archaeologists have long believed to be a processional way, aligned with the solstices. Because it now seems that Stonehenge was built to be viewed from that direction, it suggests that some sort of religious procession made its way towards the monument, along that route, probably on mid-winter's and mid-summer's day.

Detailed analysis of the data also shows that one of the stones at the now ruinous south-west side of the monument was also very deliberately worked and shaped to allow a line of sight through to the setting sun on mid-winter's day. This, along with other new evidence, suggests that the south-west side of the monument was once fully functional — and will reduce support for those who have, up till now, argued that Stonehenge was never completed.

The Independent, London