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Microtransplantation of tick transmembrane proteins as a strategy to screen new compounds and to study transmembrane proteins from arthropod vectors of diseases

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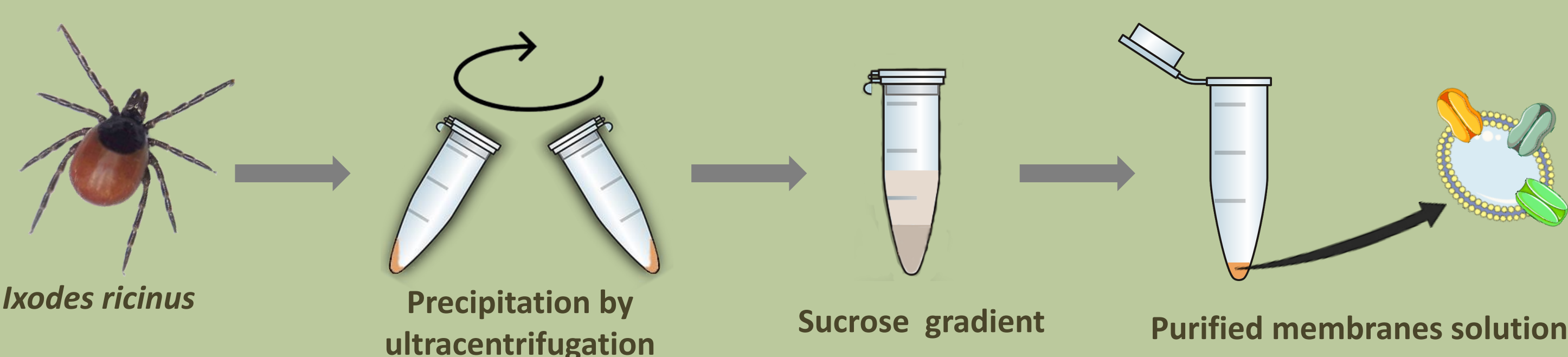
Introduction

Ticks are known as harmful parasites and vectors of many microorganisms that could be pathogenic for animal and human. For example, the castor bean tick, *Ixodes ricinus*, can transmit diseases like Lyme borreliosis and tick-borne encephalitis, becoming a worldwide threat to public health (Boulanger *et al.*, 2019). Due to the lack of effective vaccines or other non-chemical based methods, acaricides remain the main way for tick control. However, there is now increasing evidence of resistance development against currently used acaricides (Kumar *et al.*, 2019). Thus, new compounds are needed and one strategy could be to target tick neuronal nicotinic acetylcholine receptors (nAChRs). In the present work, we aim to validate the membrane microtransplantation technique as a tool to study the pharmacological profiles of the tick nAChRs expressed in the synganglion. This technique allows to microtransplant the transmembrane receptors maintained in their natural lipidic environment. Thus, the studied native receptors keep their natural properties and regulation (Eusebi *et al.*, 2009).

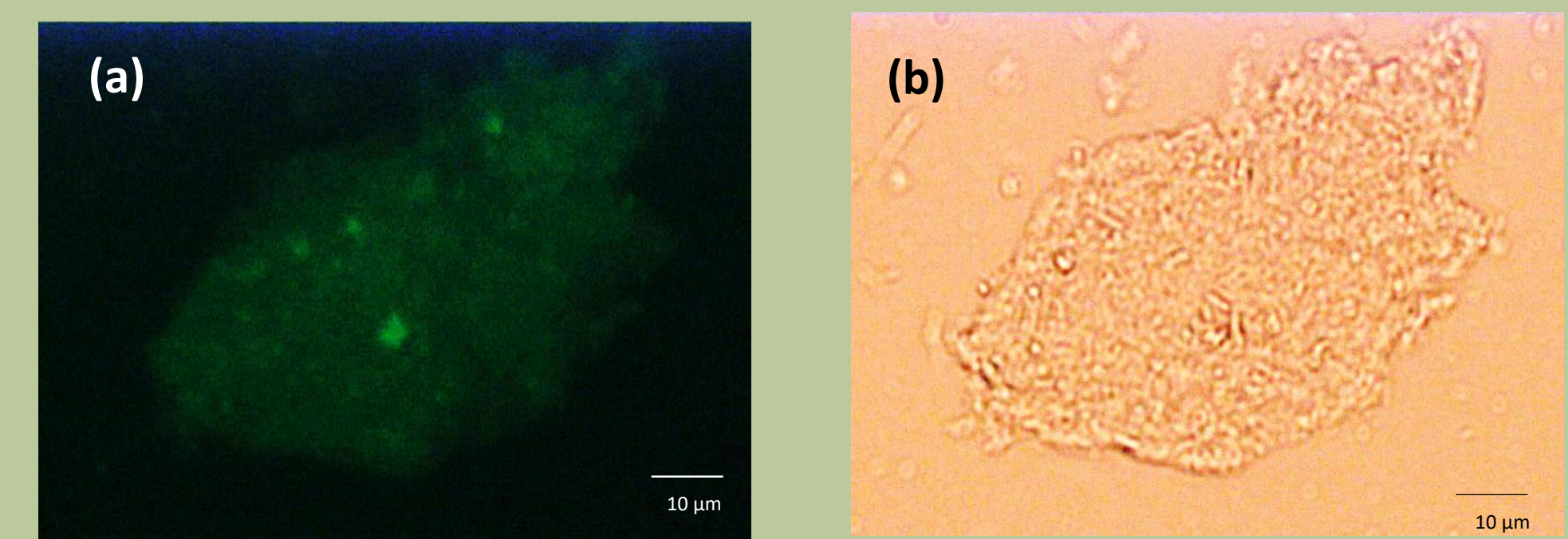
Extraction and purification of tick synganglion membranes

The membrane extraction and purification protocol consists in two crucial steps:

- The first is the dissociation of the neuronal tissues of *I. ricinus* (synganglion) and the precipitation of the transmembrane proteins by ultracentrifugation.
- The second is the purification of the membrane containing the transmembrane proteins of interest by using a sucrose gradient.

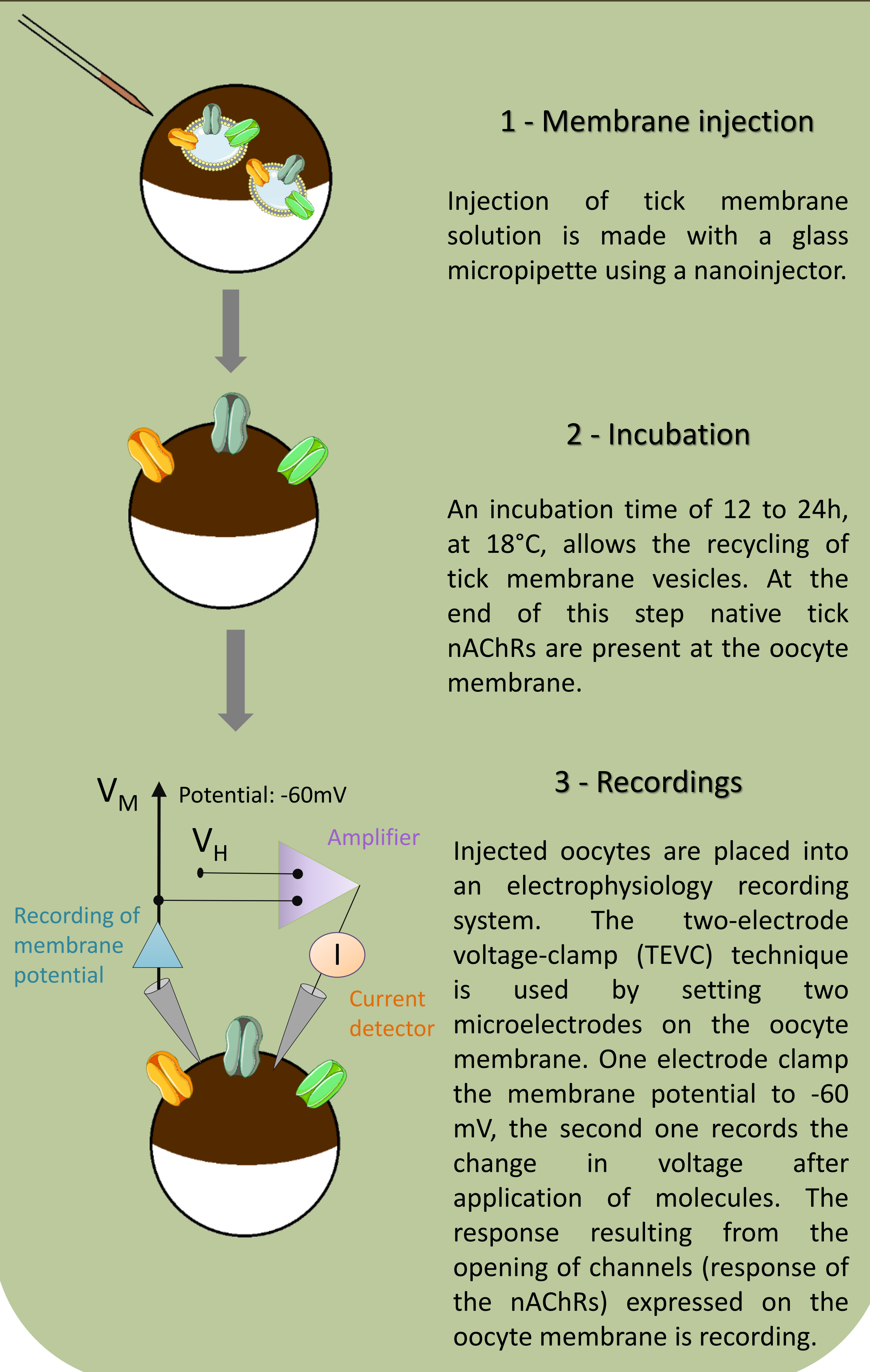


To validate the membrane preparation protocol we used α -bungarotoxin conjugated with Alexa Fluor[®]488 to label functional nAChRs.



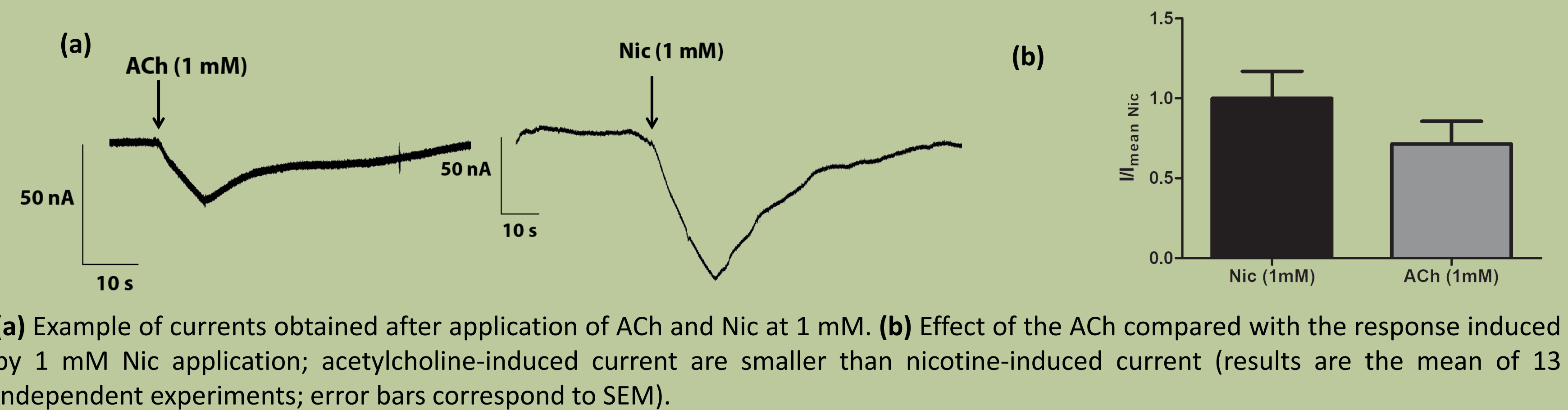
Fluorescent visualisation of the α -bungarotoxin-sensitive nAChRs using α -bungarotoxin conjugated with Alexa Fluor[®]488 in membrane preparation from the tick *Ixodes ricinus*. (a) membranes labeled with α -bungarotoxin conjugated with Alexa Fluor[®]488, each fluorescent spot highlighted the presence of functional receptors; (b) same preparation with transmitted light showing the membrane aggregates.

Electrophysiological recordings of nAChRs expressed on the oocyte membrane



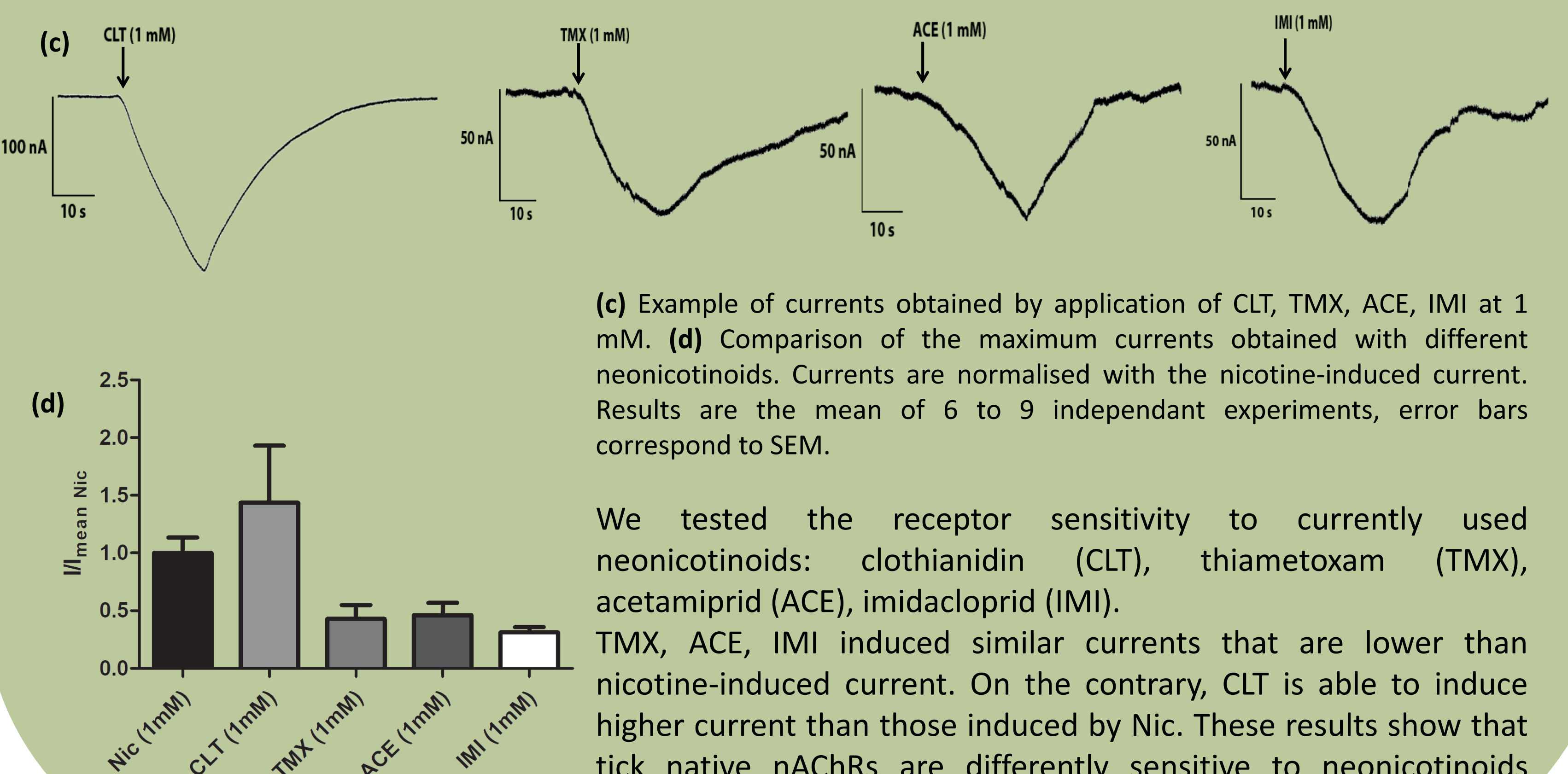
Native nAChRs of *Ixodes ricinus* are sensitive to currently used neonicotinoids

Validation of the nAChRs expression on the oocyte membrane



We tested the activation of tick nAChRs from synganglion membrane preparation by acetylcholine (ACh) and nicotine (Nic). Both molecules are able to activate the receptors, and nAChRs are more sensitive to nicotine than to acetylcholine. These pharmacological properties are typical of nAChRs.

Native nAChRs are differently modulated by neonicotinoids according to molecules.



We tested the receptor sensitivity to currently used neonicotinoids: clothianidin (CLT), thiametoxam (TMX), acetamiprid (ACE), imidacloprid (IMI). TMX, ACE, IMI induced similar currents that are lower than nicotine-induced current. On the contrary, CLT is able to induce higher current than those induced by Nic. These results show that tick native nAChRs are differently sensitive to neonicotinoids according to molecules.

Conclusion

- We validated and adapted the membrane microtransplantation protocol for the pharmacological characterisation of tick native nAChRs.
- We applied this technique for the screening of four neonicotinoids. Preliminary results highlighted that tick nAChRs are highly sensitive to clothianidin.
- To complete this study, we aim to adapt this technique to other arthropod species and for the screening of other molecules.

This study gives promising results for the screening and selection of new anti-parasitic compounds targeting arthropod transmembrane proteins.