

Title: Modification of olfactory learning and memory induced by RNA interference targeting $\alpha 7$ nicotinic acetylcholine subunit in the honeybee

Authors : LOUIS Thierry¹, AHIER Arnaud², RAYMOND DELPECH Valérie¹ & GAUTHIER Monique¹

¹-Centre de Recherches sur la Cognition Animal- Université Paul Sabatier, 118 Route de Narbonne 31062 Toulouse Cedex 9

² Institut Pasteur de Lille, 1 rue du Professeur Calmette, 59019 Lille, France

Acetylcholine is the major excitatory neurotransmitter in the central nervous system of insects and targets the numerous nicotinic acetylcholine receptors (nAChRs). The recent honeybee genome sequencing has described 11 α and non- α nicotinic subunits, but the molecular composition of the nAChRs remains unknown, in honeybees as in other invertebrates. Many studies have already demonstrated the involvement of nAChRs in olfactory learning and memory using nicotinic antagonists injection into the honeybee brain (Gauthier et al., 2006). Two nAChR subtypes have been described: α -Bungarotoxin (α -Bgt)-sensitive nAChRs necessary for long-term memory and α -Bgt-insensitive nAChRs needed for retrieval processes. In vertebrate, it is well known that α -Bgt-sensitive nAChRs are homomeric and made of $\alpha 7$ subunits that are phylogenetically conserved. The role of $\alpha 7$ nicotinic subunits was studied in the honeybee learning using siRNA to block $\alpha 7$ subunit expression. Quantitative PCR analysis revealed that siRNA reduced $\alpha 7$ expression from 3 h to 18 h after injection. Honeybees injected with siRNA 18 h before multiple-trial olfactory conditioning had poor learning and memory performance compared to controls. siRNA injected 18 h before the 24 h-retrieval test had no effect on performance, excluding an effect on retrieval processes. This result also indicates that olfactory perception was not depending on the presence of $\alpha 7$ subunit. In conclusion, deletion of $\alpha 7$ nicotinic subunit specifically impairs olfactory conditioning leading to low performances during retrieval test. As a consequence, nicotinic acetylcholine receptors including $\alpha 7$ subunit seem to be necessary for olfactory learning but not for retrieval processes. Participation of $\alpha 7$ subunit in the formation of long-term memory will be investigated by inducing $\alpha 7$ deletion during the consolidation phase of memory.