Bee afraid, bee very afraid - neonicotinoids and the nAChRs family

By Hsin-Yu Chang*, Louise Daugherty* and Alex Mitchell

Honey has been part of mankind's diet and cultural history since early civilisation; honey collection is shown in Mesolithic cave paintings, and the foodstuff's nutritional and medicinal properties, such as antiseptic and preservative abilities, are documented in ancient Chinese, Greek and Roman texts. The domestication of honey bees is a similarly ancient phenomenon, with early evidence of beekeeping depicted on the walls of Egyptian temples that are over 4,500 years old.

Picture provided by Hsin-Yu Chang

Modern beekeeping is often performed on an industrial scale, with tens of thousands of hives and hundreds of millions of honey bees (*Apis mellifera*). However, in recent years, beekeepers in Europe and the United States have begun to report significant losses in their honey bee populations. This is alarming news, not only because honey could one day disappear from our diets, but because over 80% of the world's commercial crops rely on insects such as bees for pollination [1]. Shrinking honey bee populations could therefore have serious consequences for worldwide food security and a huge economic impact.

Picture provided by Anke Liebert

'Over 80 % of the world's commercial crops rely on insects such as bees for pollination'

The reasons for the decline are currently not clear. However, some studies have linked the reduction in bee numbers to a widely-used class of pesticides, neonicotinoids, that have been broadly administered in large-scale crop production since the mid 1990's – the same time that mass bee disappearances started to be reported [2]. These nicotine-like chemicals, which include three key neonicotinoids: thiamethoxam [3], clothianidin [4,5] and imidacloprid [6,7,8], are considered safe for mammals, but are highly toxic to insects.

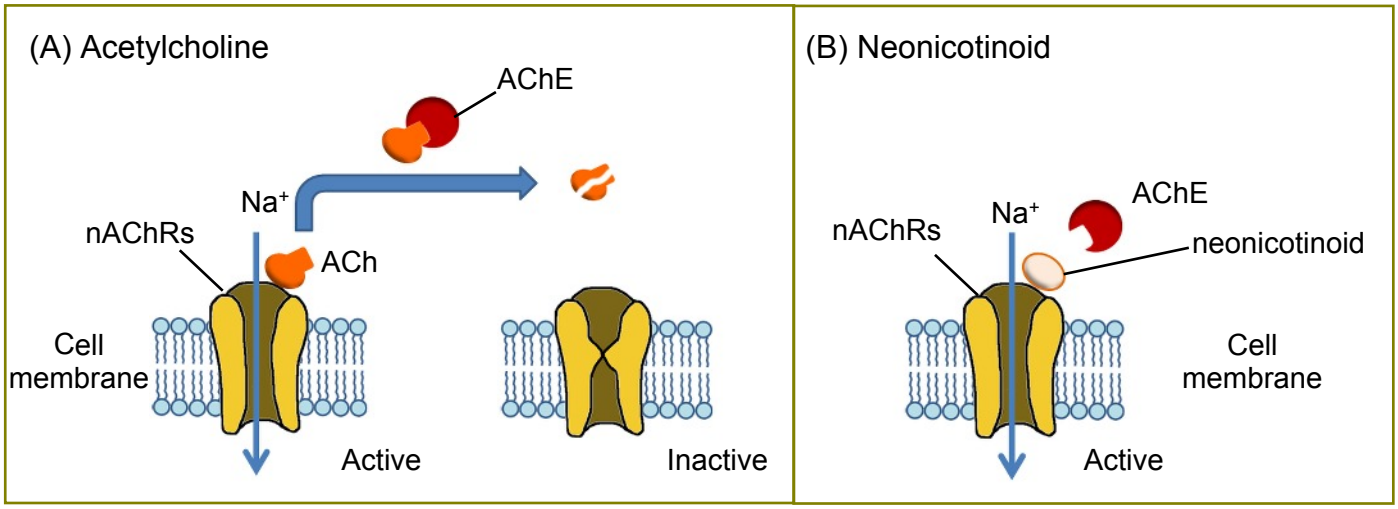

Figure1. Regulation of the nicotinic acetylcholine receptor. In the presence of acetylcholine or neonicotinoid.

Neonicotinoids act by binding to nicotinic acetylcholine receptors (nAChRs), which are found throughout the animal kingdom. nAChRs are expressed in the central nervous system (CNS) and are involved in synaptic transmission. Normally, the receptors are activated by binding a neurotransmitter known as acetylcholine (ACh). They are then inactivated when ACh is broken down by an enzyme called acetylcholinesterase (AChE), forming acetate and choline (Figure1A) [9,10,11,12].

Like ACh, neonicotinoids can bind and activate nAChRs. But, unlike ACh, they cannot be broken down by AChE [13] (Figure1 B). As a result, they cause over-stimulation of the nervous system, and eventually, death.

The different toxicity of neonicotinoids towards insects and mammals may be due to the differences in their nAChRs. Insect nAChRs have been shown to have a higher affinity for neonicotinoids than their mammalian counterparts [14,15].

To understand how ACh and neonicotinoids work, we need to examine the structure of nAChRs. In InterPro, the nAChR family (IPR002394) belongs to a wider family called the neurotransmitter-gated ion-channels (IPR006201). Members of this large family mediate chemical synaptic transmission and share similar structure, which is composed of an extracellular ligand-binding domain (IPR006202), a transmembrane domain that forms the ionic channel (IPR006029), and a cytoplasmic region (Table1 and Figure 2).

‘Neonicotinoids have been shown to have higher affinity to insect nAChRs than their mammalian counterparts’

To understand nAChRs in more detail, scientists have examined a closely-related protein, known as acetylcholine-binding

protein (AChBP), from fresh water snails (*Lymnaea stagnalis*) and from saltwater molluscs (*Aplysia californica*) [16]. AChBPs don't have the transmembrane domain and the cytoplasmic region found in nAChRs. As a result, they are soluble and can be crystallised and used in the structural studies. Moreover, AChBPs have nearly all ligand binding residues that are conserved within the nAChR family and can be used to represent the extracellular ligand-binding domain of a subtype of nAChRs.

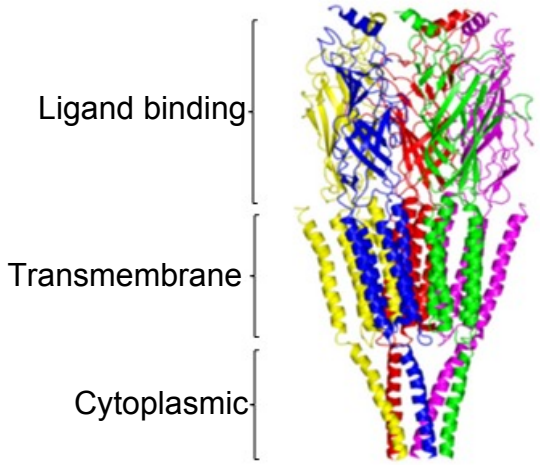

Figure 2. Structure of an neurotransmitter-gated ion-channel (PDB: 2bg9) [18].

Interestingly, the *Aplysia* AChBP has high neonicotinoid sensitivity, while *Lymnaea* AChBP has low neonicotinoid sensitivity. As a result, scientists have employed these two proteins to serve as structural surrogates, since their pharmacological profiles are reminiscent of insect and vertebrate nAChRs respectively [16,20].

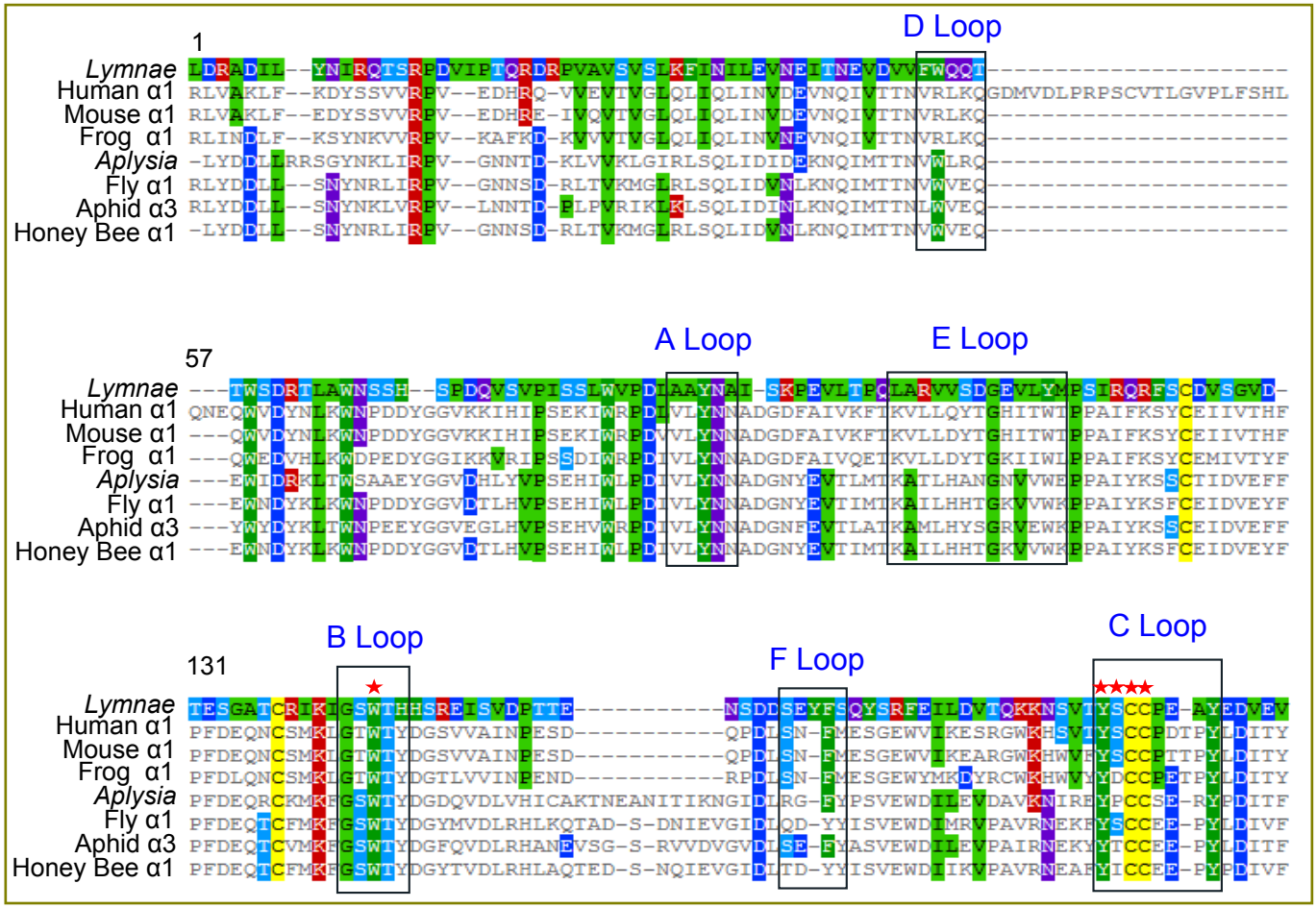

Figure 3. Sequence alignment of *Lymnae*-AChBP and *Aplysia*-AChBP with several nAChRs from different species. Potential sites that influence the interaction between AChBPs and neonicotinoids are marked (★).

In the crystallisation studies, both AChBPs form a homopentamer that exists in a least three conformational states; resting (closed), activated (open) and desensitised (closed) [17]. The open or closed states of the receptor seem to be controlled by the conformation of a C-loop found in the extracellular ligand-binding domain [18] (Figure 3). The C-loop encloses the ligand binding pocket and can be stabilised by the binding of imidacloprid (IMI), which leads to the opening of the receptor [19].

A numbers of sites in the *Aplysia* and the *Lymnaea* AChBPs have been found to be important for the interaction with IMI, including two cysteines, a serine and a tyrosine in the C-loop, and a tryptophan in the B-loop [20] (Figure 3). From photoaffinity labelling and X-ray crystallography studies [20], IMI has been found to bind to a single dominant conformation of the *Aplysia* AChBP (insect model, Figure 4B) and two conformations of the *Lymnaea* AChBP (vertebrate model, Figure 4A). The mixture of two conformations of the *Lymnaea* AChBP has been linked to the inferior affinity of neonicotinoids for this protein [20] (Figure 4). This may account for the selective toxicity of IMI to insects but not humans.

So far, most toxicity studies have been performed in the lab, since field studies assessing toxicity directly are rather challenging. Determining bee exposure to neonicotinoids in the wild is, in itself, complicated; in the agricultural industry, seeds are usually soaked in neonicotinoids before planting, with traces being found in the nectar and pollen. It is not clear how much neonicotinoid-containing nectar and

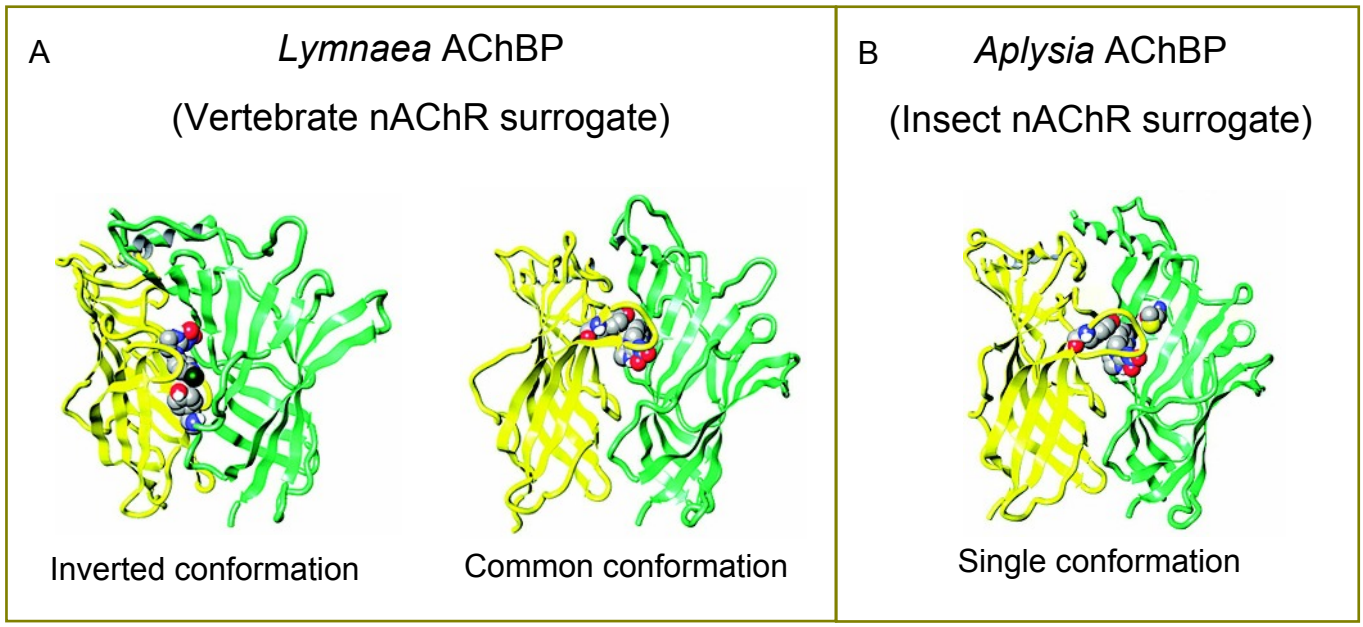

Figure 4. Binding of IMI with the *Aplysia* and the *Lymnaea* AChBPs. (A) IMI binds *Lymnaea* AChBPs in two conformations. (B) IMI binds to *Aplysia* AChBP in only one conformation. IMI is buried in the interfacial agonist binding pocket between the primary or (+)-face (yellow) and complementary or (−)-face (green) subunits. Pictures are modified from Tomizawa M. And Casida J. E. 2009. [20]

pollen is collected in bee hives, or how much can be metabolised by the bees or their larvae [21]. As a result, the effects of neonicotinoid use on bee populations have not been demonstrated conclusively.

In addition, small amount of neonicotinoids may not kill bees directly, but could impair their immune system and a number of behavioural traits, such as communication, homing and foraging abilities [22,23,24]. This, in combination with several other factors, such as fungicides, parasites and the loss of plant diversity, may all contribute to the disappearance of the bees.

The important thing is that we find out quickly what is actually killing these precious creatures and act fast to rescue the bees and our ecosystem!

Policies on neonicotinoid use:

- The EU has adopted a proposal to restrict the use of 3 pesticides belonging to the nenicotinoid family (clothianidin, imidacloprid and thiametoxam) for a period of 2 years from 1 December 2013
- The UK did not support a ban due to the lack of conclusive scientific evidence
- In USA, the EPA is not currently banning or severely restricting the use of the neonicotinoid pesticides. The neonicotinoid pesticides are currently being re-evaluated

Entry Type	InterPro ID	Entry Name	Signatures	UniProt Protein Matches	PDB Protein Structure
Family	IPR006201	Neurotransmitter-gated ion-channel	TIGR00860 PTHR18945 PR00252	~2168	 2xq3
Family	IPR002394	Nicotinic acetylcholine receptor	PR00254	~2168	 1tor
Family	IPR001445	Acetylcholinesterase, insect	PR00880	~143	 1qo9
Domain	IPR006202	Neurotransmitter-gated ion-channel ligand-binding	PF02931 G3DSA:2.70.170.10 SSF63712	~6757	 1uw6
Domain	IPR006029	Neurotransmitter-gated ion-channel transmembrane domain	PF02932 SSF90112	~6306	 1eq8
Site	IPR018000	Neurotransmitter-gated ion-channel, conserved site	PS00236	~5364	

Table 1. InterPro entries which are related to the nicotinic acetylcholine receptor.

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