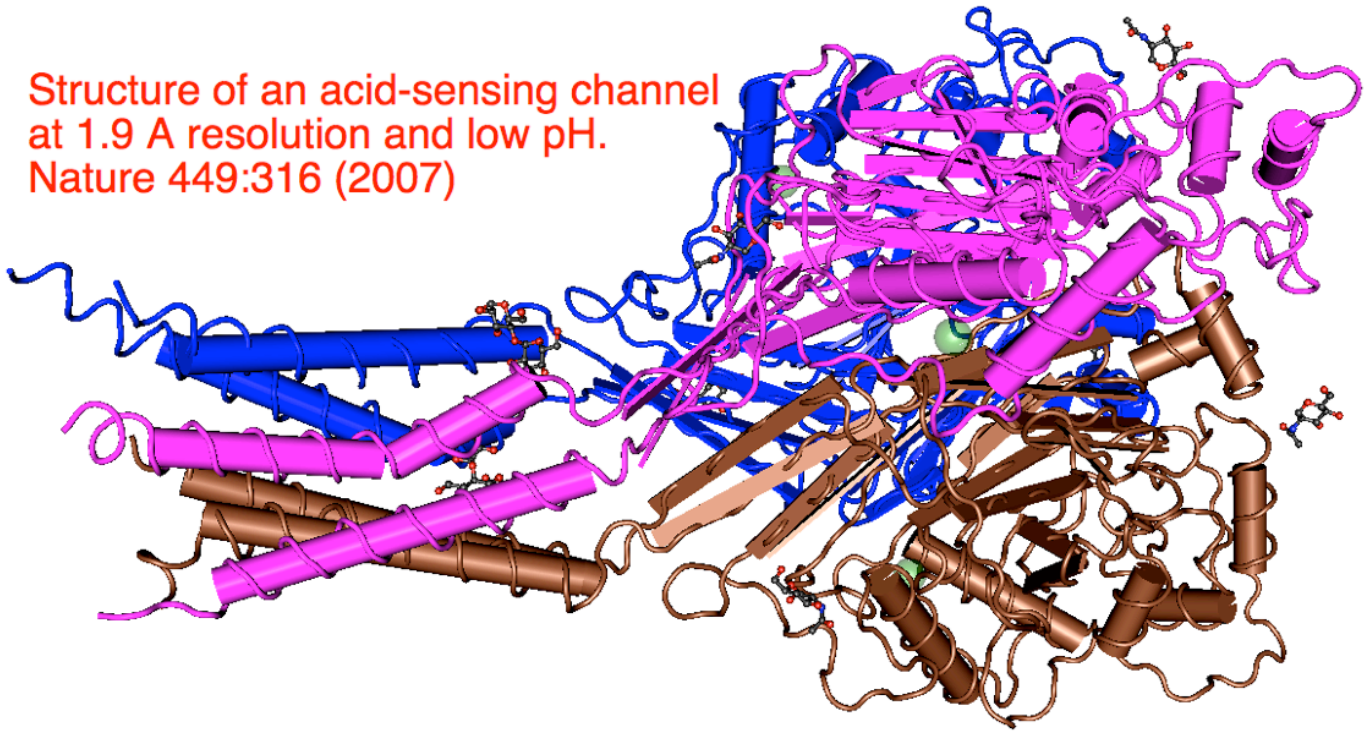


Acid-Sensing Ion Channels

Acid-sensing ion channels (ASICs) – the mamba snake venom connection.

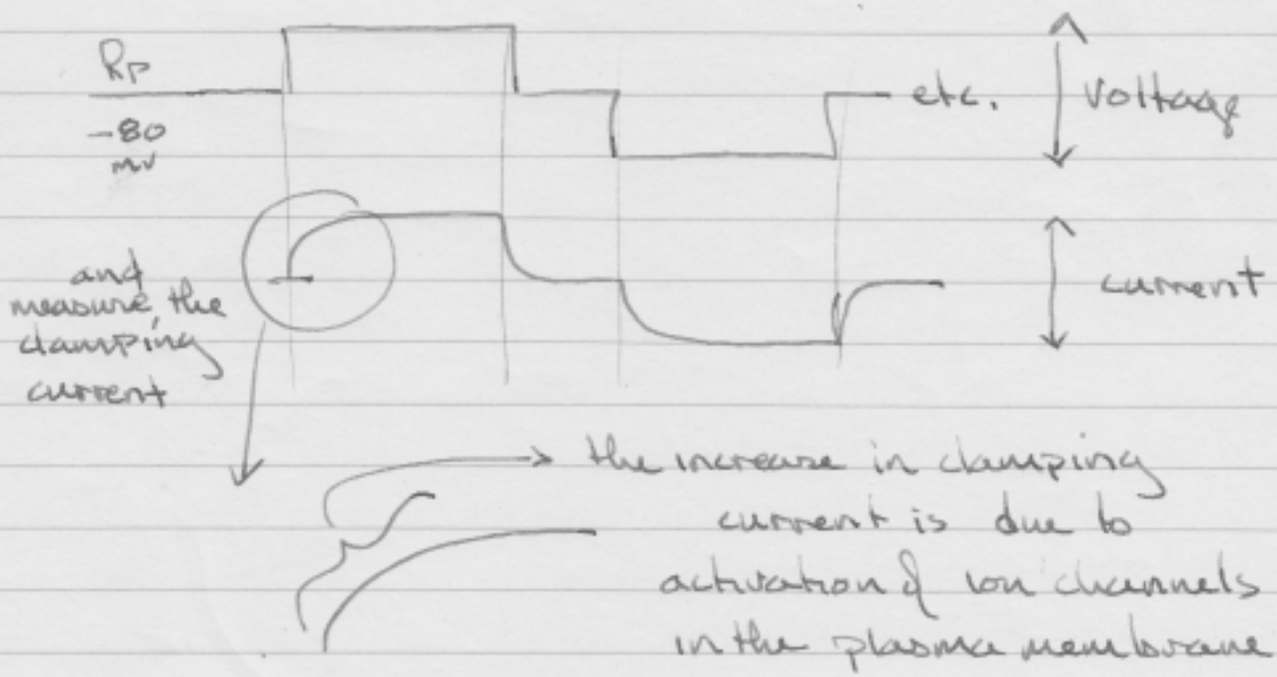
Ion channels come in a bewildering array of types.

Normally, we think of them at a fundamental level – that is, basic properties like ion selectivity.

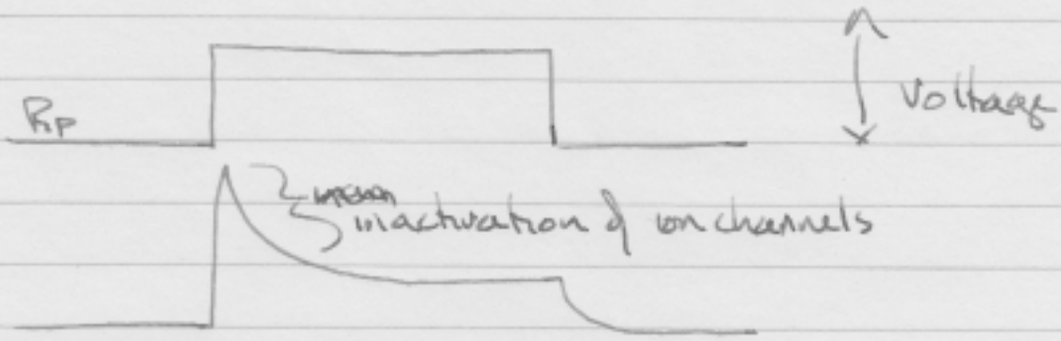
But in fact, much of their diversity is related to what causes them to open (and close, for that matter). That is, their gating properties.

One of the major effectors is voltage.

Supposing we have a cell at some resting potential. We can clamp the voltage at various levels, both +ve and -ve to the resting potential.



We can also see voltage inactivation



Sometimes, channels are activated by the voltages an inactivated at -ve voltages.
For voltage-gating alone, things get complex quickly.

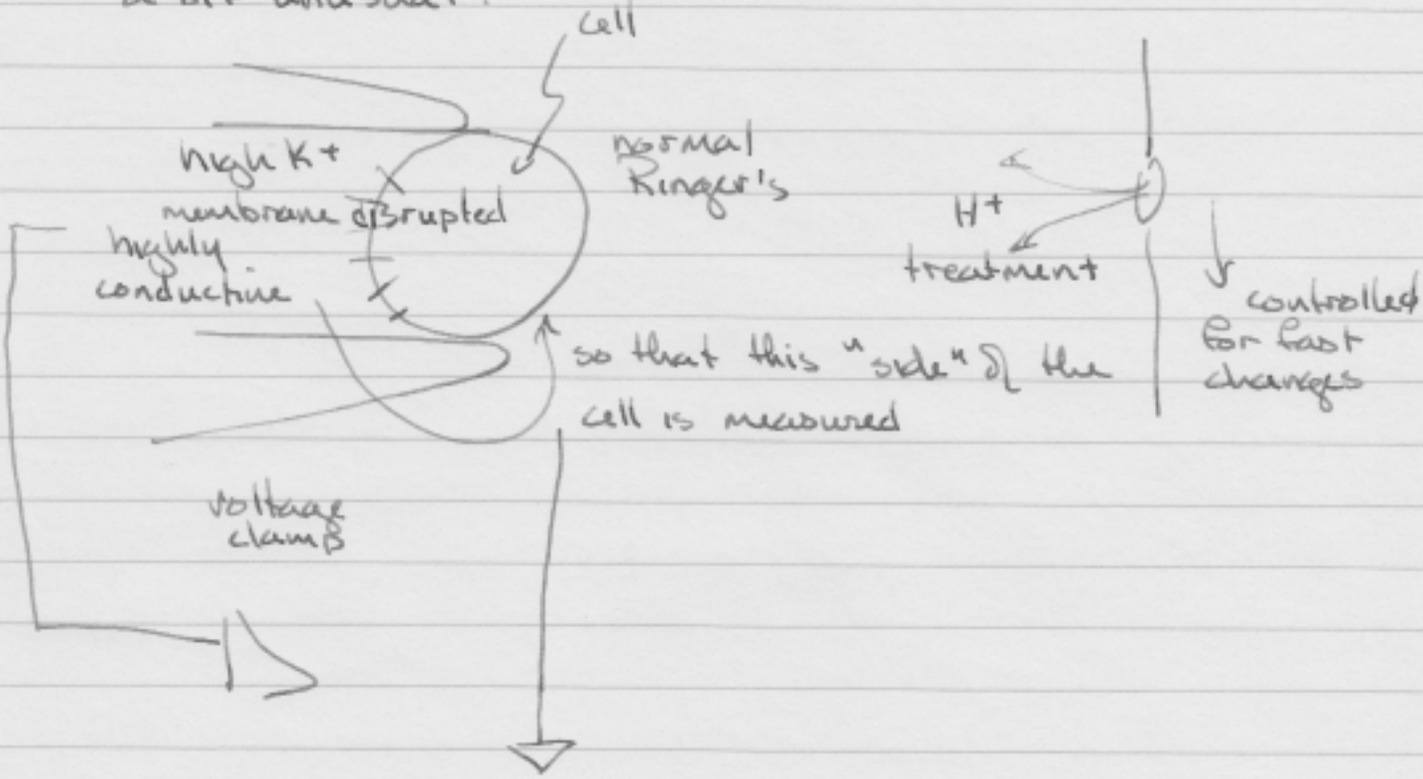
But even worse (!), a wide variety of ligands can gate channels.

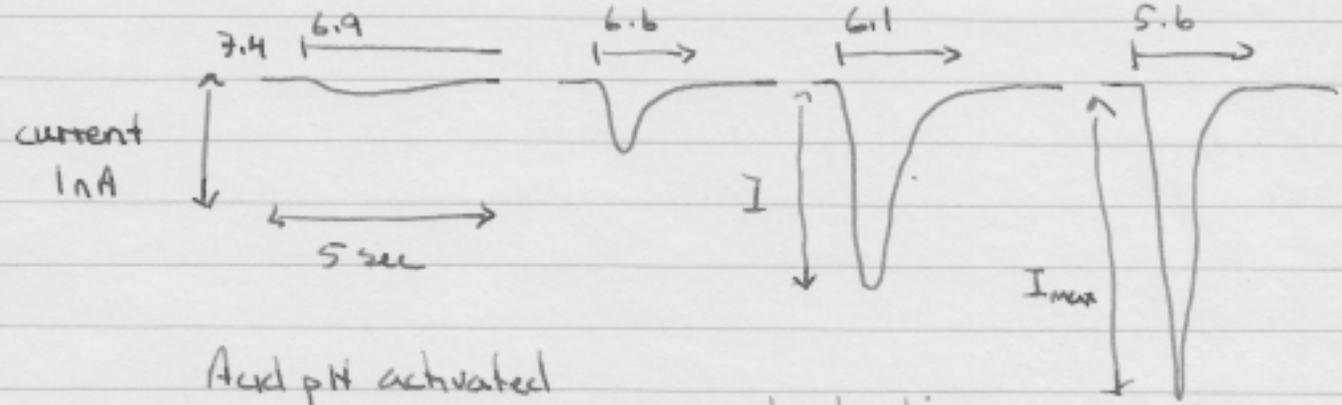
Some obvious examples are neurotransmitters Things like acetycholine. Usually, when they bind to a ~~ligand~~ receptor site on the channel, the channel opens. But of course, they can also inactivate.

In the case of the acid-sensing ion channels (ASICs), the ligand is a proton (H^+).

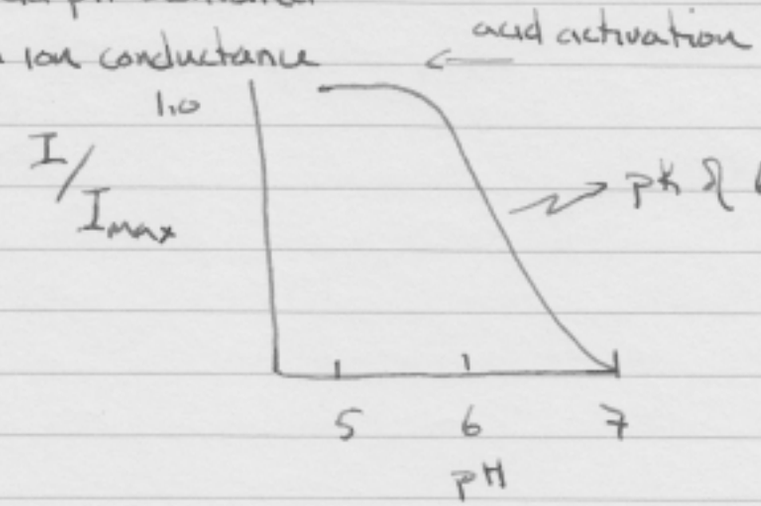
The original report was from Oleg Krishtal & VI Pidoplichko (1980) A receptor for protons in the nerve cell membrane
Neuroscience 5: 2325-2327
using rat ganglia neurons

The technique Krishtal & Pidoplichko used is a bit unusual:





Acid pH activated an ion conductance



So, a H^+-gated channel was discovered.

Neuroscience Vol. 5, pp. 2325 to 2327
Pergamon Press Ltd 1980. Printed in Great Britain

0306-4522/80/1201-2325$02.00/0

A RECEPTOR FOR PROTONS IN THE NERVE CELL MEMBRANE

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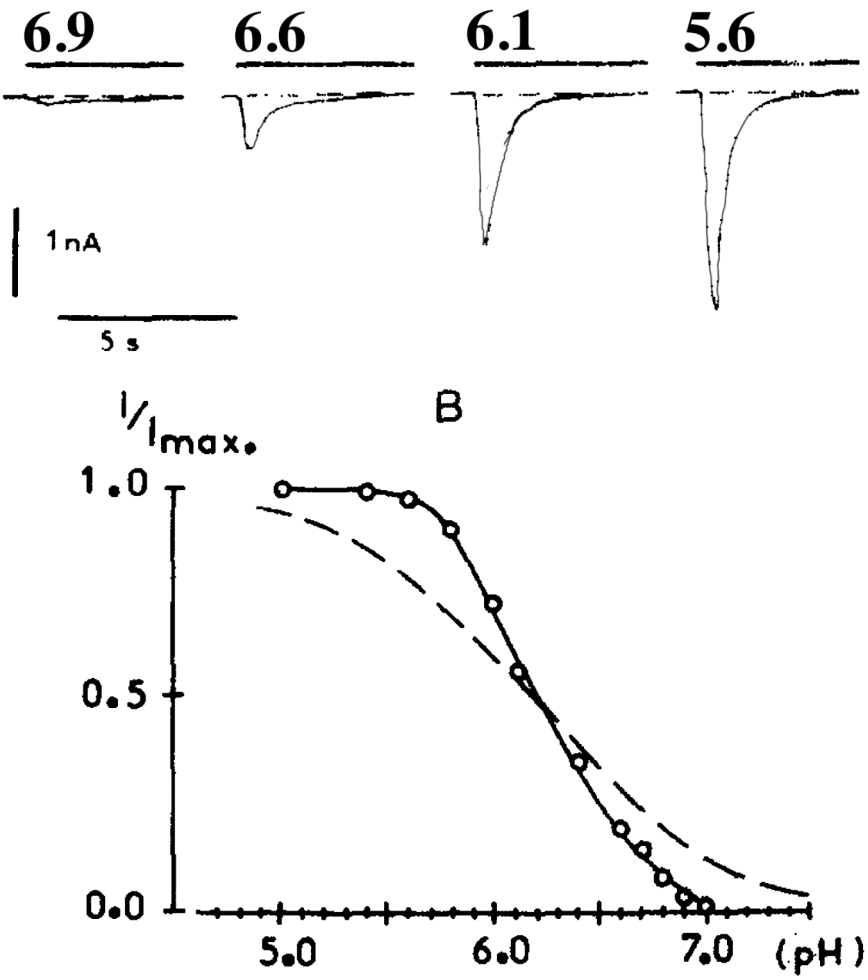
Abstract—The neurones isolated from spinal ganglia and from the ganglion of trigeminal nerve of a rat were investigated by means of intracellular perfusion and voltage clamp. The extracellular solution was replaced in 0.1 s. Many cells responded to rapid shifts of external pH from 7.4 to 6.9 and lower by a pH-dependent inward current. Its amplitude saturated at pH 5.4 (pK_a 6.2). This 'H^+-induced' current was due to the increased permeability of the membrane to Na^+ and K^+ ($P_K : P_{Na} \approx 0.1$). The H^+-induced current decay had a time constant about 0.5 s and showed a desensitization which was removed within 10 s. The H^+-induced current was also found in the cells of mouse C-1300 neuroblastoma. It had similar pH and voltage dependence but a much slower kinetics of desensitization.

It is suggested that this newly described conductance mechanism may serve as a pH-sensor in the sensory nerve endings throughout the body.



So, we have an ASIC, but, what does it do?

The first major advance was the cloning of H^+-gated channels.
Waldmann R, Champigny G, Bassilana F, Heurteaux C, and Lazdunski M (1997) A proton-gated cation channel involved in acid-sensing. Nature 386:173-177.

The gene was isolated using per to identify a cDNA. mRNA was injected into Xenopus oocytes and the functional properties characterized.

[overhead]

The general results. Yes, there is a H^+-gated ion channel. It is related to other ion channel families (that pass Na^+). The relative permeabilities are

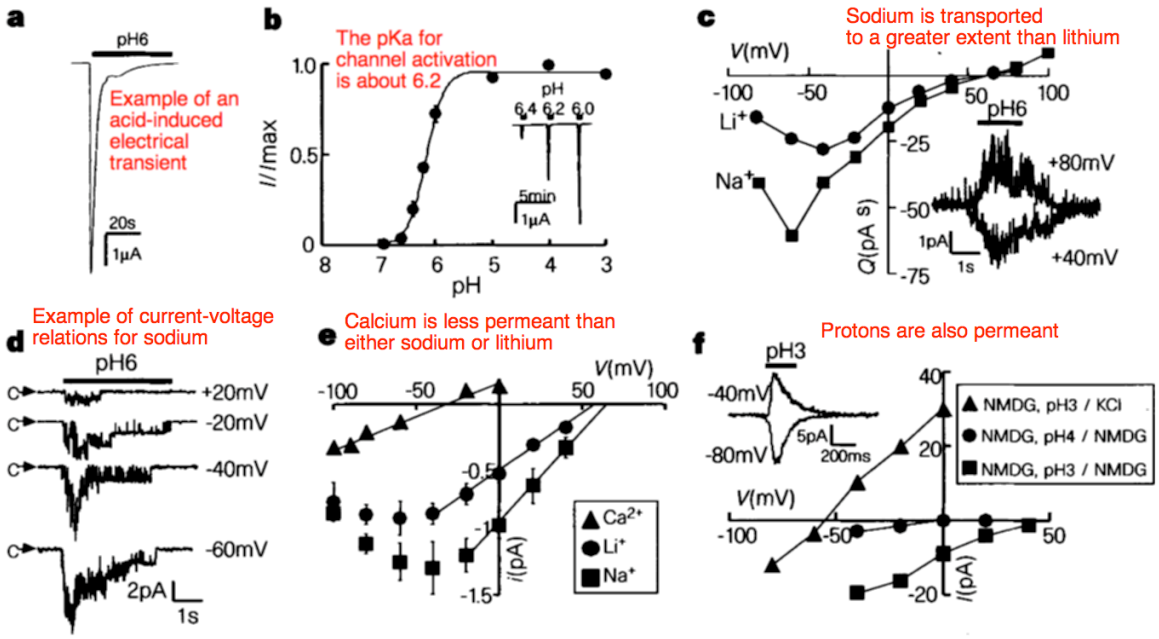
P_{Na}/P_{M^+}

$H^+ > Na^+ > Li^+ > Ca^{2+} > K^+$ [0.8 | 1.3 | 2.5 | 13]

It is found in brain and has properties consistent with it being responsible for acid-sensing in sensory neurons.

So, that (very briefly!) is the channel.

What does it do?

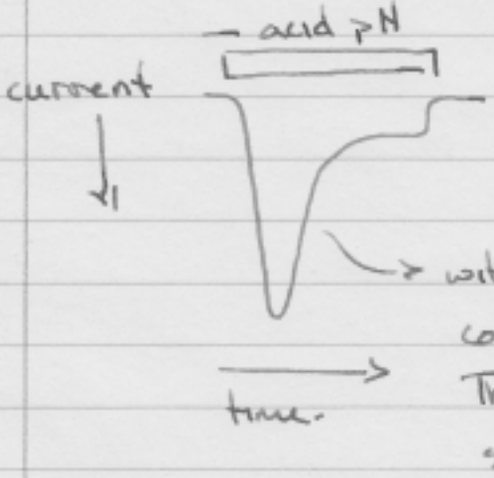


Properties of the H^+-activated channel. **a.** Examples of ion currents at –70 mV caused by a rapid change from pH 7.4 to 6.0. **b.** pH response curves showing current change normalized to the maximal current. **c.** Charge-voltage relations. The charge is calculated as the integration of the ionic current during the pH change and represents the total amount of ions (coulombs) passing through the channels. **d.** Example of an experiment to obtain the current-voltage relation. **e.** Current-voltage relations for Ca^{2+} (1.2 mM), Li^+ and Na^+ (both 140 mM) to show relative permeabilities. **f.** Proton currents through the channel.

Source: Waldmann R, Champigny G, Bassilana F, Heurteaux C and Lazdunsk M (1997) A proton-gated cation channel involved in acid-sensing. Nature 386:173–177

As a group, the ASIC tend to be observed in "higher animals". There are an annoyingly large number of isoforms, and the final channel involves multiple subunits. This means that multiple forms exist, which may (or may not) result in different physiological functions

Thinking first of their role in the peripheral nerve system (rather than the CNS - central nervous system), the idea that they play a role in pain has prevailed over decades. Pain-sensing is called nociception
Tissue damage could easily cause a shift to a more acid pH, resulting in channel activation. Arguing against this is the nature of the cation current:



→ without a sustained current, how could it measure "pain"?
Think about yourself - is the sensation of pain transient?

And, the pK_a of the channel is relatively acid, considering the normal pH is 7.2. A large drop to acidic pH is required to obtain a "strong" current through the channel.

(continued next page)

Source: Krishtal, Oleg (2003) The ASICs: Signaling Molecules? Modulators? Trends in Neuroscience 26: 477-483.

Opinion TRENDS in Neurosciences Vol.26 No.9 September 2003 477

ELSEVIER

The ASICs: Signaling molecules? Modulators?

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Acid-sensing ionic channels (ASICs) are almost ubiquitous in the mammalian nervous system, both at the periphery and in the brain. Strong evidence for the physiological function of these molecules has come from recent knockout experiments. Now it is clear that ASICs are important for certain sensory modalities (mechanoreception and nociception) at the periphery and for learning and memory in the brain. The actual mechanisms by which the acid-gated channels serve these functions remain unclear. The question of whether tissue pH is subject to quick fluctuations of a magnitude sufficient to activate ASICs is a crucial point that will determine the functional significance of these channels.

What Do They Do?

The one example where dramatic acidification could occur is in ischemia of the heart. Ischemia is due to restricted blood flow to the organ or tissue. Cardiac ischemia often results in chest pain (angina pectoris) and can cause pH of the cardiac tissue to drop to 6.7 or lower. Even though the H^+-gated channel inactivates rapidly, it could cause persistent neuronal excitation for persistent pain.

Knockout mice lacking one of the isoforms do have attenuated mechanosensation. This can be "light touch" (ASIC2a) or more noxious mechanoreception, noxious heat or acid (ASIC3).

Knockouts of ASIC1 (a and b) caused an inability to respond to acid. And, impaired hippocampal LTP (long-term potentiation)! The actual mechanism must be complex, but the excessively exaggerated notion that this would impact learning could easily be headlined....

Pain makes you learn! (not)

There's a lot of complexity here that just can't be simplified. More analysis of other knockout mice resulted in counterintuitive results (e.g., higher sensitivity to acid [2])

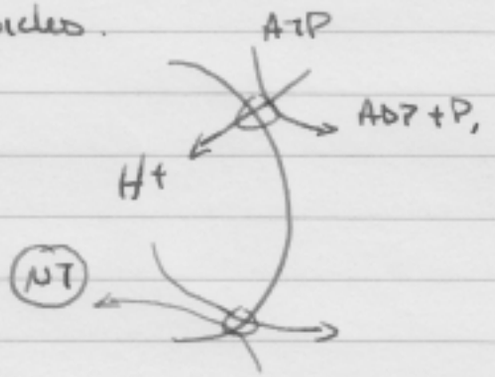
Source Krishtal Oleg (2003) Trends Neurosci. 26: 477-483.

[2] Wemmie et al. (2006) Acid-sensing channels:... Trends in Neuroscience 29: 578-586.

The complexity results in a fairly high level of speculation. Wemmie et al. (2006) traverse multiple possibilities & propose models. One example is a role of ASIC at the synapse.

[overhead]

In the pre-synapse, neurotransmitter is loaded into vesicles.



The pump is a vacuolar H^+-ATPase that normally functions as an acidifier. The neurotransmitter is taken up via an antiporter and stored for release at the pre-synaptic membrane.

When released, it could cause acidification of the synaptic gap. Wemmie et al (2006) note that proof is lacking. Is the gap acid? needs to be measured with pH sensitive dyes. Is there evidence ASIC are activated? No.

They also describe the experiments done with respect to learning in mice, shown in an [overhead]

Source Wemmie JA, Price MP & Welsh MJ (2006) Acid-sensing ion channels: advances, questions and therapeutic opportunities. Trends in Neuroscience 29: 578-586.

J. exp. Biol. **172**, 171-178 (1992)
Printed in Great Britain © *The Company of Biologists Limited 1992*

THE ROLE OF V-ATPase IN NEURONAL AND ENDOCRINE SYSTEMS

BY YOSHINORI MORIYAMA*, MASATOMO MAEDA AND MASAMITSU FUTAI

Department of Organic Chemistry and Biochemistry, Institute of Scientific and Industrial Research, Osaka University, Ibaraki Osaka 567, Japan

Summary

Synaptic vesicles have important roles in the neural transmission at nerve terminals: the storage and the controlled exocytosis of neurotransmitters. At least two different factors are responsible for the concentration process: the vacuolar-type H^+-ATPase (V-ATPase), establishing an electrochemical gradient of protons, and specific transport systems for transmitters. We will discuss our recent progress on the energy-transducing systems in synaptic vesicles: (1) structural aspects of V-ATPase; (2) energy coupling of transport of transmitters; (3) reconstitution of transporters; (4) effects of neurotoxins and neuron blocking agents; (5) function of synaptic-vesicle-like microvesicles from endocrine tissues.

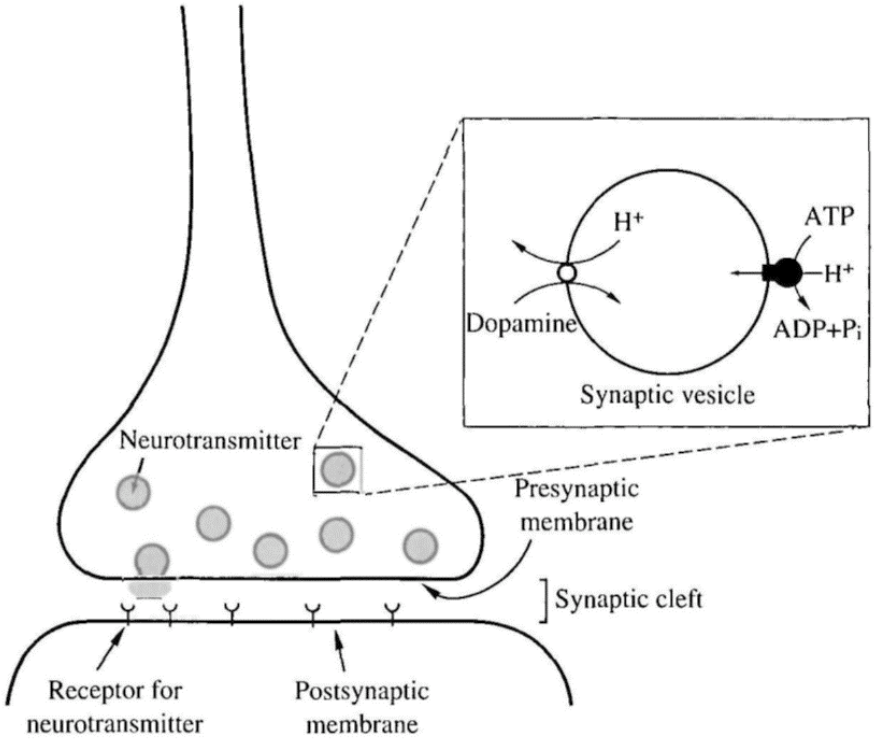
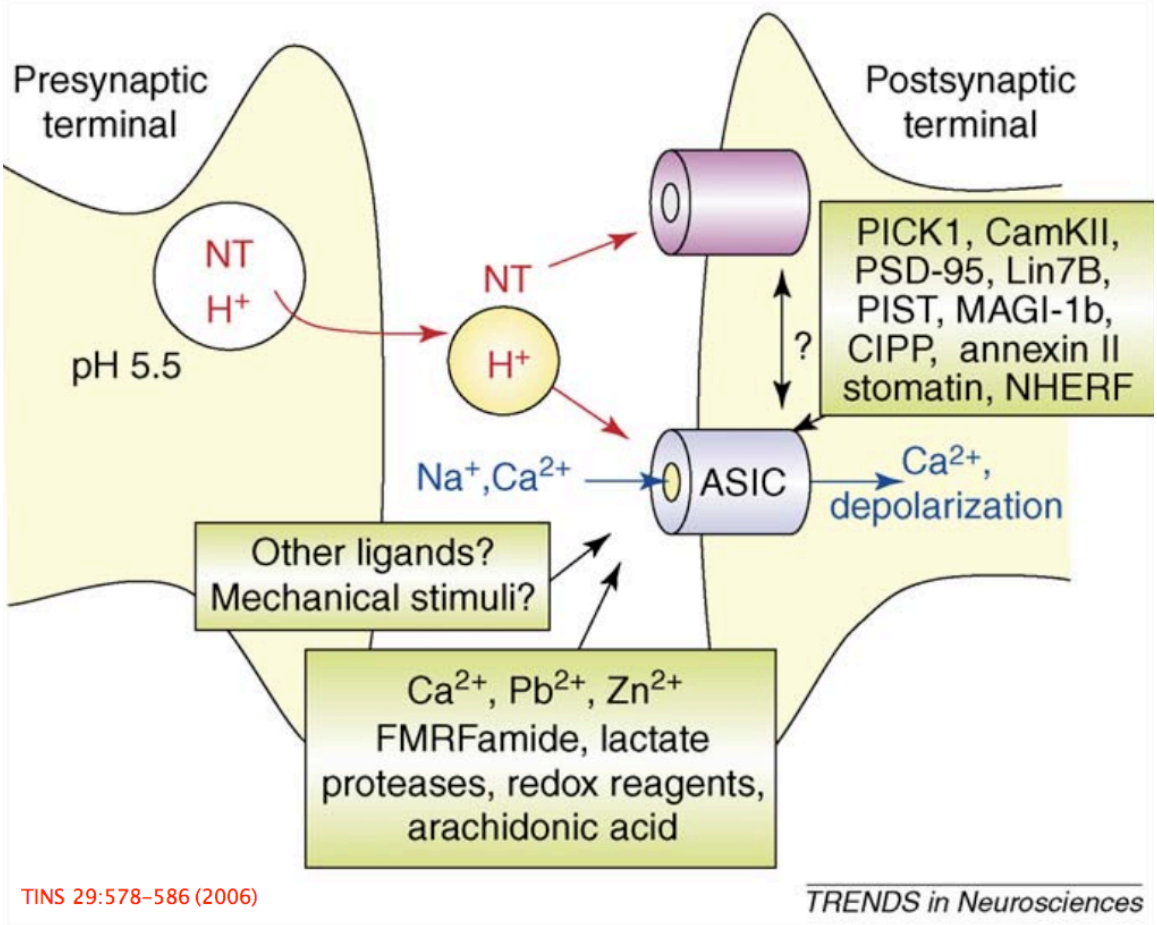


Fig. 1. Energy coupling of uptake of transmitter by synaptic vesicles.



Speculative model for the role of ASIC1a at the synapse. In the postsynaptic membrane, ASICs could respond to protons released from presynaptic neurotransmitter (NT)-containing vesicles (neurotransmitter-containing vesicles are acidic due to the activity of a H^+-ATPase). The response, which would be expected to depolarize the membrane potential and raise intracellular Ca2+ concentration, could influence other receptors and signaling proteins. This model predicts that ASIC1a currents will be activated during neurotransmission —not detected so far in brain slices and cultured neurons.

Source: Wemmie JA, Price MP, Welsh MJ (2006) Acid-sensing ion channels: advances, questions and therapeutic opportunities. Trends in Neuroscience 29:578–586.

pH Buffering

There is one major pH buffering system in animals: CO_2 / HCO_3^-

Others do exist: phosphate groups have ionizable oxygens with pK's near neutral pH and are present at significant concentrations. But, for "simplicity", we will focus on CO_2 / HCO_3^-.

First, CO_2 is present in different species.

CO_2 (g)

⇕ solubility of CO_2 in H_2O (l) is affected by the partial pressure of CO_2: pCO_2. And, by temperature

CO_2 (l)

Traditionally the solubility of CO_2 is defined by Henry's law:

$$[CO_2] = \frac{0.03\ mM}{Torr}\ pCO_2 \quad (\text{in torr, or mm Hg})$$

The "Torr" is a pressure unit equal to 1 mm Hg. This may be arcane, but it is still used...
The conversions are:

$$torr = 133.3\ Pa$$

So.

$$[CO_2] = \frac{0.23\ uM}{pascal}\ pCO_2 \quad (\text{in pascal})$$

(390 ppm CO_2)

At atmospheric pressure and room temperature (RTP) the concentration of $[CO_2](l)$ is about 10 mM
Solubility increases at cooler temperatures.

In humans, the partial pressure of CO_2 is higher than in the atmosphere: ~40 Pa (atmospheric) compared to 5-6 kiloPa in blood.
This is due to the high levels of CO_2 produced in respiration - Wikipedia says 1 kg of CO_2 per human per day.

So, now we are in solution:

$$CO_2(l)$$

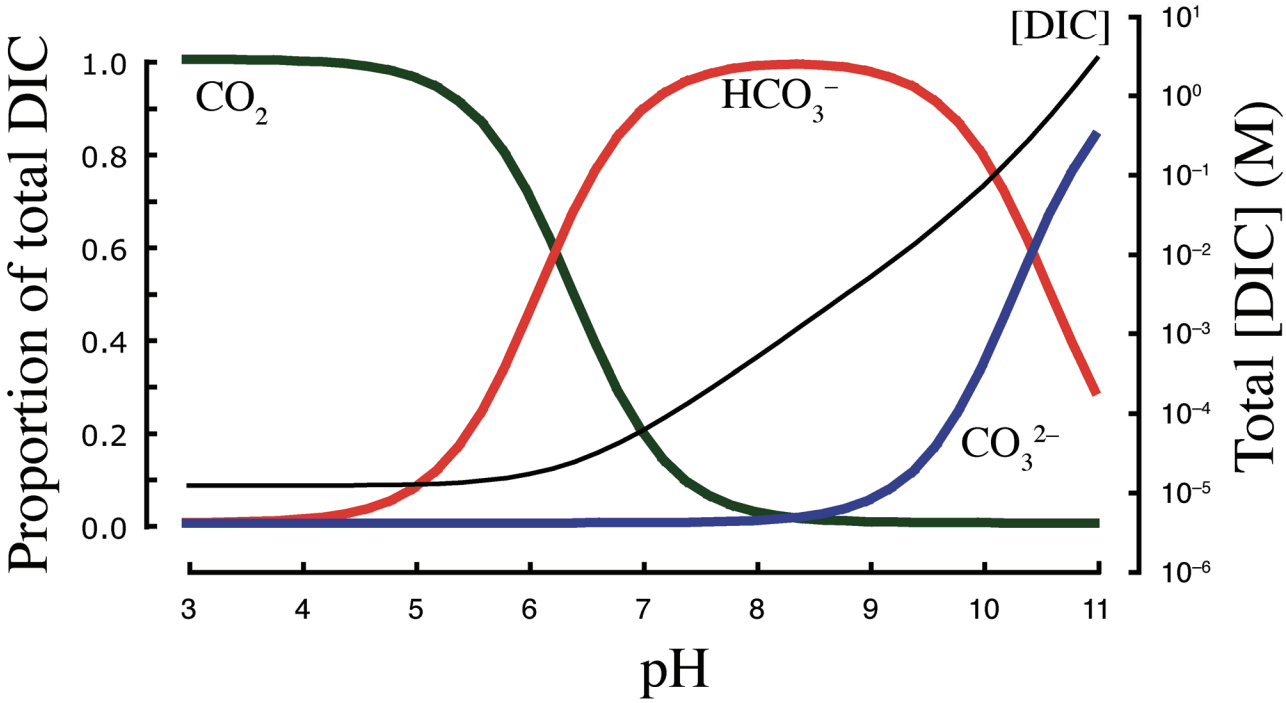
$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$
$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \quad \leftarrow \text{important at physiological pH. } pK \approx 6.1$$
$$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+$$

In these reactions, pH is determined by the ratio: $\frac{[HCO_3^-]}{[CO_2]}$

in the form of the Henderson-Hasselbach equation

$$pH = pK_1 + \log_{10} \frac{[HCO_3^-]}{[CO_2]}$$

Carbon Species Availability (as a function of aqueous pH)



The relative proportions of the various DIC (dissolved inorganic carbon) species are shown as a function of pH, based upon the equilibria shown in the chemical equation below:

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \xleftrightarrow{pK_a = 6.4} H^+ + HCO_3^- \xleftrightarrow{pK_b = 10.4} H^+ + CO_3^{2-}$$

Total [DIC] increases dramatically at alkaline pH, but the predicted concentrations shown do not account for solubility.

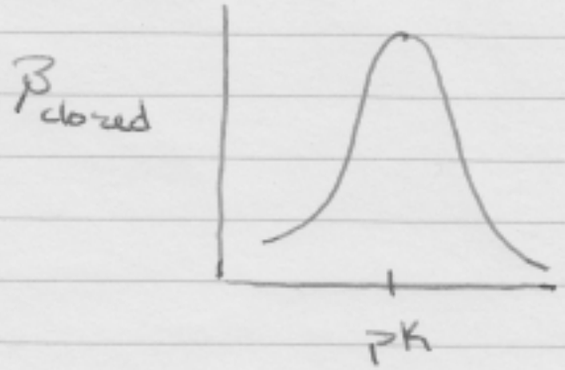
Now, buffering is defined by

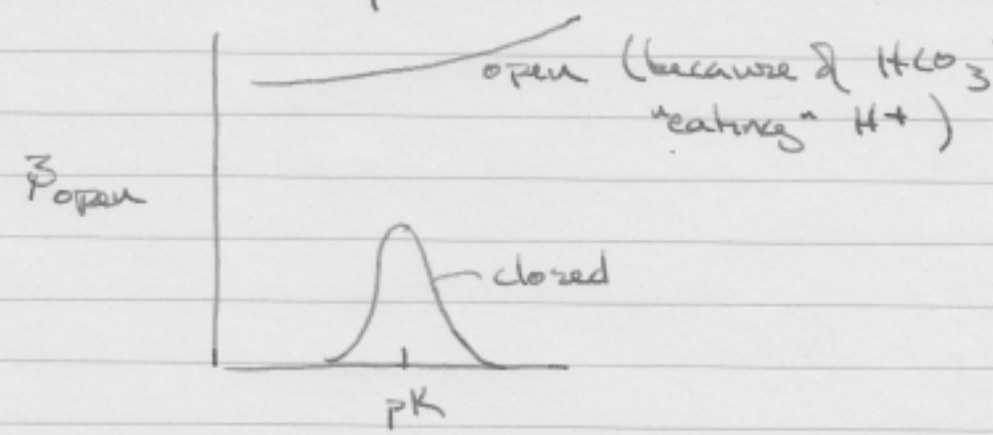
$$\beta = \frac{d(\text{Base})}{dpH} = \frac{d(\text{Acid})}{dpH}$$

In the context of CO_2 buffering:

$$\beta = \frac{d[HCO_3^-]}{dpH}$$

For a closed system (where CO_2 can't "escape" into the atmosphere)





In the "closed" system of a synaptic gap, maybe pH acidifies, but it is crucial to measure the pH to demonstrate this experimentally.

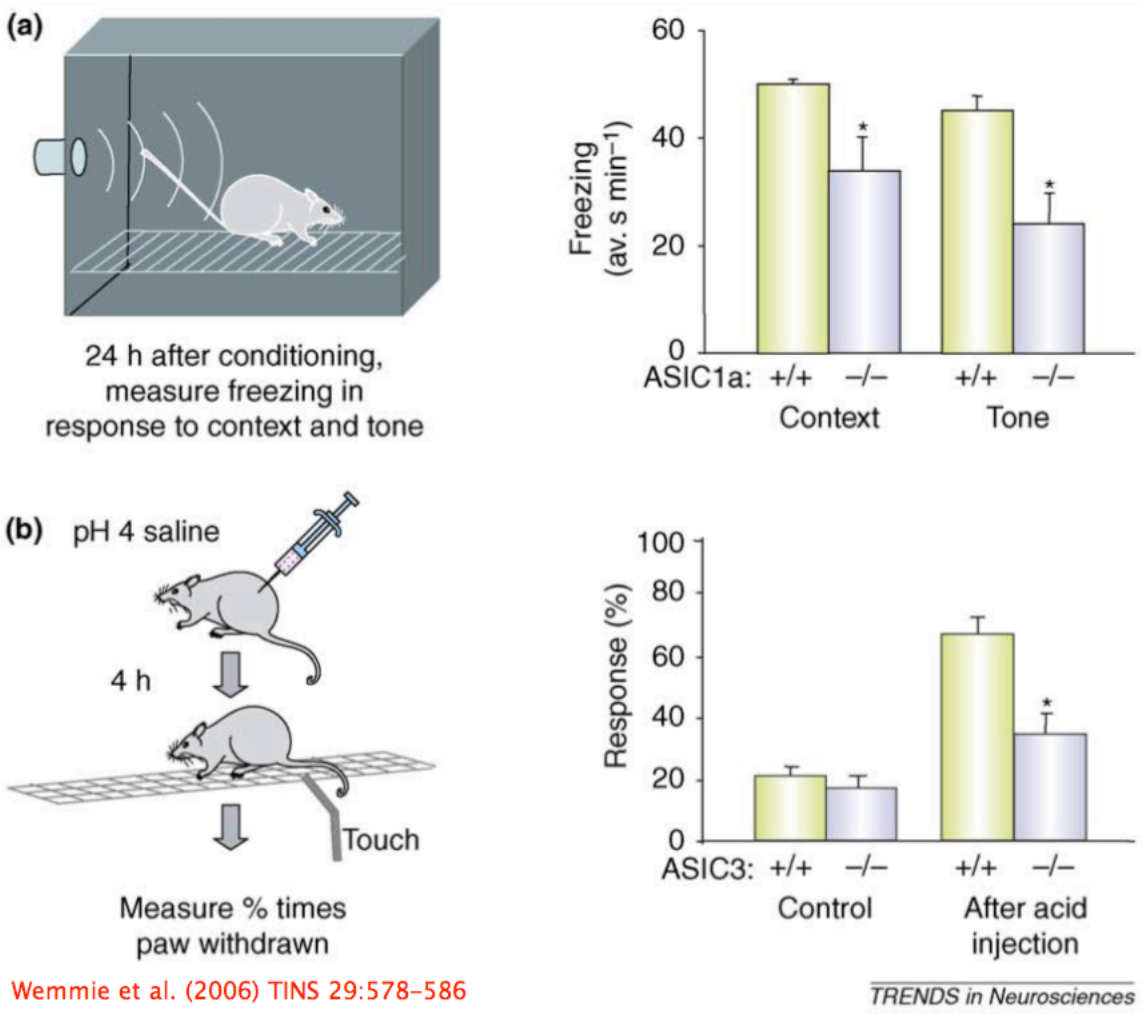


Figure 4. Loss of ASICs disrupts conditioned fear and pain. **(a)** On day 1, animals received aversive footshocks, which were paired with the training environment (context) or a tone. On day 2, the conditioned freezing responses to the context and tone were measured. Disruption of the gene encoding ASIC1a reduced the freezing response on day 2 to both context and tone. **(b)** Paw withdrawal before and after intramuscular injection of acid (pH 4.0). Disruption of the gene encoding ASIC3 reduced post-injection hyperalgesia. Asterisks indicate $P < 0.05$.

Source: Wemmie JA, Price MP, Welsh MJ (2006) Acid-sensing ion channels: advances, questions and therapeutic opportunities. Trends in Neuroscience 29:578–586.

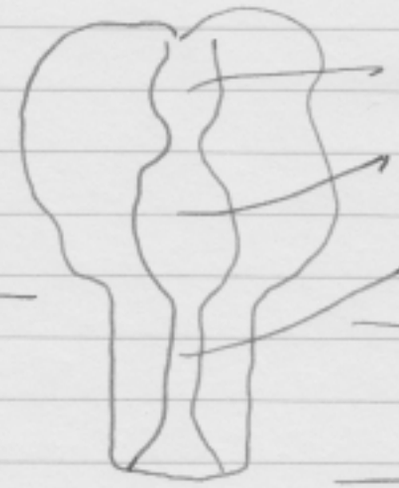
So, that's the background on the acid-sensing ion channel. Clearly, H^+-gated. Less clearly, may play roles in acid, and pain sensing, and even learning.

The next advance was the determination of the three-dimensional structure using x-ray crystallography.

[Source: Gonzales EB, Kawate T & Gouaux E (2009) Pore architecture and ion sites in acid-sensing ion channels and P2X receptors. Nature 460:599-605].

To crystallize it, the screened partial deletion mutants to identify the minimal sequence necessary for function. 466 a.a. in length, it was crystallizable. The x-ray crystallography was done using sychrotron x-rays.

The generalized structure:



[four heads]

It shares structural homology to P2X receptor ion channels which are activated by extracellular ATP.

Both channels normally exist as trimer structures in the membrane.

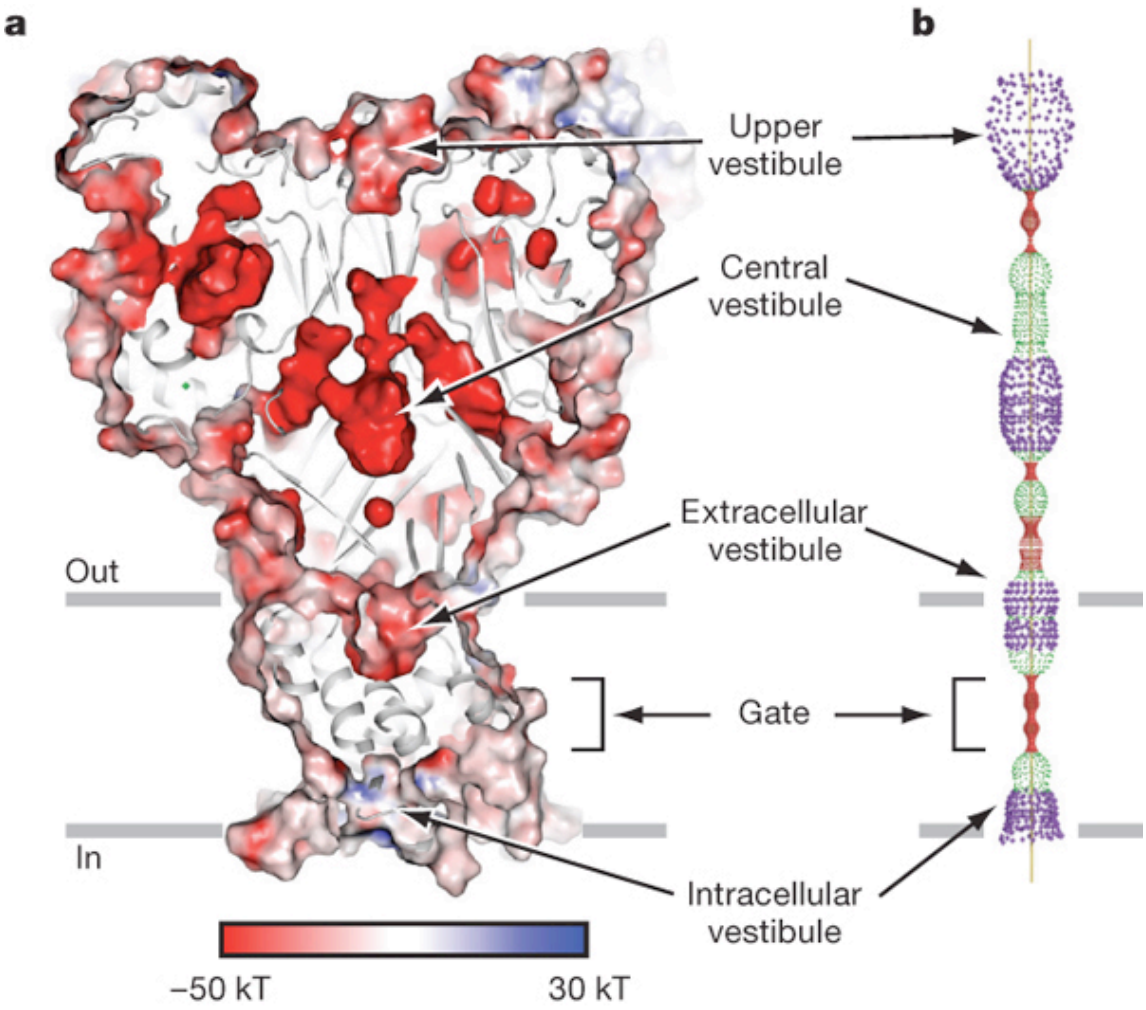
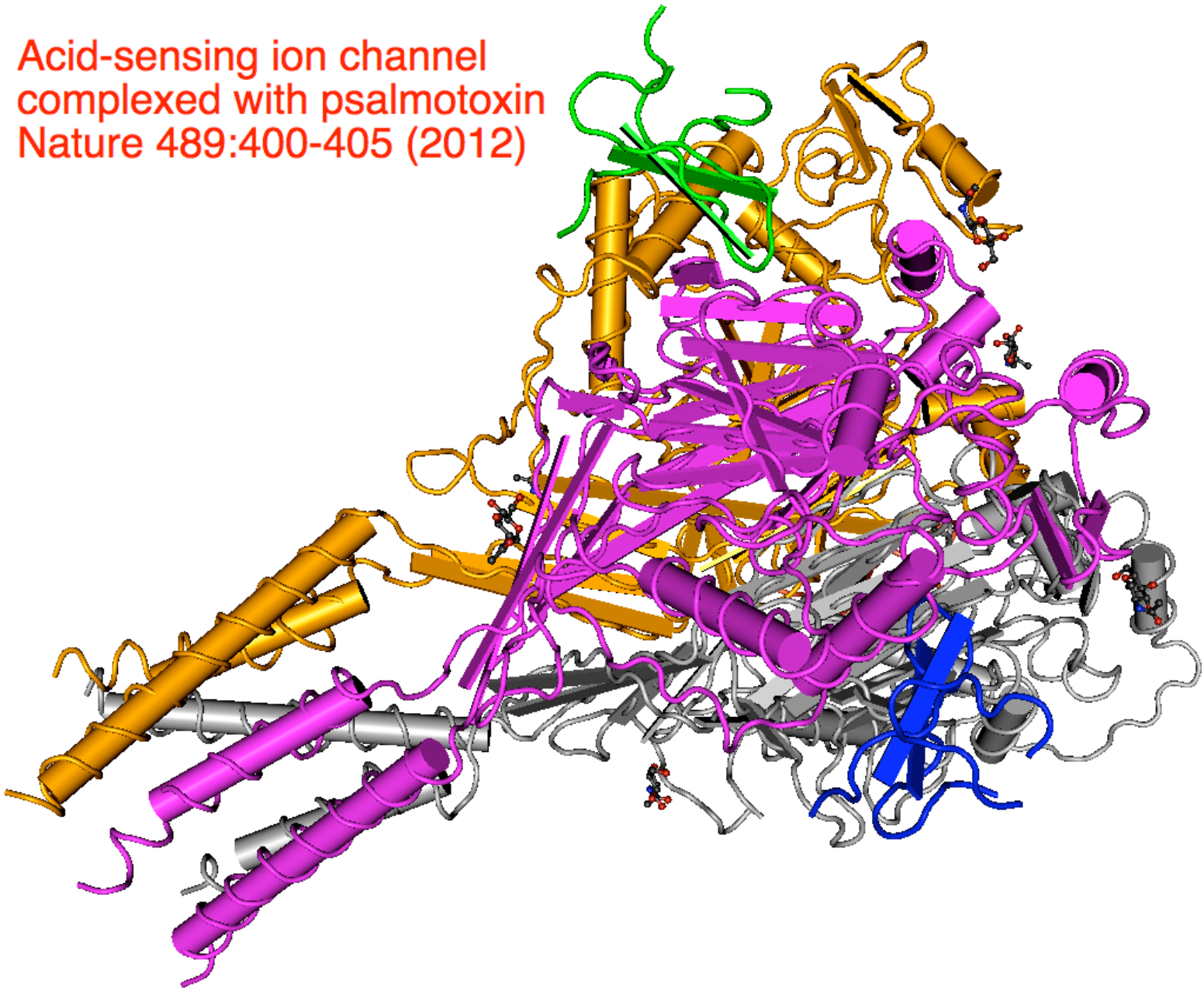


Figure 3. Vestibules and possible ion permeation pathways. a, An electrostatic potential surface and cartoon representation of ASIC1mfc sliced along the molecular three-fold axis of symmetry. **b**, Illustration of the radius of possible pathways along the three-fold axis (red < 1.4 Å < green < 2.3 Å < purple).

Source: Gonzales EB, Kawate T & Gouaux E (2009) Pore architecture and ion sites in acid-sensing ion channels and P2X receptors. Nature 460:599-604.

Acid-sensing ion channel
complexed with psalmotoxin
Nature 489:400-405 (2012)



x-ray crystallographic structures are very useful, not just for identifying the mechanisms causing ion selectivity, but also the relation between ligand binding and channel gating.

In this latter regard, there's nothing more helpful than a good toxin!

[overhead]

For acid-sensing ion channels, snakes (and spiders) are a good source.

Here are two examples....

The first is from the Texas coral snake, "whose" bite produces intense and unremitting pain" [1]. The toxin was identified in a screen of many snake venoms based on its activation of cultured neurons. To show that the toxin specifically activated the acid-sensing ion channel, ASIC mRNA was expressed in Xenopus oocytes.

The channel - expressed in oocytes - was H^+-gated and underwent sustained activation upon addition of the toxin MitTx (a dimer of MitTx-α & MitTx-β).

Injection of the MitTx causes pain responses in WT mice, but not in knockout mice. Why pain? You'll never forget the coral snake.

[1] Bohlen CJ, Chesler AT, Sharif-Naeini R, Medzihradszky KF, Zhou, S, King D, Sanchez EE, Burlingame AL, Basbaum AI, Julius D (2011) A heteromeric Texas coral snake toxin targets acid-sensing ion channels to produce pain. Nature 479: 410-414.

Pain, 1 (1975) 3–49

Review Article

TOXINS WHICH PRODUCE PAIN

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(Accepted December 18th, 1974)

INTRODUCTION

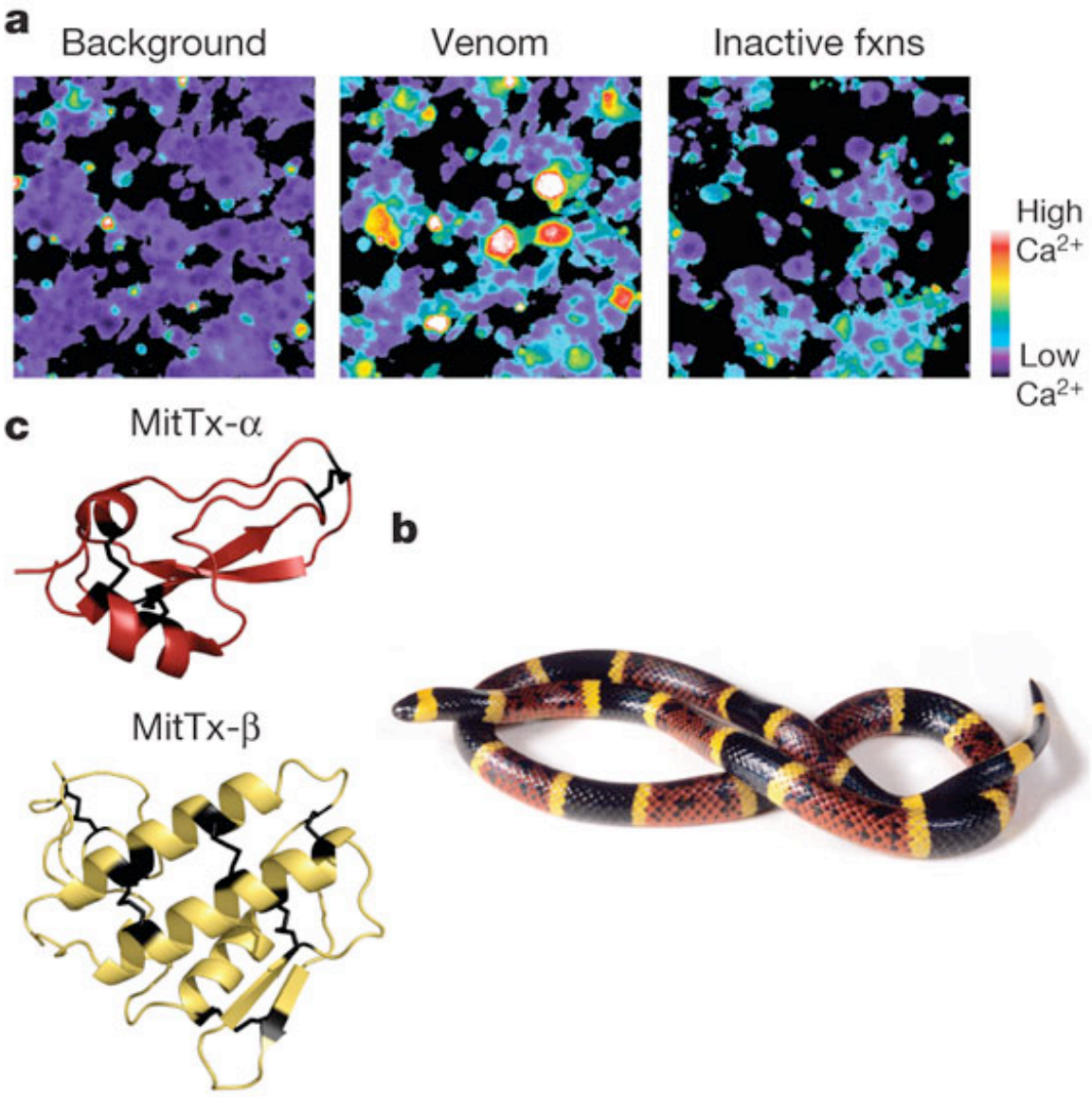
This review considers a range of toxins which produce — or are likely to produce — pain in man and other higher animals. The term 'toxin' is used, in accord with Vogt[468], to describe any naturally-occurring substance of animal or plant origin that is both foreign and damaging to the victim. Since it is common experience that many bites and stings are far more painful than is to be expected from the extent of the physical trauma produced, particular emphasis is given to venoms. The term 'venom' is used here to describe the complete secretion of the specialised venom glands of an animal, and it follows from this that some partially or completely purified fractions of venoms have been classified as toxins.

TABLE II

DISTRIBUTION OF ALGETIC AGENTS

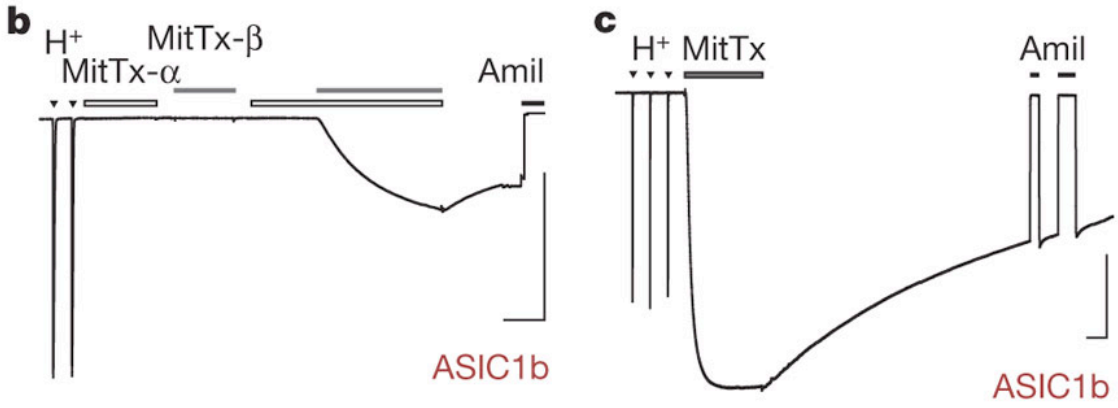
These are substances presumed to be the predominant algetic agents produced by organisms considered in this review

Agent	*Group*	*Site of action*	*Effect*
Histamine	Insects	Local	Sharp pain
5-Hydroxytryptamine	Molluscs		
	Insects		
	Arachnids	Local	Sharp pain
	Coelenterates		
	Spermatophytes		
Acetylcholine	Insects	Local	Sharp pain
	Nettles		
Kinins			
Bradykinin	Crotalid and viperid snakes	Smooth muscle	Local and abdominal pain
	Amphibia		
	Insects		
Polypeptides			
Neurotoxic	Elapid snakes	Neuro-muscular junction	Immediate pain
	Arachnids		
Non-enzymic	Amphibia	Systemic (after absorption)	Severe prolonged pain
	Octapods		
Enzymes			
Phospholipase	Crotalid and viperid snakes	Local	Prolonged pain (following autolysis)
	Insects		
Cholinesterase	Crotalid and viperid snakes	Regional	Paraesthesia
Steroids	Amphibia	Systemic	Abdominal pain
	Spermatophytes		
Glycosides	Spermatophytes	Heart	Cardiac pain
Alkaloids	Amphibia	Systemic	Autonomic stimulation
	Spermatophytes		
Saponins	Echinoderms	Local and systemic	Sharp pain
	Spermatophytes		Abdominal pain
Tetrodotoxin and saxitoxin	Amphibia	Nerve membrane	Abdominal pain (after ingestion)
	Fish		
Ciguatoxin	Food chain	Nerve membrane	Abdominal pain



Heteromeric toxin from Texas coral snake activates somatosensory neurons
a, *M. t. tener* venom (0.1 mg ml^{-1}) activates cultured neurons as assessed by ratiometric calcium imaging. Pooled venom fractions lacking neuron-specific activity (inactive fxns) produced only weak signals in non-neuronal cells (color bar indicates relative calcium levels). **b**, The Texas coral snake. **c**, Homology-based predicted structural models of MitTx subunits.

Source: Bohlen CJ, Chesler AT, Sharif-Naeini R, Medzihradszky KF, Zhou S, King D, Sánchez EE, Burlingame AL, Basbaum AI, Julius D (2011) A heteromeric Texas coral snake toxin targets acid-sensing ion channels to produce pain. Nature 479:410–414.



MitTx activates ASICs **b**, Voltage-clamp recordings show that ASIC1b-expressing oocytes respond to both extracellular acidification (H^+, pH 4) and MitTx (MitTx-α and MitTx-β combined). Toxin-evoked responses were blocked by amiloride (Amil, 1 mM). **c**, MitTx (75 nM)-evoked currents are comparable in magnitude to pH-4-evoked currents in ASIC1b-expressing oocytes. Toxin responses are non-desensitizing and persistent compared with transient proton-evoked currents. Vertical scale bars, 1 μA; horizontal bars, 1 min; V_h = –60 mV.

Source: Bohlen CJ, Chesler AT, Sharif-Naeini R, Medzihradszky KF, Zhou S, King D, Sánchez EE, Burlingame AL, Basbaum AI, Julius D (2011) A heteromeric Texas coral snake toxin targets acid-sensing ion channels to produce pain. Nature 479:410–414.

Do, at least with the Texas coral snake, the pain caused by MitTx is something memorable. But in another example of a toxin produced by a (Black mamba) snake, the opposite effect is observed: Inhibition of the acid-sensing ion channels [1]

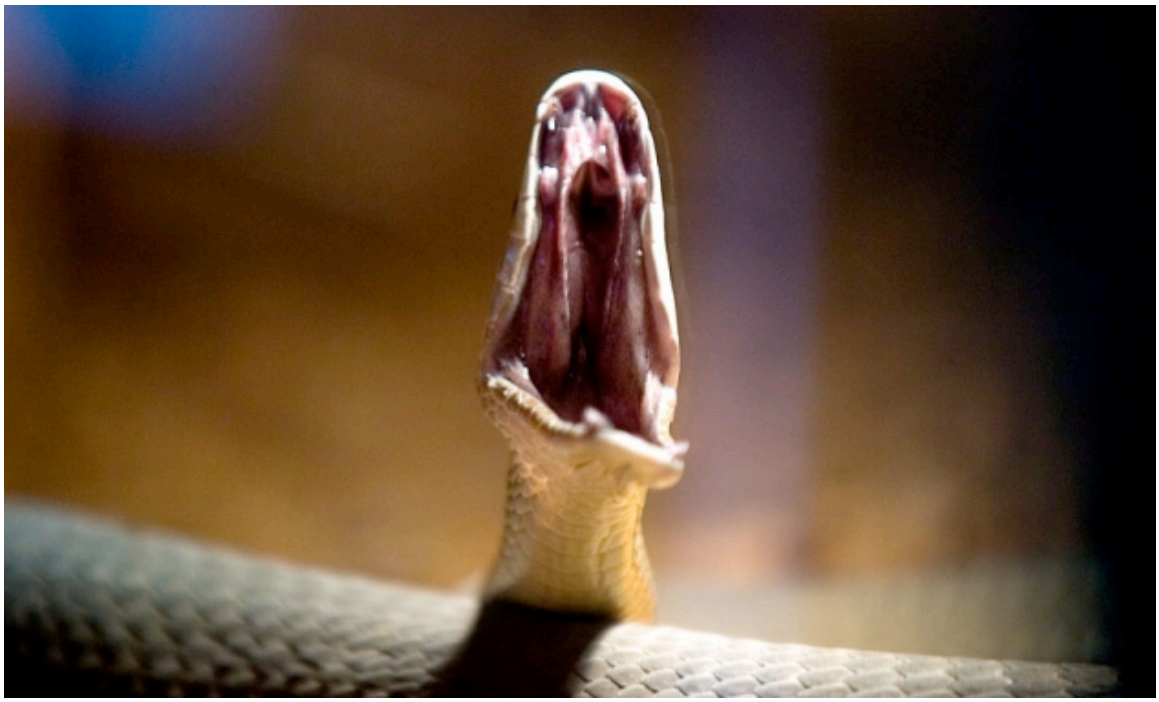
Again, the technique of choice is heterologous expression of ASICs in Xenopus oocytes, followed by perfusion assays. In the case of the Mambalgin toxin, the normal acid-ogation of the ASIC channel disappears. this true for different ~~of~~ isoforms & combinations thereof.

[Overhead]

The "newsy" aspect of the research is that - in a mouse model - the new mambalgins are as efficacious as morphine, but without the side effects

[Overhead]

[1] Diochot S, Baron A, Salinas M, Douguet D, Scarzello S, Dabert-Gay A-S, Debayle D, Friend V, Alloui A, Lazdunski M, Lingueglia E (2012) Black mamba venom peptides target acid-sensing ion channels to abolish pain. Nature 490:552-555.



“Deadly snake venom delivers pain relief. Proteins from the black mamba could inspire painkilling drugs.” By Helen Shen

“With a series of swift bites, the black mamba injects a toxic cocktail that can kill a human within 20 minutes. But among the compounds that squirt from the snake’s fangs, two proteins can block pain in mice as effectively as morphine — and with fewer side effects, according to a study published today in Nature1.

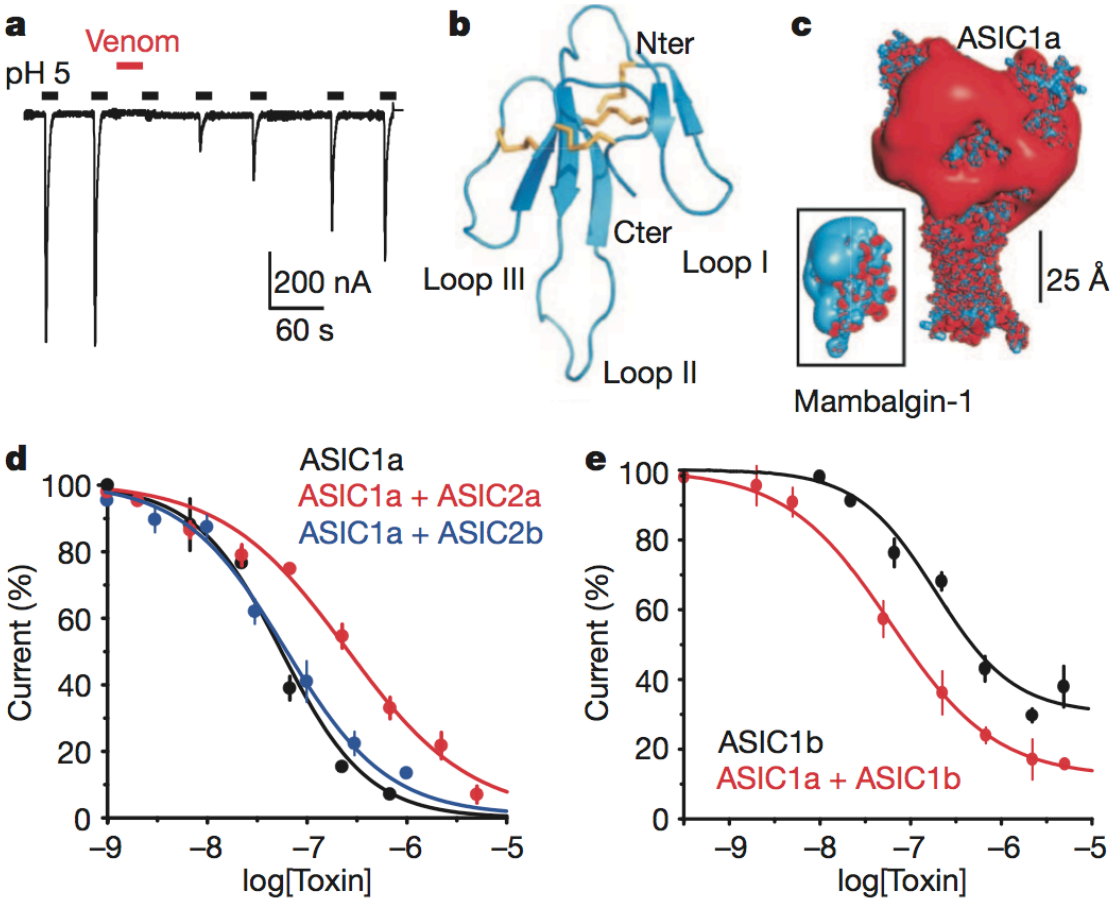
The snake proteins — called mambalgins — were discovered as part of a search for alternatives to opiate drugs such as morphine. Many patients grow tolerant of opiates, requiring higher doses over time, and the drugs often cause side effects such as nausea, constipation and drug dependency.

“It’s important to try to develop new drugs that can have complementary or different types of action,” says Eric Lingueglia, a molecular physiologist at the Institute of Molecular and Cellular Pharmacology in Valbonne, France. He and his colleagues identified the proteins from the black mamba (Dendroaspis polylepis) after testing about 50 different animal venoms.

The team found that mice injected with mambalgins could withstand hot water on their tails and paws for about twice as long as untreated animals. The snake proteins also reduced hypersensitivity to pain following tissue inflammation. Over 5 days of repeated treatment the mice developed a tolerance for both opiates and mambalgins, but the effect was less pronounced with the snake-venom proteins.

Mambalgins also did not slow the mice's breathing rate, a potentially dangerous side effect of opiods that can complicate their use.”

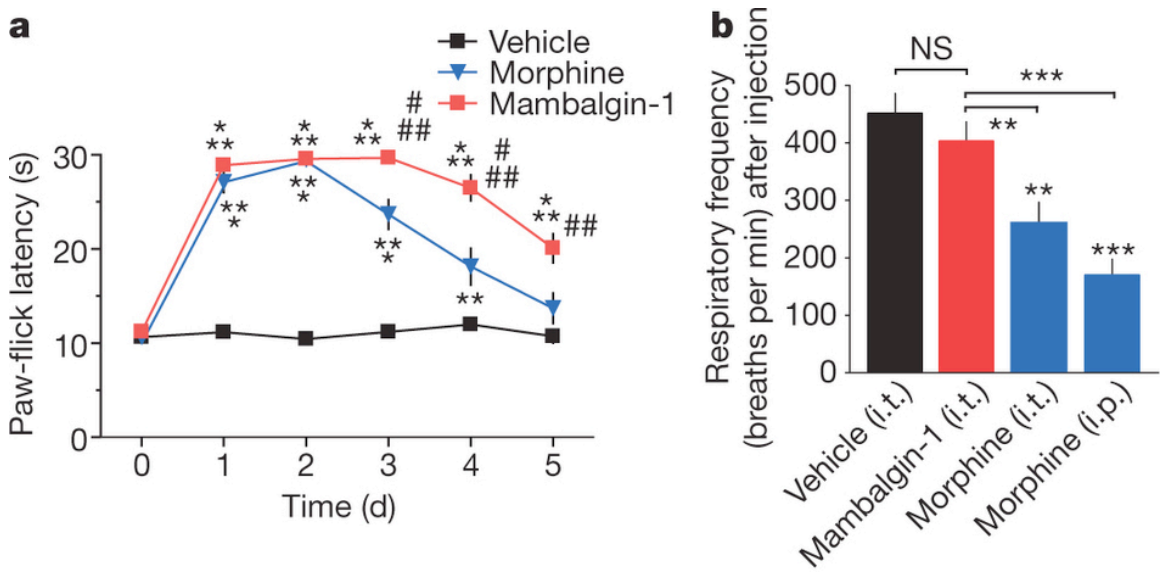
Source: Nature doi:10.1038/nature.2012.11526.



Mambalgins represent a new class of three-finger toxins targeting ASIC channels.

a, Black mamba venom (0.1 mg ml−1) reversibly inhibits rat ASIC1a current expressed in Xenopus oocytes. **b,** Three-dimensional model of mambalgin-1 (disulphide bridges in yellow). **c,** Electrostatic properties of mambalgin-1 and human ASIC1a channel (on the basis of the three-dimensional structure of chicken ASIC1a29) with positive and negative isosurfaces in blue and red, respectively. **d, e,** Inhibition of rat ASIC channels expressed in COS-7 cells (applied before the pH drop as in a).

Source: Diochot S, Baron A, Salinas M, Douguet D, Scarzello S, Dabert-Gay A-S, Debayle D, Friend V, Alloui A, Lazdunski M, Lingueglia E (2012) Black mamba venom peptides target acid-sensing ion channels to abolish pain. Nature 490:552–555.



The central analgesic effect of mambalgin-1 shows reduced tolerance compared with morphine, no respiratory depression and involves the ASIC2a subunit.

a, Repeated intrathecal injections of mambalgin-1 induce less tolerance than morphine at concentrations giving the same analgesic efficacy (n = 10, comparison with vehicle (*) or morphine (#)). **b**, Mambalgin-1 (i.t., intrathecal) induces no respiratory depression unlike morphine (i.t., intrathecal or i.p., intraperitoneal), 0.01 and 0.4 mg per mouse, respectively; n = 4–7, comparison with vehicle unless specified).

Source: Diochot S, Baron A, Salinas M, Douguet D, Scarzello S, Dabert-Gay A-S, Debayle D, Friend V, Alloui A, Lazdunski M, Lingueglia E (2012) Black mamba venom peptides target acid-sensing ion channels to abolish pain. Nature 490:552–555.